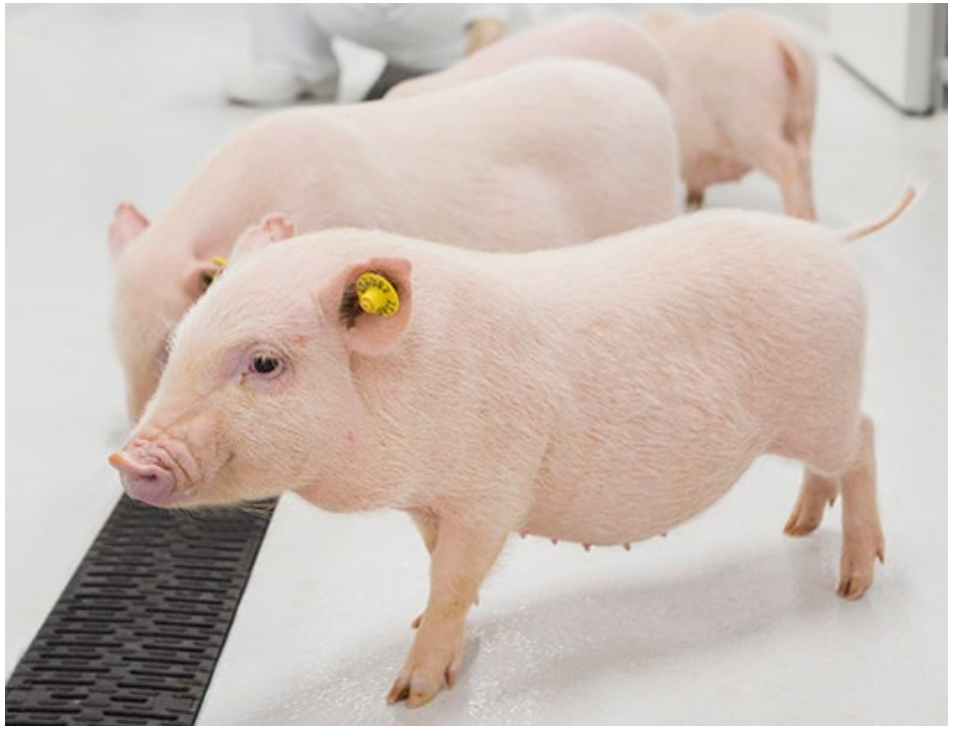

Göttingen Minipig MASH model

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RESEARCH ARTICLE

Translational Physiology

Choline-deficient, high-fat diet-induced MASH in Göttingen Minipigs: characterization and effects of a chow reversal period

