Animal Model Sharing – NASH, or Non-Alcoholic Steatohepatitis

Introduction:

NASH, or Non-Alcoholic Steatohepatitis, is the manifestation of a metabolic disorder in the liver. Clinical symptoms are often absent or non-specific, why patients may not be aware of the condition until the late stages of the disease.
It is characterised by hepatic steatosis and inflammation not derived from alcohol consumption or other competing aetiologies.
In its most severe manifestation, it can progress to cirrhosis or hepatic carcinoma.

Semaglutide is a long-acting GLP-1 receptor agonist currently being evaluated in clinical trials for NASH.

Aim:

We aim to help you find the relevant doses and preclinical models to evaluate semaglutide in NASH and hepatic fibrosis.
With this leaflet we present the effect of 2-3 doses semaglutide (0.3-30 nmol/kg) in two preclinical mouse models:

- metabolic NASH with mild fibrosis in the DIO NASH model, and
- hepatic inflammation and fibrosis in in the mechanistic CDA HFD model.

Methods:

Male C57BL/6J mice (7-8 weeks old) were allowed one week to acclimatise after arrival from vendor.
Mice were induced to liver disease on either

- a high-fat diet (60%) lacking choline with 0.1% methionine (A060711302 from Research Diets, CDA-HFD) for 6 weeks prior to treatment start, or
- a 40% fat, 2% cholesterol and 22% fructose diet (D09100310 from research Diets, GAN diet) for at least 28 weeks.

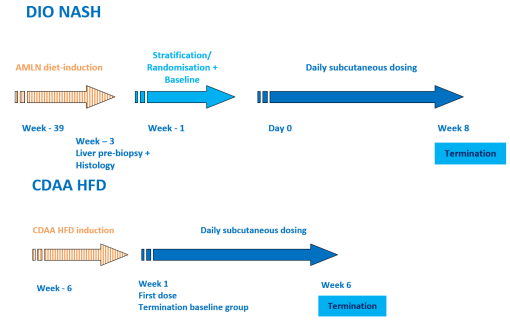
Mice on chow diet were included for reference.
To reflect the disease state at the start of treatment, either a liver biopsy[2] was taken or a group was terminated a dose start (baseline group), and analysed for histology and other endpoints. (n=15-16 per group).

Semaglutide was uptitrated according to the schedule in Refinement section to avoid dehydration and GI discomfort.

Semaglutide or vehicle was administered s.c daily for 8-12 weeks (DIO NASH) or 6 weeks (CDA HFD), while body weigh was monitored.

At study termination blood was sampled via the tail vein for analysis of liver enzymes and plasma lipids (ALT, AST, TG and TC).
Animals were euthaised in isofluorane anastesia, liver tissue was collected weighed and subjected to TG and TC xtraction, or embedded for histology.

Fig. 1



Results:

Semaglutide dose-dependently reduced body weight, ALT and AST in both models (Fig 2) while reducing hepatic triglyceride only in the DIO-NASH model (-24, -31 and -50%) but as expected not in the mechanistic CDA-HFD model. (Table 1)

Fig. 2

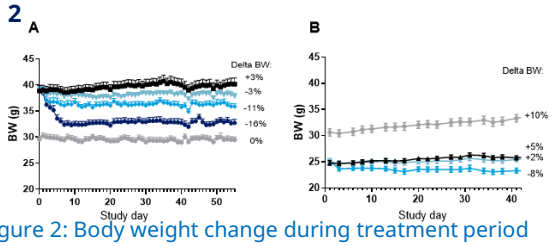


Figure 2: Body weight change during treatment period A) DIO NASH B) CDA HFD

Table 1

Model	Chow	DIO-NASH	DIO-NASH semaglutide	DIO-NASH semaglutide	DIO-NASH semaglutide	Chow	CDA-HFD	CDA-HFD	CDA-HFD semaglutide	CDA-HFD semaglutide
Dose	vehicle (n=10)	vehicle (n=12)	0.3 nmol/kg (n=12)	1.8 nmol/kg (n=12)	30 nmol/kg (n=12)	vehicle (n=13)	Baseline (n=15)	Vehicle (n=10)	0.3 nmol/kg (n=14)	1.8 nmol/kg (n=12)
Liver weight (g)	1.3 ± 0.1***	3.8 ± 0.3	3.1 ± 0.2*	2.4 ± 0.1***	2.1 ± 0.1***	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.2 ± 0.1***
Liver index (% of BW)	4.5 ± 0.1***	9.3 ± 0.4	8.2 ± 0.3*	6.6 ± 0.3***	6.2 ± 0.3***	4.9 ± 0.1***	6.7 ± 0.2	6.8 ± 0.1	6.2 ± 0.1*	5.4 ± 0.2***
Liver TG (mg/g tissue)	8.7 ± 0.8***	113 ± 7	86.0 ± 7.2*	78.0 ± 3.8***	55.8 ± 4.6***	11.6 ± 0.8***	132 ± 9.0***	57 ± 7.4	64 ± 7.4	55.0 ± 5.4
Liver TC (mg/g tissue)	2.5 ± 0.2***	10.4 ± 0.7	9.7 ± 0.9	9.2 ± 0.6	6.9 ± 0.5***	2.0 ± 0.1	2.7 ± 0.1***	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
terminal plasma ALT (U/L)	24.2 ± 1.5***	197.3 ± 26.2	143.6 ± 16.8*	75.5 ± 15.4***	34.7 ± 2.9***	74.0 ± 13.8** *	518 ± 21.5** *	325 ± 20.6	293 ± 14.5	222 ± 20.7***
Terminal plasma AST (U/L)	68.2 ± 18.8***	224 ± 26	183 ± 19	136 ± 14**	88.0 ± 5.0***	55.8 ± 5.4***	337 ± 13.0*	287 ± 15.4	257 ± 14.3	214 ± 14.7***

Table 1: TG: triglyceride ALT: alanine aminotransferase AST: Aspartate transaminase Oneway ANOVA with Dunnett's correction, all groups tested against NASH vehicle *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001

Semaglutide reduced hepatic inflammation based on morphometry of CD11b positive leukocytes (Fig. 3) in both models.

