TNO Ldlr-/-.Leiden MASH mouse model

Metabolic dysfunction-associated steatohepatitis (MASH; formerly known as NASH) is one of the most prevalent chronic liver diseases and is closely associated with obesity, insulin resistance and dyslipidemia. These important features therefore need to be reflected in a preclinical model. Based on 25 years of research on translational metabolic disease models, TNO has developed the Ldlr-/-.Leiden mouse that accurately mimics the etiology and pathology of MASH and fibrosis in humans. By using a high-fat diet, with a macronutrient composition comparable to that of human diets (e.g., without added cholesterol), Ldlr-/-.Leiden mice develop obesity, insulin resistance, adipose tissue inflammation, increased gut permeability with altered microbiota composition, and MASH with bridging fibrosis (F3).

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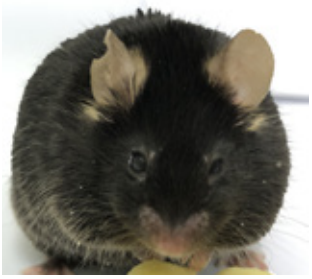
TRANSLATIONAL MODEL CHARACTERISTICS

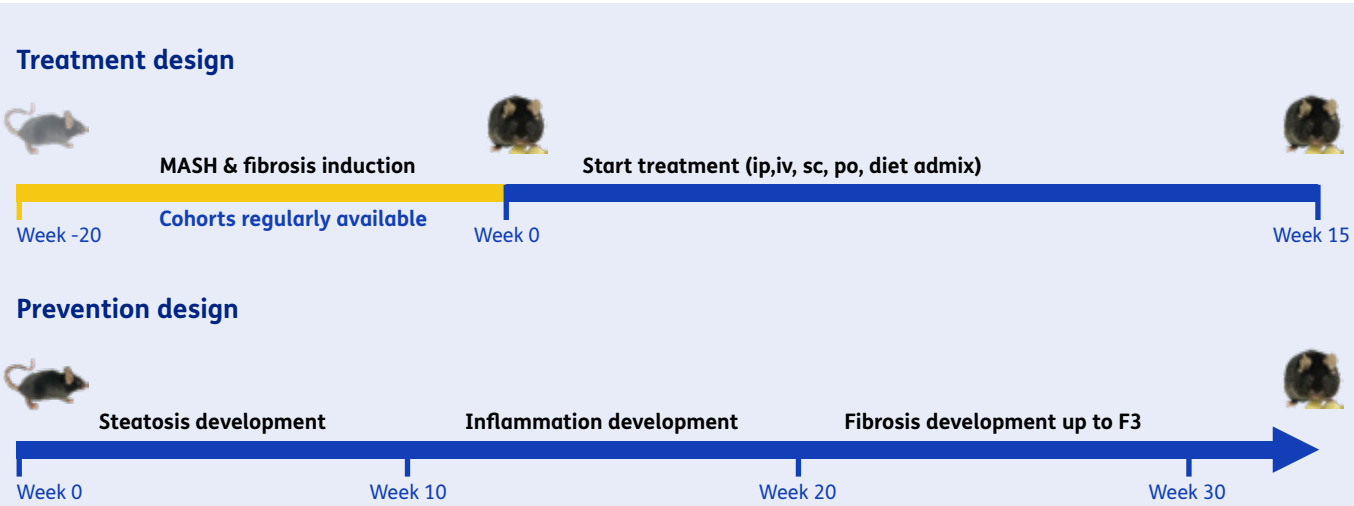
- Translational disease induction and clinical phenotype
- Severe obesity (with adipose tissue inflammation)
- Insulin resistance
- Hyperlipidemia (humanized lipoprotein profiles: high triglycerides, high (V)LDL, low HDL)
- Translational histology: steatosis, inflammatory aggregates and bridging fibrosis (up to F3)
- Atherosclerosis development
- Translational underlying pathways (verified on transcriptomics, proteomics and metabolomics level)
- Extensive validation with multiple interventions
- Metabolic overload-induced complications in multiple organs.