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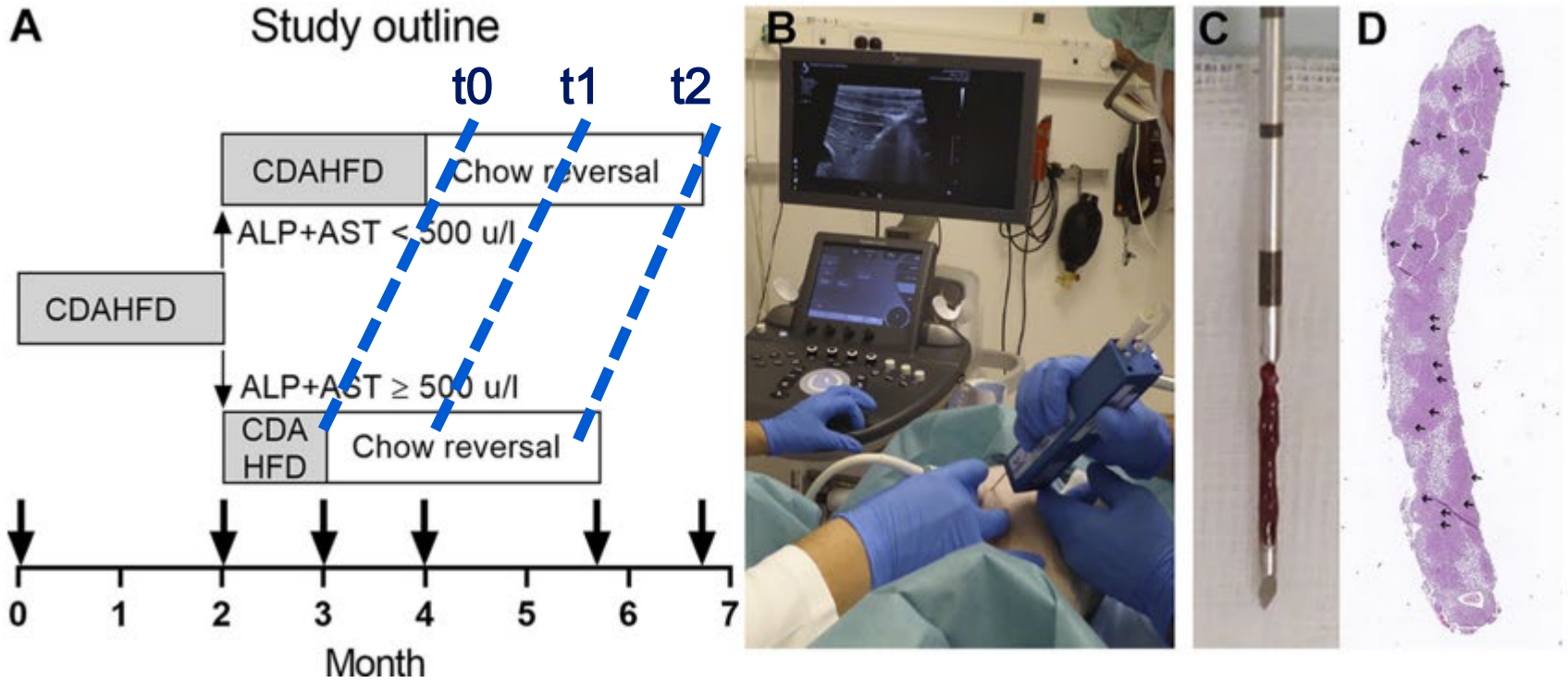
Performed as a part of the IMI-funded LITMUS consortium
(**L**iver **I**nvestigation: **T**esting **M**arker **U**tility in **S**teatohepatitis)

Background

- Metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction associated steatohepatitis (MASH): Prevalent and serious disease, characterized by steatosis, inflammation and fibrosis in the liver.
  - MASLD patients: Higher all-cause mortality.
  - MASH: Common reason for need for liver transplantation in the US.
- To develop treatments, animal models of MASLD/MASH are needed!
- There are many rodent models of MASLD/MASH available, however, in some scenarios non-rodent models are needed.
- Our hypothesis: Göttingen Minipigs on a choline-deficient high fat diet (CDAHFD) is a useful model of MASLD/MASH.
- Aim of study: Thorough characterization of phenotype in CDAHFD-fed Göttingen Minipigs, with special focus on the effects of a chow-reversal period and comparison to human disease and other models.

