

# TNO LDLR-/-LEIDEN NASH MOUSE MODEL



**TNO** innovation  
for life

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

#### TRANSLATIONAL MODEL CHARACTERISTICS

- Translational disease induction and clinical phenotype
- Severe obesity (with adipose tissue inflammation)
- Insulin resistance
- Hyperlipidemia (humanized lipoprotein profiles: high triglycerides, high 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.

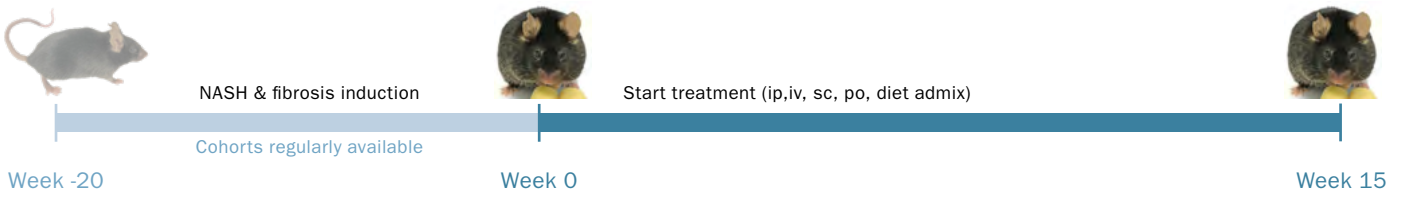
*The TNO Ldlr<sup>-/-</sup>-Leiden NASH mouse: a diet-induced obesity model for study of all aspects of non-alcoholic fatty liver disease (NAFLD) including non-alcoholic steatohepatitis (NASH) and fibrosis.*



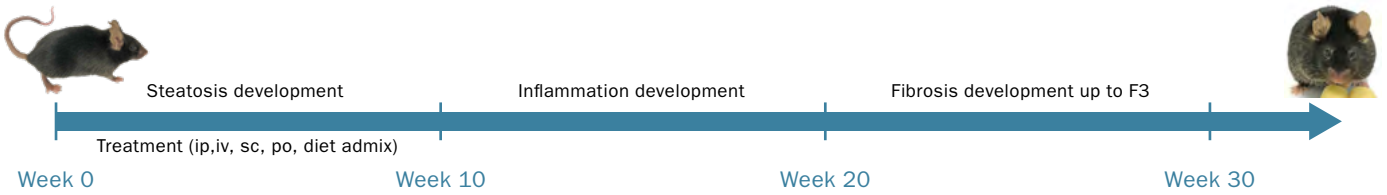
**TIME-LINES**

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:

**TREATMENT DESIGN**



**PREVENTION DESIGN**



**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 AND**

**BIOCHEMISTRY:**

**Standard:**

- Continuous NASH 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/Collagen fibers/a-SMA
- Newly synthesized collagen
- Collagen characteristics (2nd Harmonic imaging)

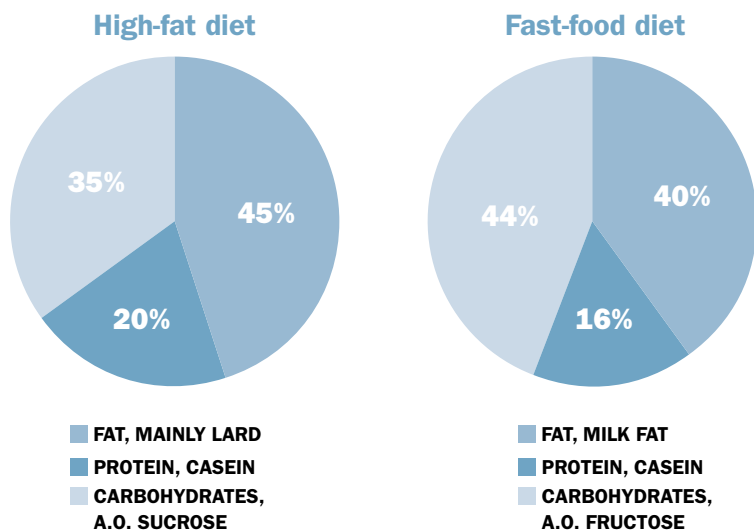
- Omics (NGS, lipidomics, oxylipins etc.)
- Oxidative stress
- Mitochondrial damage

**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 NASH and fibrosis, Ldlr<sup>-/-</sup>-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 insulin resistance and WAT inflammation, whereas the FFD diet results in more pronounced hepatic bridging fibrosis.