The TNO Ldlr-/-.Leiden MASH mouse: a diet-induced obesity model for studying all aspects of metabolic dysfunction-associated steatotic liver disease (MASLD; formerly known as NAFLD) including metabolic dysfunction-associated steatohepatitis (MASH) and fibrosis.

Time lines

TNO studies are tailor-made, based on what is known from time-resolved data.
Starting point of intervention depends on the targeted pathway/process. Typical study designs are shown here:



Read-out parameters

Blood/plasma:

Standard:
- Glucose
- Insulin
- Cholesterol, Triglycerides
- ALT, AST

Optional e.g.:
- Lipoprotein profile (VLDL, LDL, HDL)
- Inflammation markers (SAA, MCP-1)
- Cytokine/chemokine panels
- Adiponectin, leptin
- CK18m30, TIMP-1
- Short chain fatty acids
- Bile acids

Liver histology & Biochemistry:

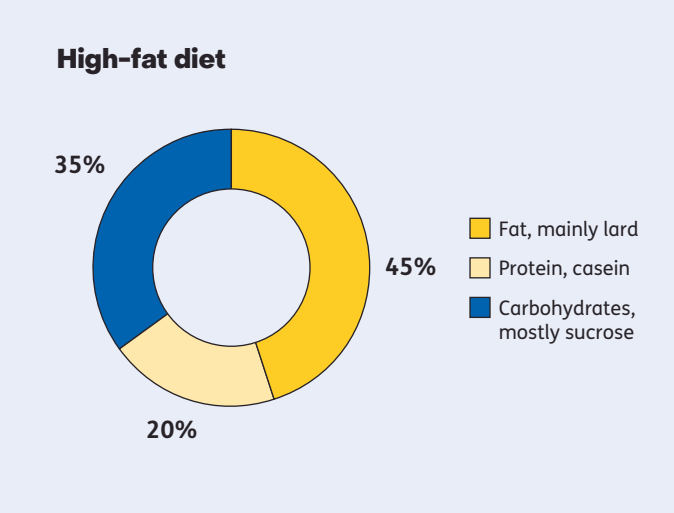
Standard:
- Continuous MASH score (HE staining)
- Fibrosis percentage/stage (SR staining)
- Collagen content (hydroxyproline)

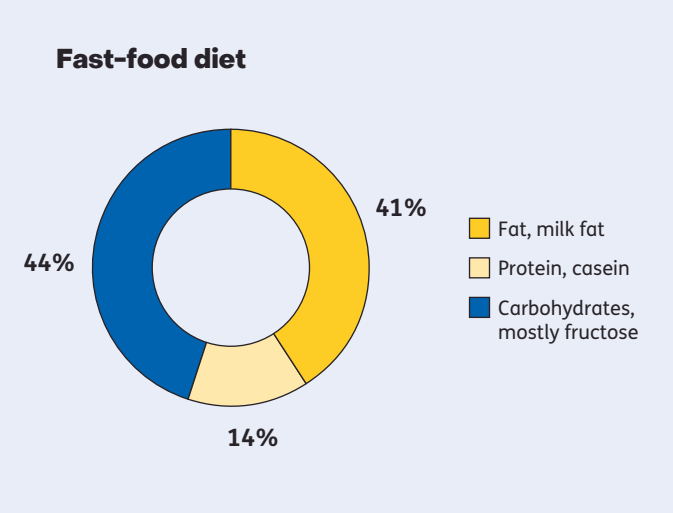
Optional e.g.:
- Liver lipids (TG, FC, CE)
- Specific inflammatory cells (F4/80, GR-1)
- Collagen fibers/a-SMA
- Newly synthesized collagen
- Collagen characteristics (AI technology)
- Omics (NGS, lipidomics, oxylipins etc.)
- Oxidative stress
- Mitochondrial damage