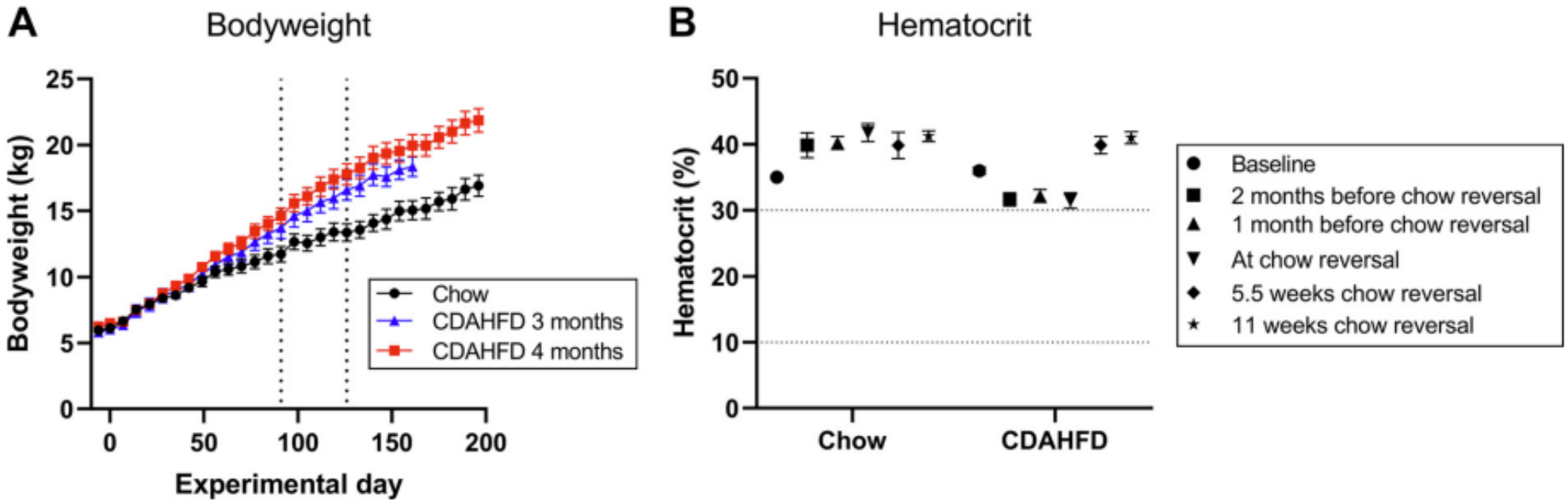
Study design

- Göttingen Minipigs on CDAHFD for 3 or 4 months, followed by 11-week chow reversal period.
- Plasma levels of ALP and AST at 2 months used to determine duration of CDAHFD feeding
- Ultra-sound guided needle biopsies collected from the liver during the experiment



Results

- Minipigs on CDAHFD gained weight, and maintained their bodyweight during the chow-reversal period
- No anaemia



