# *Comparative metabolic and hepatic effects of liraglutide, elafibranor and obeticholic acid in diet-induced and genetically obese mouse models of biopsy-confirmed NASH*

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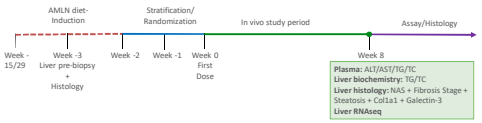
### *Introduction and Aim*

The GLP-1 analogue liraglutide, the peroxisome proliferator activated receptor (PPAR) α/δ agonist, elafibranor, and the farnesoid x receptor agonist, obeticholic acid (OCA), are currently undergoing clinical investigation for the treatment of nonalcoholic steatohepatitis (NASH). The aim of the present study was to compare metabolic and histopathological effects of liraglutide, elafibranor and OCA in a diet-induced and a genetically obese animal model of biopsy-confirmed NASH – the Gubra DIO-NASH and the Gubra ob/ob-NASH mouse.

### *Methods*

Male C57BL/6J and leptin-deficient lep$^{ob/ob}$ mice were either fed chow or a diet high in trans-fat, fructose and cholesterol for a total of 26 and 12 weeks to induce DIO-NASH and ob/ob-NASH mice, respectively. After diet-induction, a liver biopsy was performed for histological evaluation of individual disease progression. Only biopsy-confirmed steatotic and fibrotic animals (Steatosis Score ≥2; Fibrosis Stage ≥1) were included and randomized into treatment groups receiving either vehicle, liraglutide, elafibranor or OCA for 8 weeks. Primary endpoints included a blinded histological assessment for individual and combined components of the NAFLD Activity Score (NAS) (steatosis, inflammation, ballooning degeneration) including Fibrosis Stage. Secondary endpoints included metabolic parameters and histological quantitative assessment.

### *Study Design*




| Group # | Animal | Gender | Strain | Number of animals | Treatment | Administration form | Dosing volume | Daily Dosing concentration |
|---|---|---|---|---|---|---|---|---|
| 1 | DIO-NASH/ ob/ob-NASH | Male | C57BL6J/ Lepᵒᵇ/ᵒᵇ | 10-12 | **Vehicle** | QD, PO | 5 ml/kg | - |
| 2 | DIO-NASH/ ob/ob-NASH | Male | C57BL6J/ Lepᵒᵇ/ᵒᵇ | 10-12 | **Liraglutide** | BID, SC | 5 ml/kg | **0.4 mg/kg** |
| 3 | DIO-NASH/ ob/ob-NASH | Male | C57BL6J/ Lepᵒᵇ/ᵒᵇ | 10-12 | **Elafibranor** | QD, PO | 5 ml/kg | **30 mg/kg** |
| 4 | DIO-NASH/ ob/ob-NASH | Male | C57BL6J/ Lepᵒᵇ/ᵒᵇ | 10-12 | **OCA** | QD, PO | 5 ml/kg | **30 mg/kg** |
| 5 | LEAN-CHOW ob/ob-CHOW | Male | C57BL6J/ Lepᵒᵇ/ᵒᵇ | 10 | **Vehicle** | QD, PO | 5 ml/kg | - |

### *The biopsy procedure*

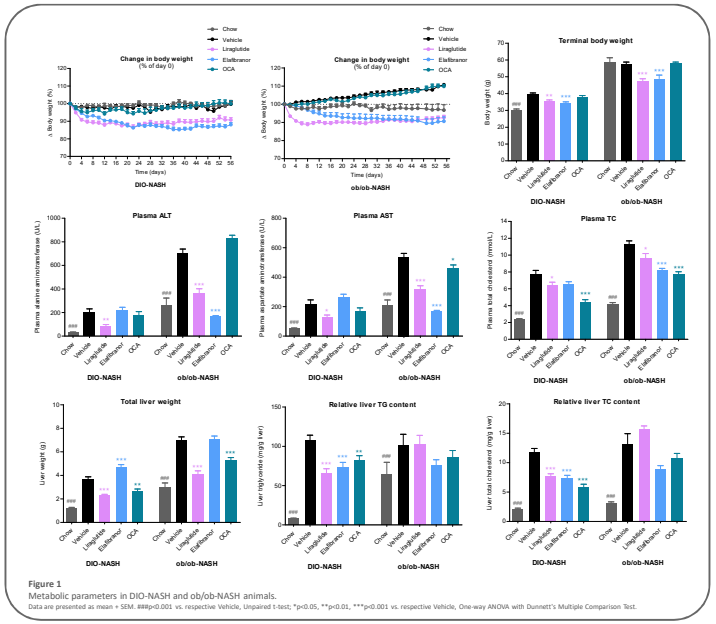



**Figure 1**
Metabolic parameters in DIO-NASH and ob/ob-NASH animals.
Data are presented as mean + SEM. ###p<0.001 vs. respective Vehicle, Unpaired t-test; *p<0.05, **p<0.01, ***p<0.001 vs. respective Vehicle, One-way ANOVA with Dunnett's Multiple Comparison Test.