Fig. 3

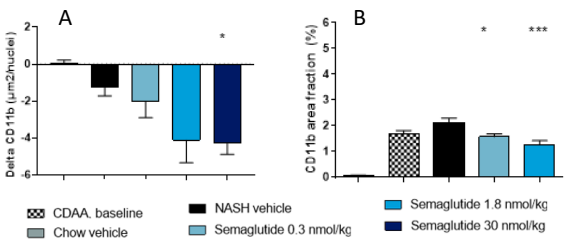


Figure 3 - A) Delta CD11b area% (pre- and post treatment biopsy) in DIO NASH mice. B) CD1B area% in CDA HFD mice

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Results cont.

Fig. 4

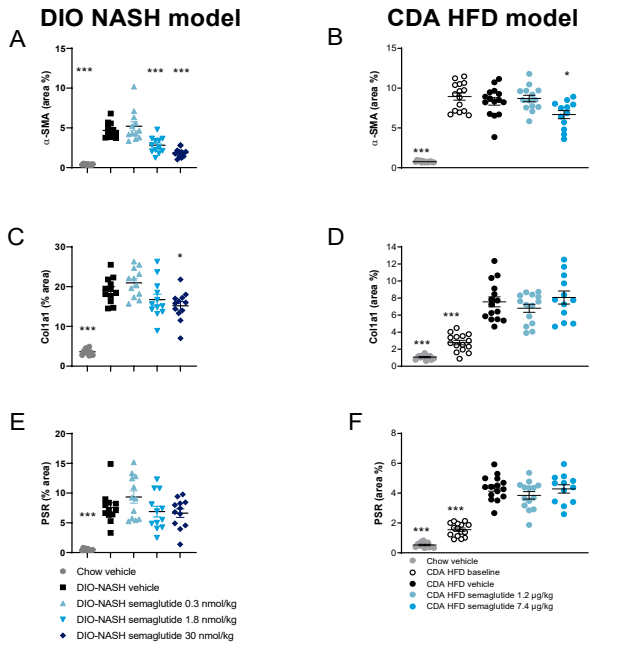

Figure 4 - Immunohistochemistry of fibrogenesis in DIO NASH and CDA HFD mice. A) and B): aSMA area%. C) and D): Collagen 1a area%. E) and F): PSR area%

6-8 weeks treatment with 1.8-30 nmol/kg semaglutide reduced aSMA, a marker of hepatic stellate cell activation (Fig. 4, A and B) and reduced tissue collagen 1a1 (Fig 4 C) in the DIO NASH using 30 nmol/kg semaglutide.

Conclusions:

We have presented two different mouse models of NASH, and their response to GLP-1 treatment.
In both models, we saw improvement in hepatic inflammation and stellate cell activation (fibrogenesis) after 6-8 weeks treatment with semaglutide.

A summary of the expected effects of GLP-1 treatment in the models is illustrated below:

Endpoint	DIO NASH mouse		CDA HFD mouse	
	Vehicle vs chow	GLP-1 Tx vs vehicle	Vehicle vs chow	GLP-1 Tx vs vehicle
Body weight	↑	↓	↓ Reduced weight gain in growth phase	↓ Monitor for excess weight loss
ALT	↑	↓	↑	↓
Insulin sensitivity	↓ Mildly decreased	↑	↑	← Small window
Steatosis	↑	↓	↑	←
Hepatic inflammation	↑	↓	↑	↓
HSC activation	↑	↓	↑	↓
PSR fibrosis	↑	← Need longer Tx	↑	← Need longer Tx

Animal welfare considerations:

Replacement:
Has non-animal methods been applied before using the animal model, e.g. 3D primary human cell-based in vitro model[1]

Reduction:
In this animal model, a group size of 15-16 has been calculated with a power of 0.8.
Please consider if all control grous always are required if you perform multiple studies.

Refinement:
The NASH animal model requires studies of longer duration as the progression of hepatic steatosis is slow. The duration of a study must be adjusted according to the individual study aim and scientific endpoints are met as early as possible.
Animals must be group-housed. Single-housing may be justified if exessive cage-aggression occours.
Environmental enrichment (bedding, hides, nesting material, gnawing objects) must be provided.
Mice sholuld preferably be handled by cupping or tunnel, not picked up by the tail.
Micro sampling of blood should be utilised.
GLP-1s, including semaglutide, should be uptitrated over several days to avoid dehydration and GI discomfort, se schedule below:

Day	1	2	3	4	5
Dose (nmol/kg)	1	4	6	15	30
Konc (nmol/ml)	2	2	2	15	15
Vol (ml/kg)	0,5	2	3	1	2

Humane Endpoints (HE) must be established – general as well as model specific.
One example of a model specific HE:

- Blood glucose monitoring in diabetic animals with a cut-off value for euthanasia.

In this model ALT and AST are not increased to a level which makes them suitable for biomarkers.
Ascites and anaemia is normally not seen if the described model set-up is applied.

References:

1. Ströbel, S et al: A 3D primary human cell-based in vitro model of non-alcoholic steatohepatitis for efficacy testing of clinical drug candidates. Scientific Reports volume 11
2. Baandrup Kristiansen, M N et al: Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy - PMC (nih.gov)

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