Plasma parameters

Table 2. *Plasma and liver parameters measured in study 2*

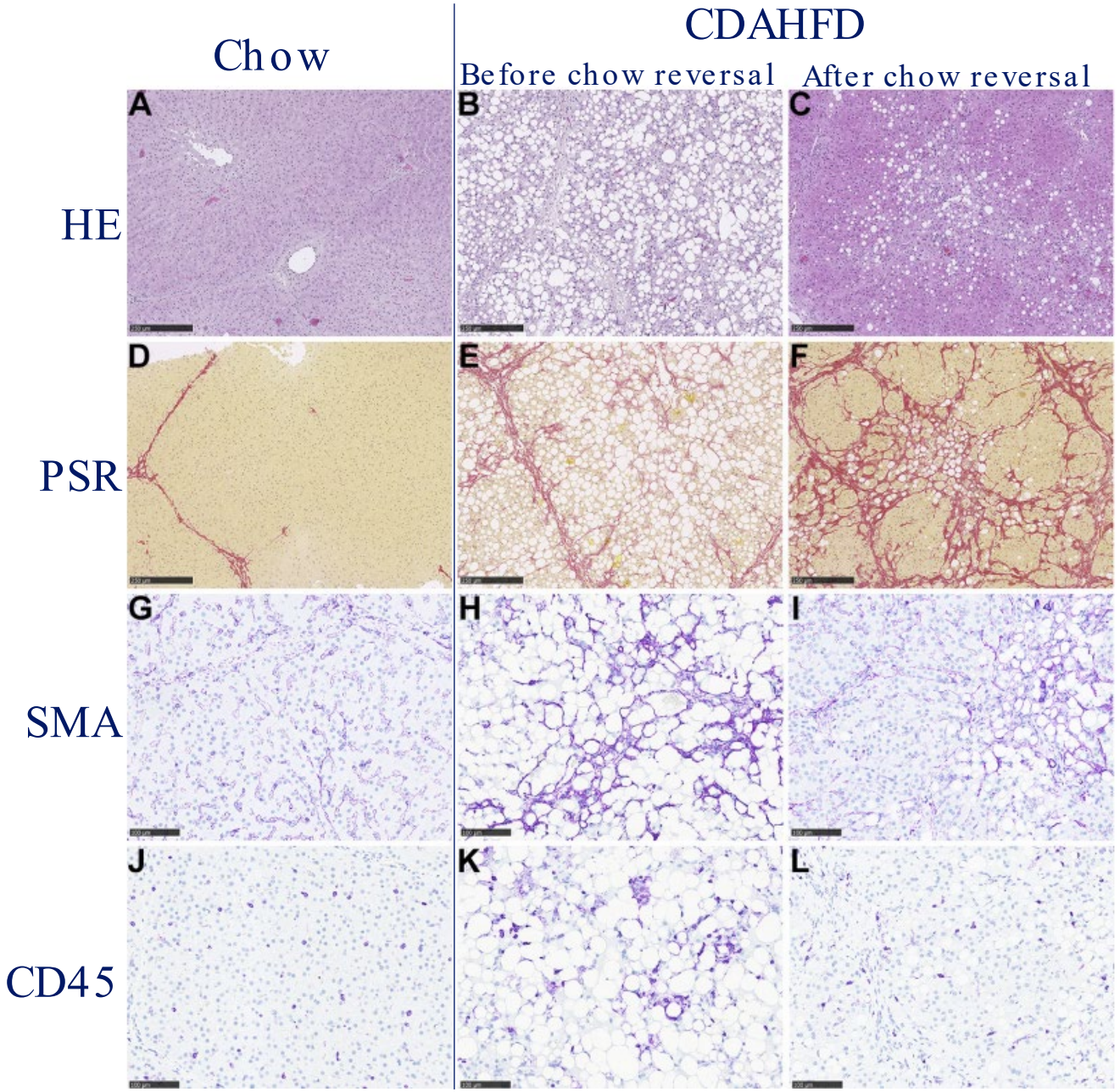
	Baseline		2 Mo		Time Point t0 (3 or 4 Mo on CDAHFD)		End of Study	
	Chow	CDAHFD	Chow	CDAHFD	Chow	CDAHFD	Chow	CDAHFD + 11 wk chow reversal
Total protein, g/L	61.6 ± 1.4	59.9 ± 0.7	63.6 ± 0.9	60.6 ± 0.8	65.4 ± 0.4	64.6 ± 1.1	68.4 ± 0.6	68.6 ± 0.8
Albumin, g/L	39.6 ± 1.2	40.5 ± 0.5	48.8 ± 0.6	45.0 ± 1.0	52.0 ± 0.9	46.0 ± 1.3*	52.7 ± 0.8	50.0 ± 0.8
Urea, mmol/L	1.6 ± 0.2	1.4 ± 0.1	2.2 ± 0.1	4.3 ± 0.2***	2.2 ± 0.2	2.3 ± 0.1*	2.1 ± 0.1	2.0 ± 0.2
Creatinine, µmol/L	62 ± 3	62 ± 1	67 ± 2	54 ± 1**	76 ± 3	60 ± 2**	75 ± 4	82 ± 3
Iron, µmol/L	9.5 ± 1.0	12.0 ± 0.7*	22.6 ± 0.7	19.0 ± 0.6	21.3 ± 2.8†	14.9 ± 1.3†	21.7 ± 1.2	20.2 ± 0.7
Total bilirubin, µmol/L	0.44 ± 0.12	0.76 ± 0.11	0.55 ± 0.12	0.63 ± 0.19	0.87 ± 0.29	3.02 ± 1.30***	0.53 ± 0.11	0.67 ± 0.08
Conjugated bilirubin, µmol/L	0.23 ± 0.04	0.37 ± 0.06	0.32 ± 0.05	0.86 ± 0.16**	0.41 ± 0.16	0.97 ± 0.58*	0.38 ± 0.05	0.34 ± 0.06
ALP, U/L	25.3 ± 4.7	20.0 ± 5.3	32.3 ± 7.7	233.4 ± 59.7***	29.1 ± 11.7†	382.5 ± 82.7***†	16.3 ± 6.5	15.5 ± 5.4