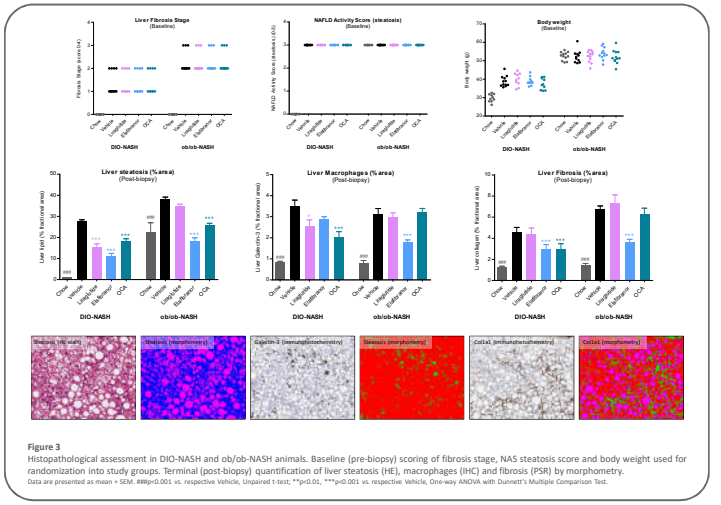



**Figure 3**
Histopathological assessment in DIO-NASH and ob/ob-NASH animals. Baseline (pre-biopsy) scoring of fibrosis stage, NAS steatosis score and body weight used for randomization into study groups. Terminal (post-biopsy) quantification of liver steatosis (HE), macrophages (IHC) and fibrosis (PSR) by morphometry.
Data are presented as mean + SEM. ###p<0.001 vs. respective Vehicle, Unpaired t-test; **p<0.01, ***p<0.001 vs. respective Vehicle, One-way ANOVA with Dunnett's Multiple Comparison Test.

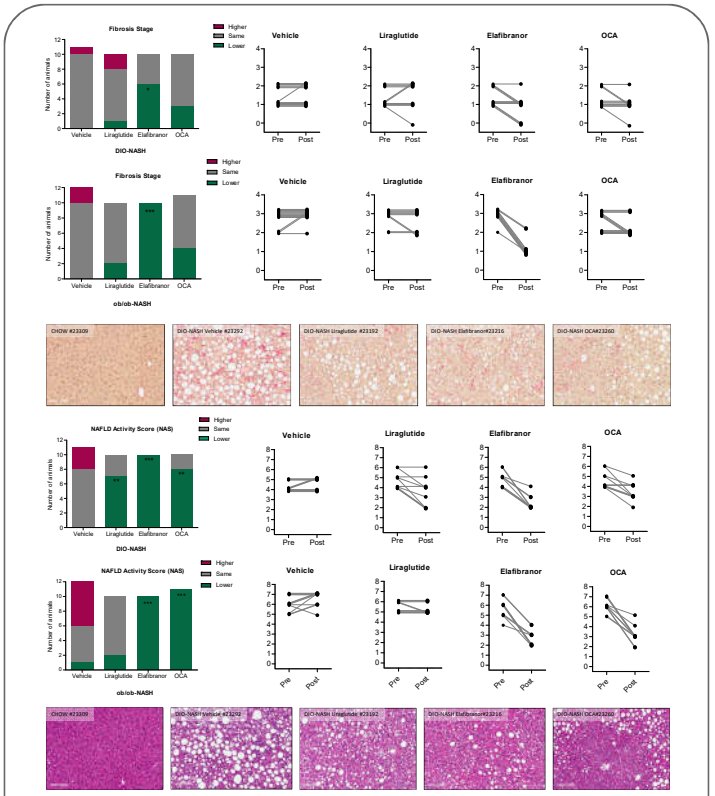



**Figure 2**
Summary of histopathological scoring of the pre- and post-study biopsies for all DIO-NASH and ob/ob-NASH animals separated on groups.
For each compound group, significance of number of animals with a lower score versus respective Vehicle was assessed using Fisher's exact test followed by adjustment for multiple correction using the Bonferroni method. *p<0.05, **p<0.01, ***p<0.001.

### *Conclusion*

- The Gubra DIO-NASH mouse model exhibits hallmark features of biopsy-confirmed NASH (hepatosteatosis, inflammation and fibrosis) and metabolic disease.
- The Gubra ob/ob-NASH mouse exhibits an accelerated and aggressive metabolic fibrotic NASH model.
- Pharmacological intervention with liraglutide, elafibranor and OCA induced a diverse metabolic profile.
- All treatments exerted an anti-steatohepatitis action and improved liver histopathology by reducing NAFLD Activity Score in DIO-NASH animals. In conjunction, elafibranor and OCA reduced NAFLD Activity Score in ob/ob-NASH mice.
- OCA reduced liver fibrosis by morphometric analysis in DIO-NASH mice and trended to reduced Fibrosis Stage in DIO-NASH and ob/ob-NASH animals.
- Elafibranor exerted anti-fibrotic effect and reduced Fibrosis Stage in DIO-NASH and ob/ob-NASH animals .