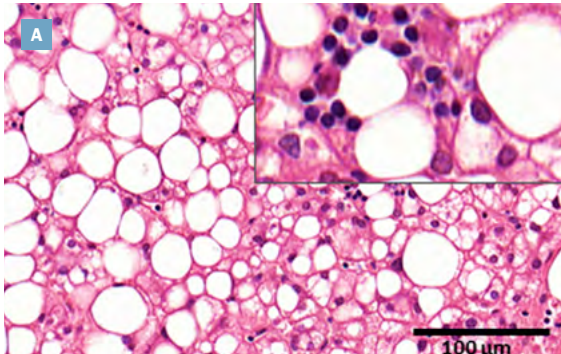
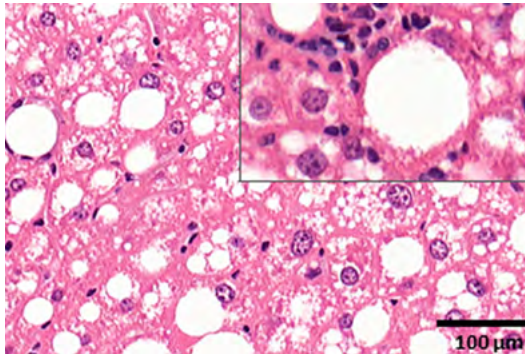
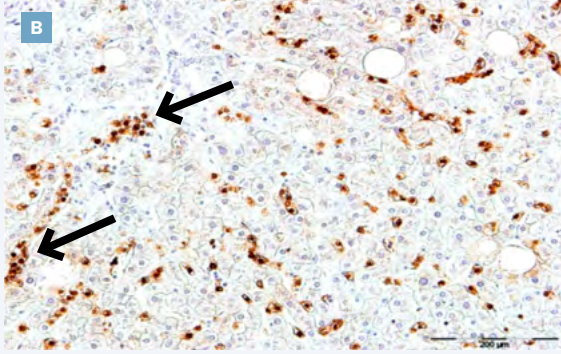
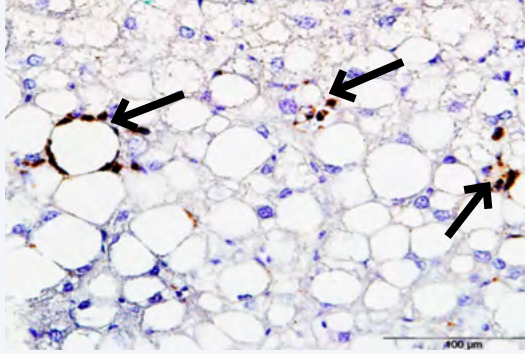
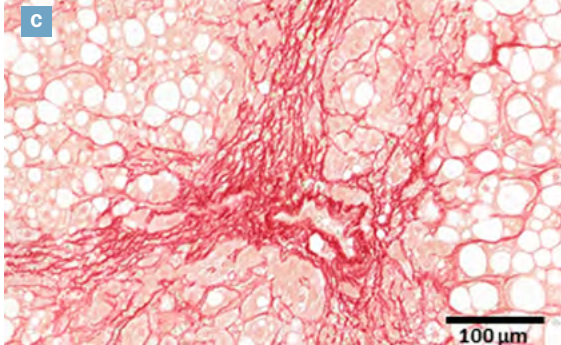
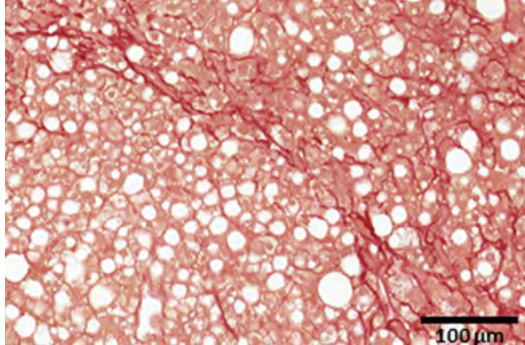
**TRANSLATIONAL METABOLIC CONTEXT**