ALT, U/L	30.6 ± 4.6	39.0 ± 1.8	74.6 ± 3.5	61.4 ± 4.8	75.1 ± 3.1	100.6 ± 8.4	78.3 ± 3.5	60.8 ± 2.9
AST, U/L	37.7 ± 5.2	36.4 ± 4.6	28.7 ± 1.8	77.6 ± 15.9***	30.5 ± 3.0	192.0 ± 61.5***	36.9 ± 4.4	48.2 ± 6.9
GGT, U/L	227.5 ± 53.1	175.6 ± 33.6	99.1 ± 9.3	130.7 ± 23.8	173.3 ± 64.6†	335.4 ± 44.1†	185.5 ± 30.4	175.9 ± 19.5
Glucose, mmol/L	4.9 ± 0.1	5.2 ± 0.2	5.1 ± 0.1	5.7 ± 0.2	4.6 ± 0.2	4.5 ± 0.1	4.1 ± 0.1	4.4 ± 0.1
Insulin, pmol/L	27.4 ± 12.1	42.0 ± 6.21	71.2 ± 11.6	128.0 ± 18.7	71.0 ± 22.7	46.0 ± 4.7	37.2 ± 8.9	84.2 ± 10.6*
Phosphate, mmol/L	2.7 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	2.6 ± 0.1*	2.4 ± 0.03†	2.3 ± 0.03†	2.1 ± 0.04	2.2 ± 0.05
Triglyceride, mmol/L	0.45 ± 0.04	0.37 ± 0.02	0.41 ± 0.04	0.64 ± 0.13*	0.52 ± 0.04	0.83 ± 0.08*	0.42 ± 0.01	0.38 ± 0.03
Cholesterol, mmol/L	2.1 ± 0.1	2.2 ± 0.1	1.7 ± 0.1	7.4 ± 1.0***	1.7 ± 0.1	12.2 ± 1.1***	1.7 ± 0.1	1.9 ± 0.1
Liver								
Glycogen, µmol/g tissue							203.7 ± 36.7	128.6 ± 14.9*
Triglyceride, µmol/g tissue							7.6 ± 0.9	12.0 ± 2.1
Cholesterol, µmol/g tissue							5.5 ± 0.5	17.3 ± 1.7***