Other organs:
- Atherosclerosis (lesion area and severity)
- Adipose tissue expansion (adipocyte size) and inflammation (crown-like structures)
- Gut microbiota and permeability (FD4)
- Brain inflammation (microglia, astrocytes)
- Muscle atrophy (myofiber diameter and type)

Translational diets

To induce MASH and fibrosis, Ldlr-/-.Leiden mice are fed high-caloric diets with a macronutrient composition and cholesterol content comparable to human diets. Mice are given a high-fat diet (HFD) or fast-food diet (FFD) both without cholesterol supplementation. By working with two different diets, different characteristics of the model (that are critical for human pathogenesis) can be emphasized to allow the best combination of model and diet suitable for your research question. For instance, the HFD emphasizes multi-organ pathophysiology with insulin resistance and WAT inflammation, whereas the FFD diet results in more pronounced hepatic bridging fibrosis.





Translational metabolic context

In TNO's view the best way to mimic MASH development in animals is by ensuring that the disease induction takes place within the same metabolic context as in MASH patients. This means mice should develop severe obesity, with multi-organ involvement, insulin resistance and hyperlipidemia. The latter cannot be studied in wild-type mice and ob/ob mice, because in these mice cholesterol circulates as HDL and not as VLDL/LDL as it is the case in humans. Hence, Ldlr-/-.Leiden mice mimic the human lipoprotein profile in which the plasma cholesterol is confined to the atherogenic VLDL/LDL lipoprotein particles.

	Chow	HFD	FFD
Body weight (g)	38.3 ± 1.5	52.3 ± 1.1***	48.7 ± 1.5***
Food intake (kcal/mouse/day)	13.5 ± 0.8	12.5 ± 0.6	14.9 ± 1.9
Liver weight (g)	1.8 ± 0.1	3.1 ± 0.2***	5.9 ± 0.4*
Blood glucose (mM)	7.7 ± 0.4	7.7 ± 0.2	6.5 ± 0.3**
Plasma insulin (ng/mL)	2.9 ± 0.6	14.7 ± 4.2***	3.9 ± 0.4
HOMA-IR	1.0 ± 0.2	4.9 ± 1.4**	1.1 ± 0.1
Plasma cholesterol (mM)	8.0 ± 0.7	32.2 ± 3.7***	41.3 ± 4.7***
Plasma triglycerides (mM)	1.5 ± 0.3	6.5 ± 1.3**	8.3 ± 1.2***
Plasma ALT (U/L)	53.3 ± 7.1	363.7 ± 64.8**	348.6 ± 33.0***
Plasma AST (U/L)	88.2 ± 8.2	449.1 ± 69.1***	342.9 ± 58.4***
Plasma SAA (µg/mL)	39.6 ± 4.2	244.6 ± 54.6***	318.6 ± 73.3***
Plasma E-selectin (ng/mL)	61.3 ± 3.7	83.0 ± 10.1*	83.7 ± 3.4***
Plasma MCP-1 (pg/mL)	33.2 ± 6.5	75.4 ± 11.2**	70.7 ± 6.3**

Ldlr-/-.Leiden mice were fed a chow diet (healthy reference group) or high-fat diet (HFD) containing lard or fast-food diet (FFD) containing milk fat for 28 weeks. Data represent mean ± SEM for n≥8 mice/group. * $p<0.05$.

