Clinical translatability of a diet-induced obese mouse model of non-alcoholic steatohepatitis

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INTRODUCTION AND AIM

The Amylin (AMLN) diet-induced obese (DIO) mouse model has become one of the preferred preclinical animal models of non-alcoholic steatohepatitis (NASH). The translatable value of preclinical data in this model is however still not properly validated. Our aim was to evaluate AMLN DIO-NASH mouse liver samples with the results from NASH patients.

METHODS

Liver samples were obtained from C57Bl/6J mice fed the AMLN diet (40% fat, 20% fructose, 2% cholesterol, AMLN DIO-NASH mice), a 60% high-fat diet (DIO mice) or chow (lean mice) for at least 30 weeks, and compared to human samples collected from lean (BMI <25 kg/m2), obese (BMI > 30 kg/m2) and patients with histology-proven NASH (n=12). Histopathological assessment of NAFLD activity score (NAS) and fibrosis stage was performed, and quantitative histology was used to analyse steatosis (H&E staining), inflammation (galectin-3 immunohistochemistry), and fibrosis (Pico-Sirius red staining).

STUDY DESIGN AND GROUPS

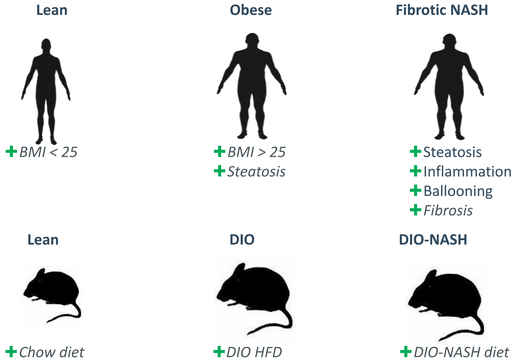

Figure 1 | Liver samples was collected from Lean, Obese and NASH diagnosed individuals with fibrosis. C57Bl/6J mice were fed either standard chow, high-fat diet or AMLN DIO-NASH to generate groups matching the disease spectrum from healthy to fibrotic NASH.

RESULTS

Anthropomorphic characteristics and plasma markers

Group	n	m/f	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	ALT (U/l)	AST (U/l)
Lean	14	14/0	39.5 ± 12.0	181.7 ± 5.4	76.6 ± 7.6	23.1 ± 1.6	31.8 ± 8.9	33.4 ± 9.0
Obese	12	12/0	36.6 ± 10.2	186.8 ± 8.1	115.2 ± 12.1	33.2 ± 1.3	39.7 ± 15.8	41.2 ± 15.4
Fibrotic-NASH	16	12/4	38.9 ± 17.0	173.8 ± 8.9	102.9 ± 22.6	33.9 ± 6.2	115.0 ± 50.0	54.8 ± 20.6

Figure 2 | Summary of patient cohort characteristics expressed as mean +/- standard deviation.

Liver histology

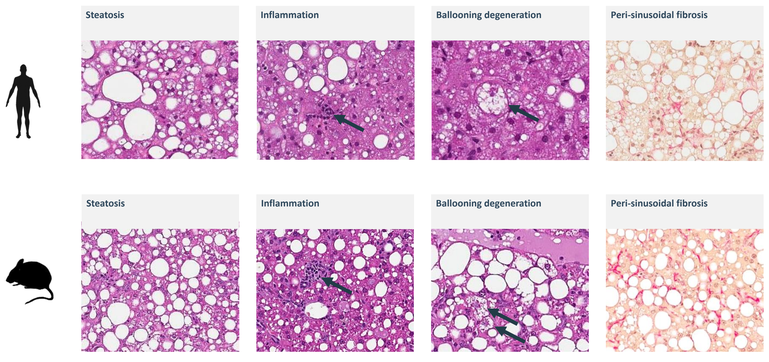

Figure 3 | *Top panel* | Representative images of liver morphology of human NASH patient biopsy after hematoxylin and eosin stain (three leftmost images) or picro sirius red (rightmost images). | *Bottom panel* | Same as above for DIO-NASH mouse biopsy. Arrows indicate position of either inflammatory foci or ballooning hepatocytes.

Histopathological scoring

		Steatosis				Inflammation			Ballooning			Fibrosis stage				
Group	n	0	1	2	3	0	1	2	0	1	2	0	1	2	3	4
Lean	14	13	1	-	-	14	-	-	14	-	-	14	-	-	-	-
Obese	12	6	5	1	-	12	-	-	12	-	-	12	-	-	-	-
Fibrotic NASH	16	-	-	2	14	-	16	-	-	10	6	3	12	1	-	-

		Steatosis				Inflammation			Ballooning			Fibrosis stage				
Group	n	0	1	2	3	0	1	2	0	1	2	0	1	2	3	4
Lean	51	51	-	-	-	50	1	-		51	-	-	51	-	-	-
DIO-NASH	57	-	22	33	2	-	8	29	20	25	32	-	-	22	33	2

Figure 4 | *Top panel* | Summary of histopathological scoring of human cohorts| *Bottom panel* | Same as above for Lean and DIO-NASH mouse biopsies.

Quantitative morphometry

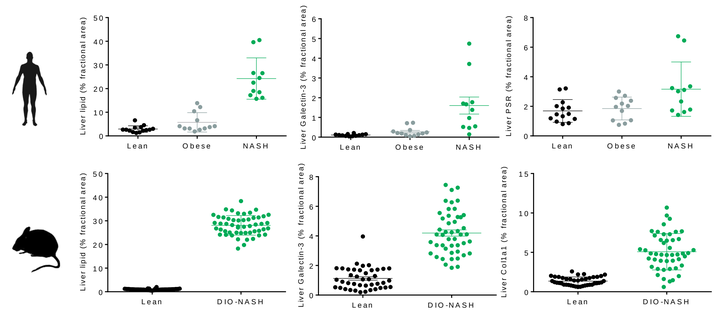

Figure 5 | *Top panel* | Degree of steatosis, inflammation and fibrosis in human biopsies was assessed by morphometry. Scanned slides were analysed in two steps: Crude detection of tissue at low magnification and detection of steatosis, Collagen 1A1, PSR and or Galectin-3 at high magnification. All values are given as percent of total tissue area. Average and SD are indicted plots.

Gene expression

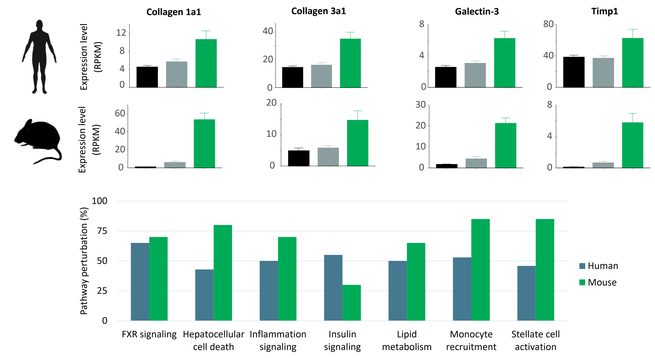

Figure 6 | *Top panel* | mRNA levels in human biopsies was analysed using RNAseq. | *Middle panel* | Same as above for Lean and DIO-NASH mouse biopsies. Values are presented as mean RPKM + SEM. | *Bottom panel* | Degree of perturbation of prototypical NASH associated pathways

CONCLUSION

- **Liver histopathology is highly comparable between human and mice samples**
- **Gene expression analysis showed similar regulation pattern in human and mice, albeit with a clearer separation between lean and NASH in mice.**
- **In conclusion, the AMLN DIO-NASH models show a liver phenotype highly similar to that of human NASH patients with moderate fibrosis.**