Values are mean plasma concentration ± SE. *, **, and *** indicate $P < 0.05$, 0.001, and 0.0001, respectively, when CDAHFD was compared with chow at each time point. †Geometric mean values calculated on $n = 3$ chow animals and $n = 5$ CDAHFD animals.

Liver histology

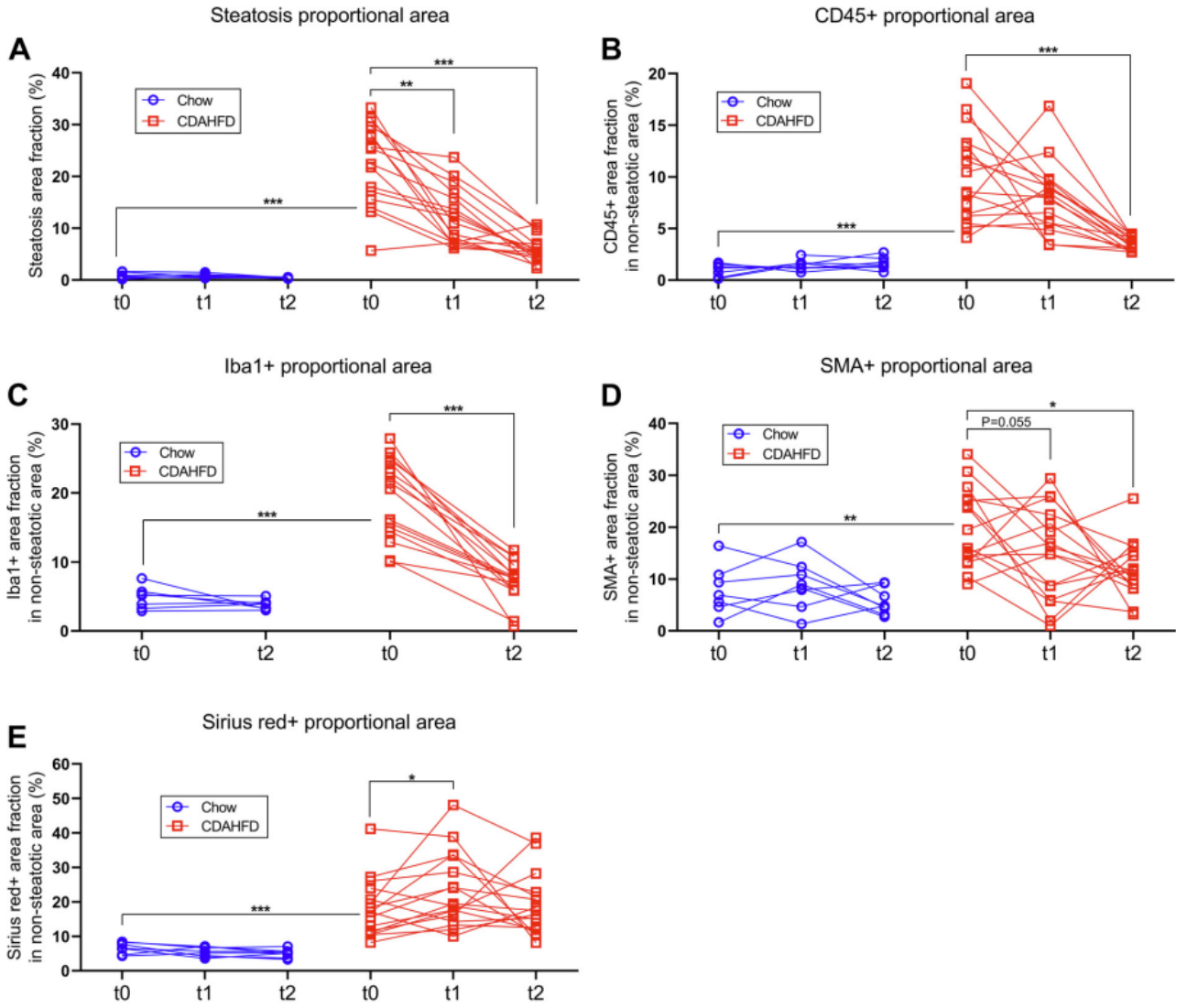
- Pronounced steatosis, inflammation and fibrosis in the liver after 3 or 4 months on CDAHFD