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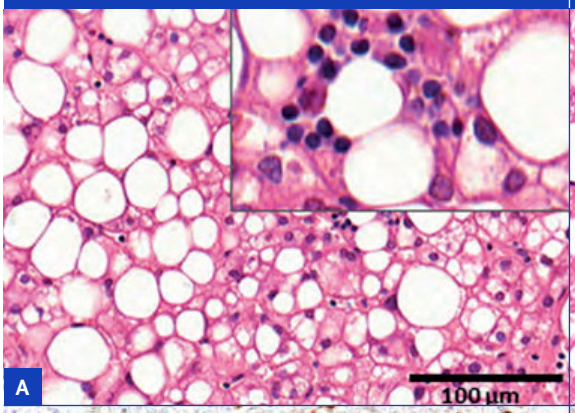
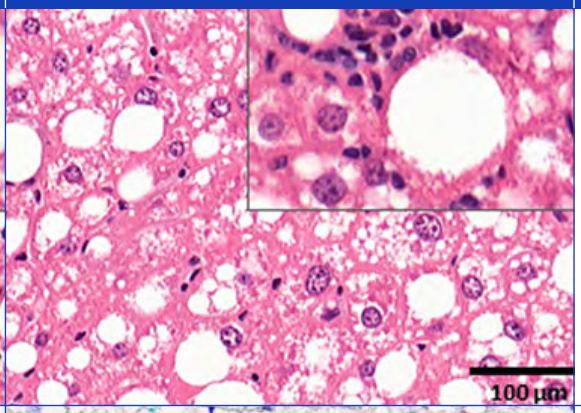
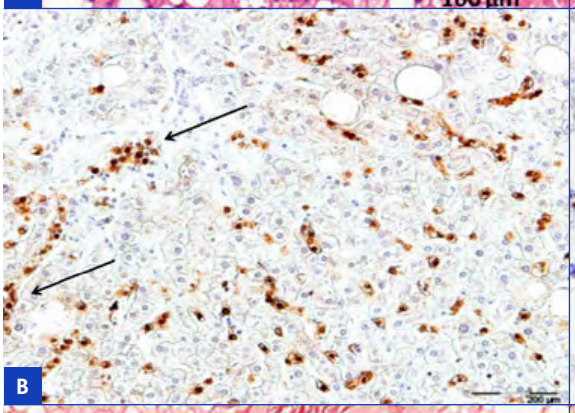
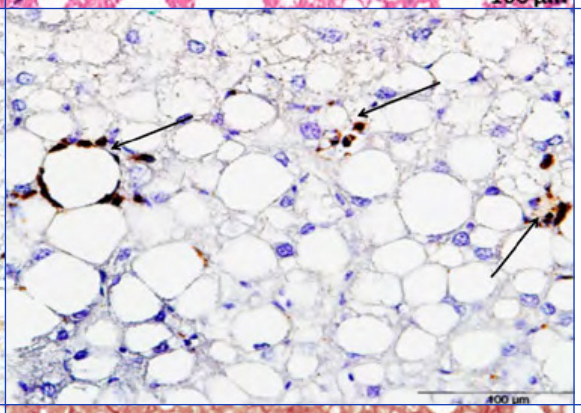
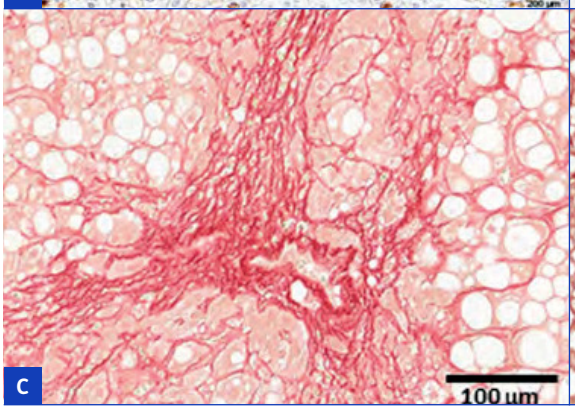
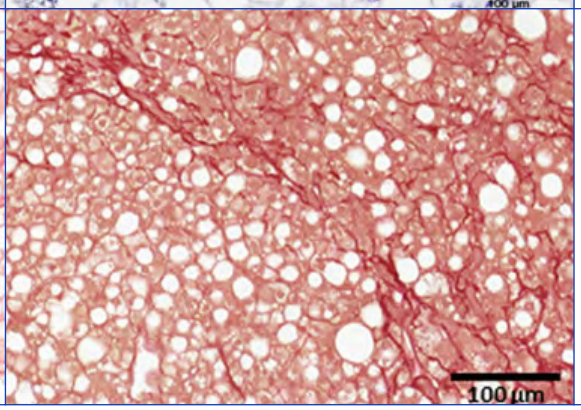
Translational MASH and fibrosis history