- Chow reversal led to:
  - Marked reduction of steatosis
  - Reduction of inflammation
  - No reduction of fibrosis



Liver histology

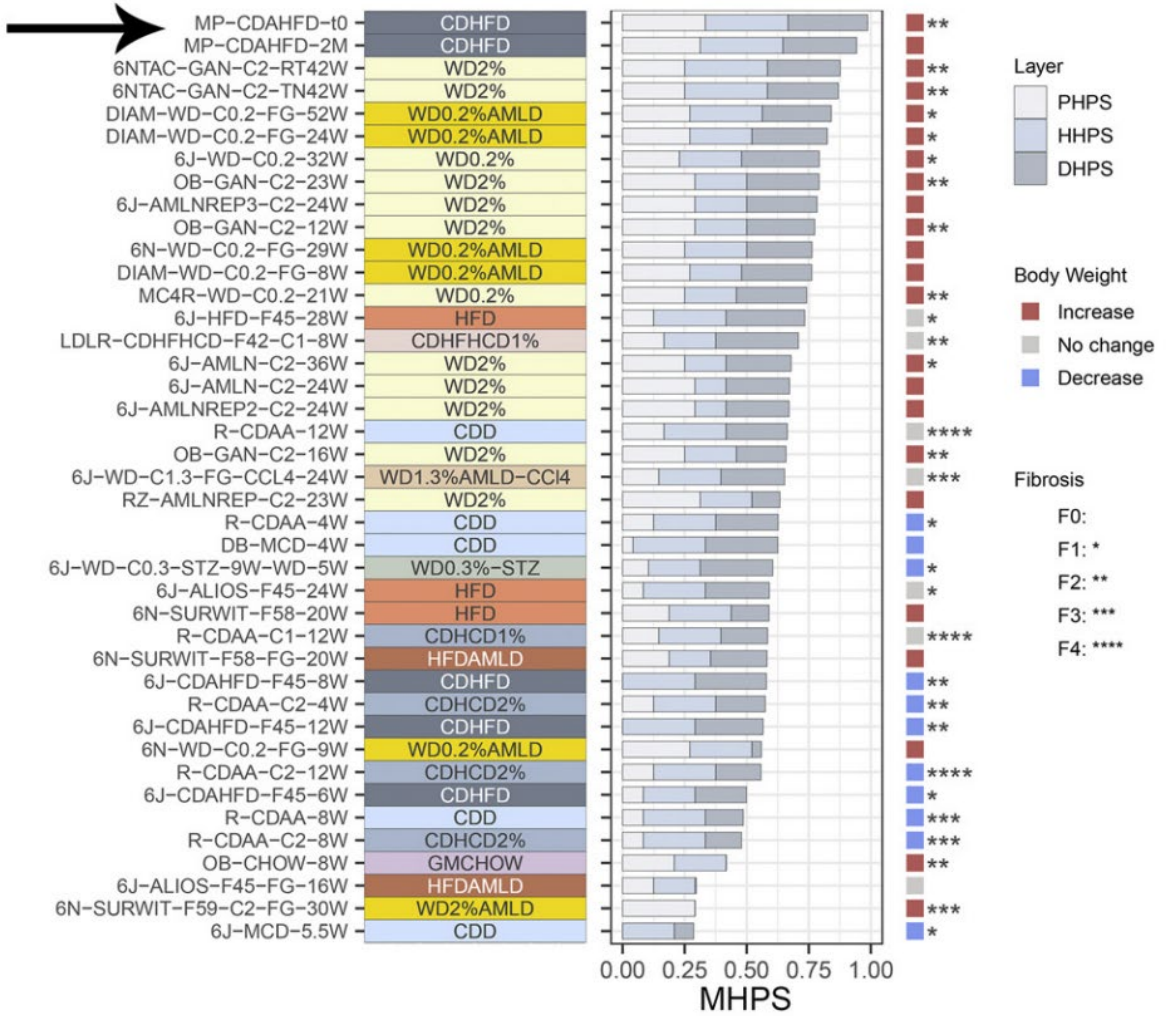
Quantitative image analysis



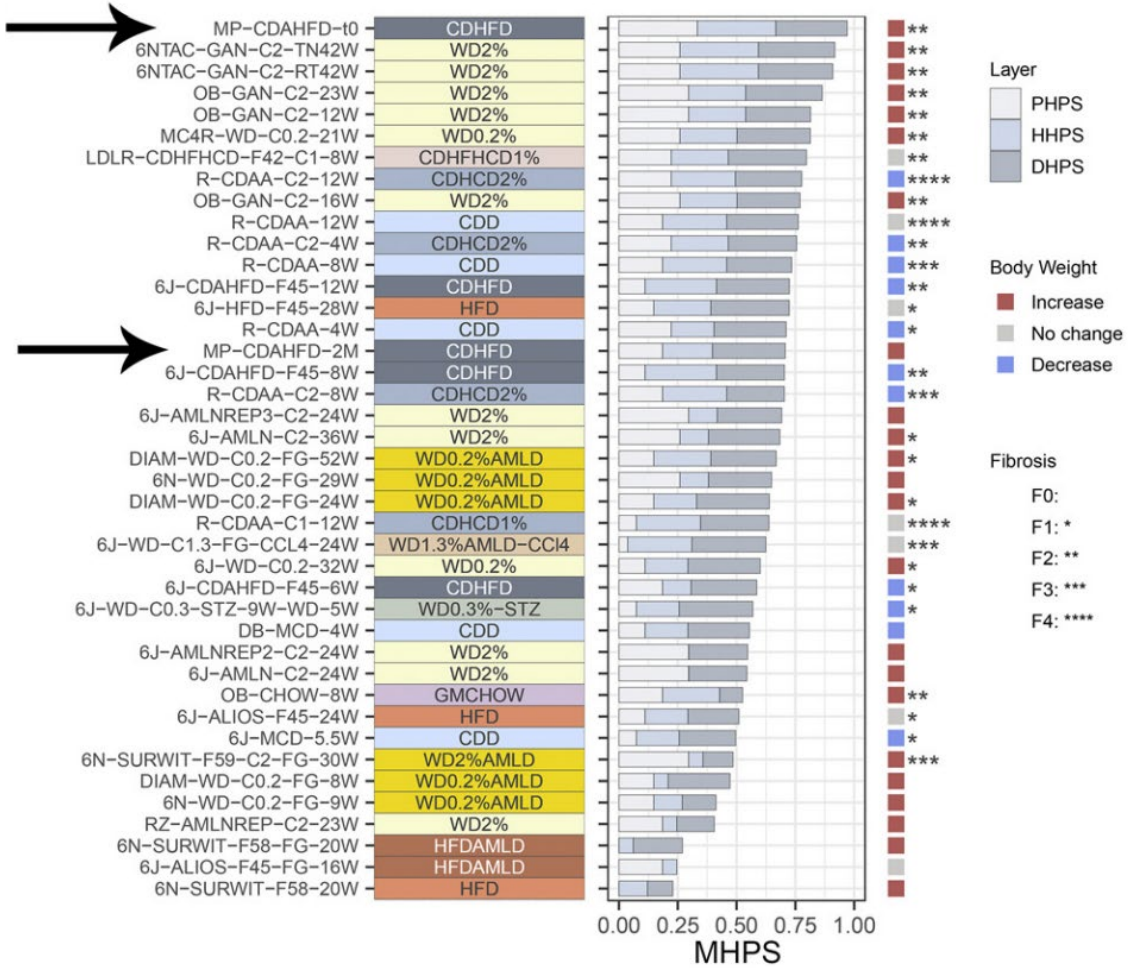
Comparison of minipig and rodent models to human disease

- Combined evaluation of in vivo parameters, liver histology and RNAseq
- This approach and panel of rodent models described in detail by Vacca et al., Nat Metab, 2024 Jun;6(6):1178-1196