Human	Ldlr-/-.Leiden MOUSE	
		**Steatosis** Inflammatory aggregates (mixed cell types)
		**Infiltration of neutrophils** (other cell types also present: a.o. macrophages and T-cells).
		**Periportal, pericentral and bridging fibrosis**

Human-like MASH characteristics in Ldlr-/-.Leiden mice on a high-fat diet. A. HE staining; B. MPO staining; C. Sirius red staining. Human photos A and C from study described in Vreeken et al., BMJ Open 2019.

TNO Ldlr-/-.Leiden MASH mouse model

Ldlr-/-.Leiden mice develop key features of MASH when fed a HFD or FFD: steatosis, i.e., macrovesicular steatosis and microvesicular steatosis, lobular inflammation, as well as hepatic fibrosis. MASLD histology is scored by a board-certified pathologist using a continuous scoring system for rodent models (Liang et al., PLoS One 2014) that is based on the human NAS scoring system (Kleiner & Brunt, Hepatology 2005).

The histological scoring system was applied on mouse liver sections to:

- quantify the area percentage covered with cells displaying steatosis (Figure A);
- count the number of inflammatory aggregates expressed per mm² (Figure B);
- determine the fibrosis stage (F0-F4) (Figure C).

These figures demonstrate typical MASH and liver fibrosis induction in the Ldlr-/-.Leiden mouse model. Many different interventions have been applied in TNO's Ldlr-/-.Leiden mouse model of which an overview is provided in the table on page 7.

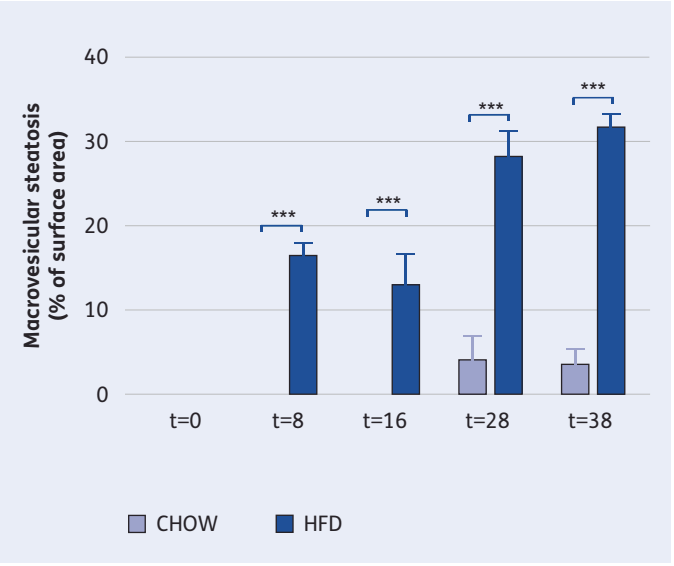


Figure A: Quantification of steatosis in Ldlr-/-.Leiden mice after different periods of HFD feeding

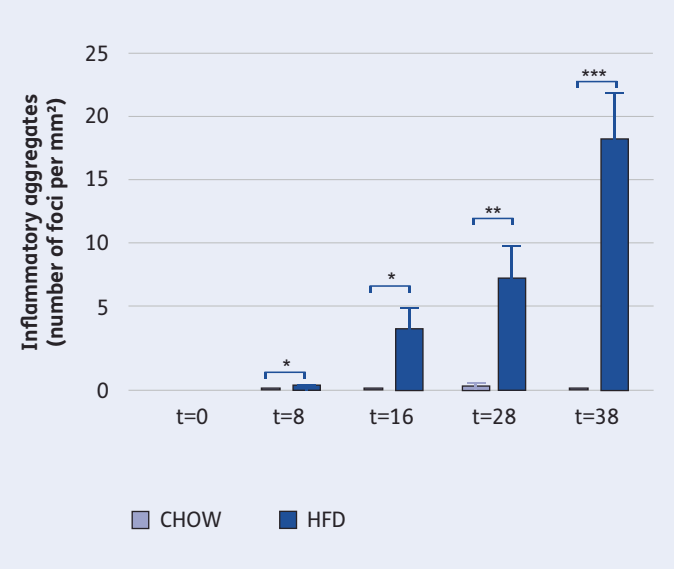


Figure B: Quantification of hepatic inflammation in Ldlr-/-.Leiden mice after different periods of HFD feeding

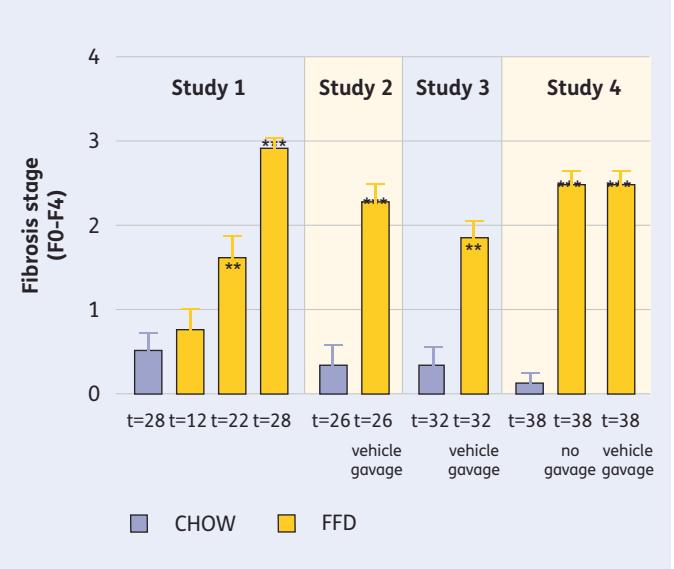
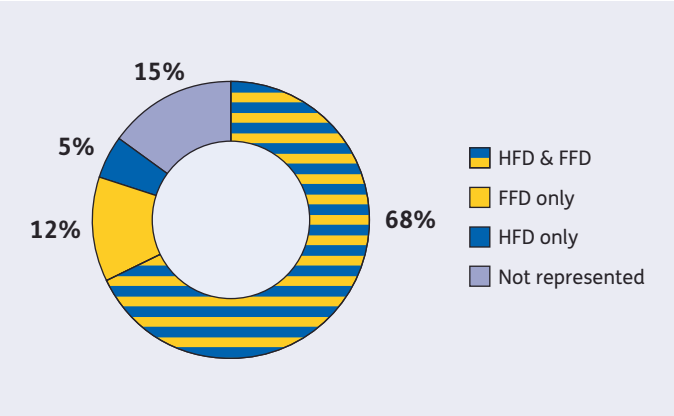


Figure C: Robust induction of fibrosis in Ldlr-/-.Leiden mice

Translational underlying pathways

A very high percentage of differentially expressed pathways that characterize MASH patients is recapitulated in the Ldlr-/-.Leiden mouse (73% on HFD, 80% on FFD), this is in clear contrast with other diet-induced and genetic mouse models (Teufel et al., Gastroenterology 2016).