Comparison to human patients with mainly metabolic alterations



Comparison to human patients with pronounced liver fibrosis



Conclusion

- Göttingen Minipigs fed CDAHFD for 3-4 months gain weight and develop a disease which in several aspects resembles MASLD/MASH, with steatosis, inflammation, hepatocellular ballooning and fibrosis.
- It is possible to repeatedly collect liver biopsies from Göttingen Minipigs to monitor disease progression during an experiment.
- Hepatic gene-expression in CDAHFD-fed minipigs has relatively high resemblance to human MASLD/MASH patients.
- During an 11-week chow reversal period, liver fibrosis persisted but steatosis, SMA-, Iba1- and CD45-positive proportional areas were decreased, indicating that the induced disease to some extent regressed when CDAHFD was removed.
- **What about response to pharmacological treatment?**

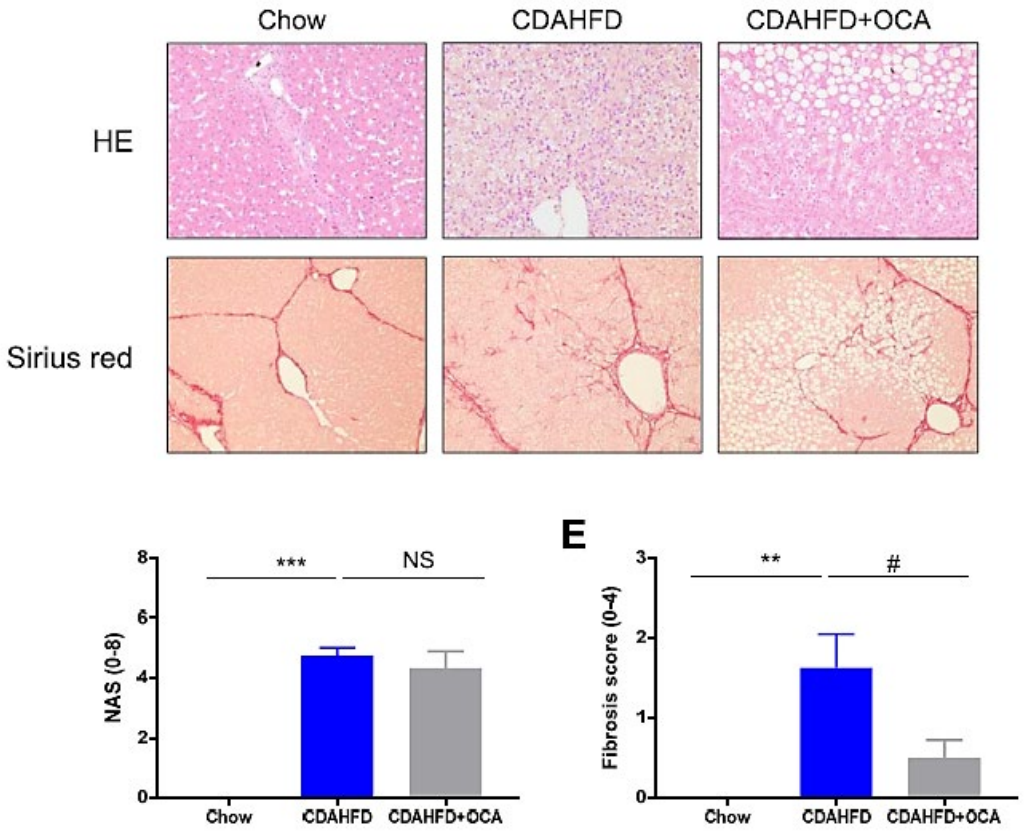
Pharmacological validation

Characterization and Pharmacological Validation of a Preclinical Model of NASH in Göttingen Minipigs

Valérie Duvivier *, Stéphanie Creusot *, Olivier Broux *, Aurélie Helbert *, Ludovic Lesage *, Kevin Moreau *, Nicolas Lesueur *, Lindsay Gerard *, Karine Lemaitre *, Nicolas Provost *, Edwige-Ludiwyne Hubert *, Tania Baltauss *, Angelique Brzustowski †, Nathalie De Preville *, Julia Geronimi *, Lucie Adoux ‡, Franck Letourneur ‡, Adel Hammoutene *,§, Dominique Valla ‖, Valérie Paradis §, Philippe Delerive *

J Clin Exp Hepatol, 2021 Sep 8;12(2):293–305

- Similar findings was reproduced by a group at Servier, that in addition validated the effect of pharmacological treatment with obeticholic acid (OCA)
- The treatment effects largely resembled the effects demonstrated in a placebo-controlled phase 3 trial in patients with MASH and fibrosis (F2–F3), where an improvement of fibrosis with no worsening of the MASH was observed[1]



[1] *Younossi et al, Lancet 2019; 394: 2184–96*

Questions ?