Representation of human MASH pathways



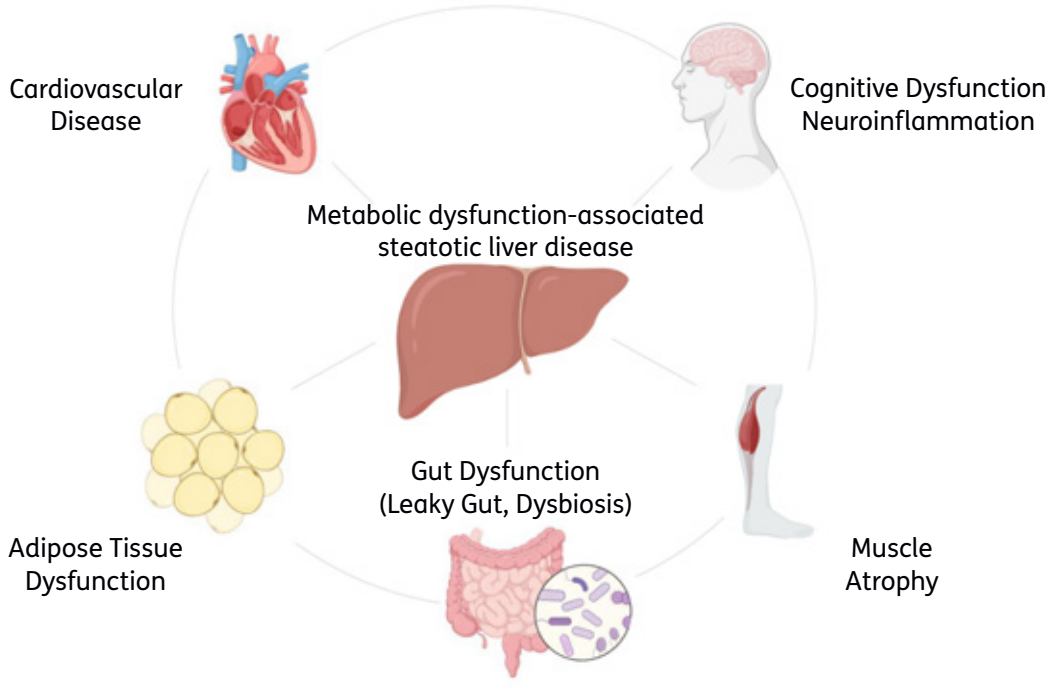
Among the pathways that were represented by both diets were important pathways such as hepatic fibrosis/stellate cell activation, several inflammatory pathways, mitochondrial dysfunction, oxidative stress induced pathways and also atherosclerosis signaling pathways.
Translation of the model was verified on the molecular level by transcriptomics and metabolomics analyses and in a head-to-head comparison with NASH patients (Morrison et al., Hepatol Commun 2018, Martinez-Arranz et al., Hepatol 2021).

Metabolic overload in ldlr-/-.Leiden mice leads to multiple organ dysfunction

Ldlr-/-.Leiden mice differ from other models in that they allow study of dysregulation of pathogenic processes in many different organs that contribute to MASH pathogenesis. Multiple metabolically active organs interact with the liver during MASH development such as gut, WAT and muscle, as well as brain and heart. Ldlr-/-.Leiden mice can provide more insight into this inter-organ cross-talk and the mechanistic pathways underlying MASH development.

At TNO we have experience with studying organ dysfunction in relation to MASH, for example:

- An early increase in gut permeability together with microbiota dysbiosis and adipose tissue inflammation is observed during NASH (Gart et al., Int J Mol Sci 2019).
- Simultaneous loss of muscle strength and relative muscle mass emerge together with increased intramuscular TG levels during development of NASH (van den Hoek et al., Metabolism 2021).
- Cardiovascular disease (CVD) is the major overall cause of mortality in NASH patients. Ldlr-/-.Leiden mice develop NASH in association with atherosclerosis allowing investigation of therapeutics for NASH and simultaneously on CVD (van den Hoek et al., Cells 2020).
- Cognitive dysfunction and brain inflammation develop in parallel with NASH in Ldlr-/-.Leiden mice (Seidel et al., Front Cell Neurosci. 2023; Arnoldussen et al., Int J Obes 2017 and 2021).
- Surgical removal of inflamed WAT attenuates NASH development in obese mice (Mulder et al., Int J Obes 2016).



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TNO Ldlr-/-.Leiden MASH mouse model

The Ldlr-/-.Leiden MASH model has been validated with many different interventions

Intervention	Target	Publication
Caspase-1 inhibitor	Caspase 1	Morrison et al., Int J Obes 2016
Cenicriviroc	CCR2 & CCR5	In preperation
Antioxidants (olive oil polyphenols)	Oxidative stress	Luque-Sierra et al., Mol Nutr Food Res, 2018 | Alvarez-Amor et al., Nature Sci Rep. 2021
Rosi-, pioglitazone	PPAR-γ	Mulder et al., Nat Sci Rep 2016 & in-house
Lanifibranor	PPARs	In preparation
EPA, DHA from krill oil	PPARs, oxylipins, resolvins	Gart et al., Nutrients 2021
Obeticholic acid	FXR	Morrison et al., Hepatol Commun, 2018 | van den Hoek et al., Cells 2020
Volixibat	IBAT	Salic et al., PLoS One 2019
Butyric acid	i.a. TGF-β fibrosis sign.	Arnoldussen et al., Int J Obes 2017 | Gart et al., Biomedicines 2021
Propionic acid	TCA cycle	Gart et al., FASEB J 2020
Semaglutide	GLP-1	Inia et al., Int J of Mol Sci 2023
DGAT2 inhibitor	DAGT2	In preparation
L-carnitine and nicotinamide riboside	β-oxidation, mitochondria	Salic and Gart et al., Int J Mol Sc 2019
Casein hydrolysate	i.a. lipid modulators	Schoemaker et al., PLoS One 2017
Branched chain amino acids; exercise	Mitochondria, TCA cycle	Gart et al., FASEB J. 2022 | van den Hoek et al., Metabolism 2021
Milk-fat globule membrane	Metabolism	Arnoldussen et al., Int J Obes 2021
Natural cholesterol lowering compounds	Neutrophils	Morrison et al., Front Endocrinol 2021
Resmetirom	THR-β	In preperation

Selected references

- Gart et al., Translational characterization of the temporal dynamics of metabolic dysfunctions in liver, adipose tissue and the gut during diet-induced NASH development in Ldlr−/−.Leiden mice. Heliyon (2023).
- Inia et al., Semaglutide Has Beneficial Effects on Non-Alcoholic Steatohepatitis in Ldlr-/-.Leiden Mice. Int J of Mol Sci (2023).
- van den Hoek et al., Diet and exercise reduce pre-existing NASH and fibrosis and have additional beneficial effects on the vasculature, adipose tissue and skeletal muscle via organ-crosstalk. Metabolism (2021).
- Gart et al., Krill oil treatment increases distinct PUFAs and oxylipins in adipose tissue and liver and attenuates obesity-associated inflammation via direct and indirect mechanisms. Nutrients (2021).
- van den Hoek et al., A translational mouse model for NASH with advanced fibrosis and atherosclerosis expressing key pathways of human pathology. Cells (2020).
- Morrison et al., Obeticholic acid attenuates fibrosis development in a high fat diet induced NASH model (Ldlr-/-. Leiden mice). Hepatol Commun (2018).
- Morrison et al., Key inflammatory processes in human NASH are reflected in Ldlr -/-.Leiden mice: a translational gene profiling study. Front Physiol (2018).
- van Koppen et al., Uncovering a predictive molecular signature for the onset of NASH related fibrosis in a translational NASH mouse model. Cell Mol Gastroent Hepatol (2017).
- Mulder et al., Surgical removal of inflamed epididymal white adipose tissue attenuates the development of non-alcoholic steatohepatitis in obesity. Int J Obes (2015).
- Liang et al., Establishment of a general NAFLD scoring system for rodent models and comparison to human liver pathology. PLoS ONE (2014).

The Ldlr-/-.Leiden mouse model can be used in contract research and public private partnerships (e.g. studying MASH and liver fibrosis, as well as organ cross-talk or body-brain interactions). Contact us to discover the opportunities.

TNO Ldlr-/-.Leiden MASH mouse model

TNO HEALTHY LIVING AND WORK
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