In our view the best way to mimic NASH development in animals is by ensuring that the disease induction is within the same metabolic context as in NASH patients. This means mice should develop severe obesity with white adipose tissue (WAT) inflammation, 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<sup>-/-</sup>.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/m/d)	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<sup>-/-</sup>.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

**TRANSLATIONAL NASH AND FIBROSIS HISTOLOGY**

HUMAN	Ldlr <sup>-/-</sup> .Leiden MOUSE	
		Steatosis Inflammatory aggregates (mixed cell types)
		Infiltration of neutrophils (other cell types also present: a.o. Mø and T-cells).
		Periportal, pericentral and bridging fibrosis

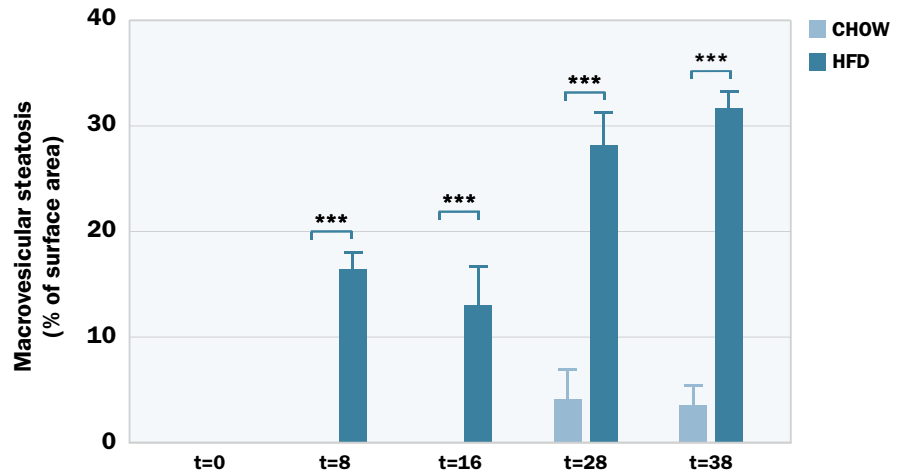
Human-like NASH characteristics in Ldlr<sup>-/-</sup>.Leiden mice on a high-fat diet. A. HE staining; B. MPO staining; C. Sirius red staining  
Human photos A and C from study described in Vreeken et al., BMJ Open 2019.

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

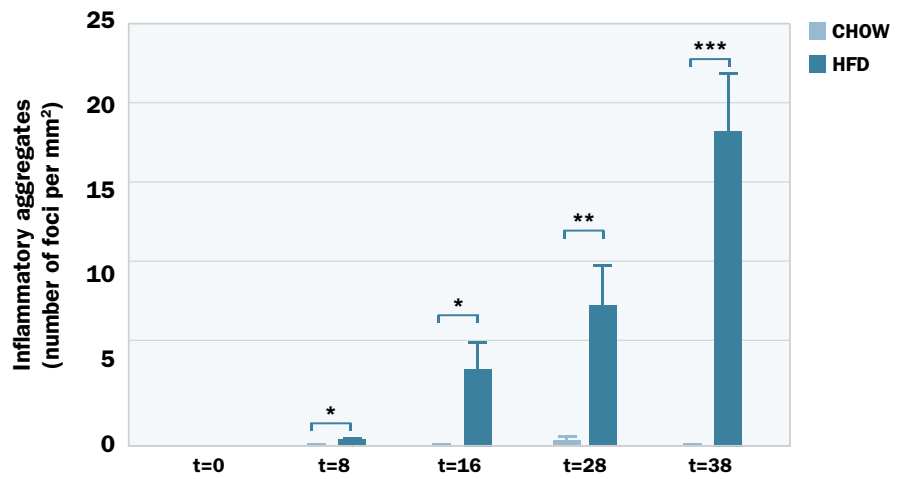
The NAFLD histology scoring system was applied on mouse liver sections to:

1. quantify the area percentage covered with cells displaying steatosis (top figure);
2. count the number of inflammatory aggregates expressed per mm<sup>2</sup> (middle figure);
3. determine the fibrosis stage (F0-F4) (bottom figure).

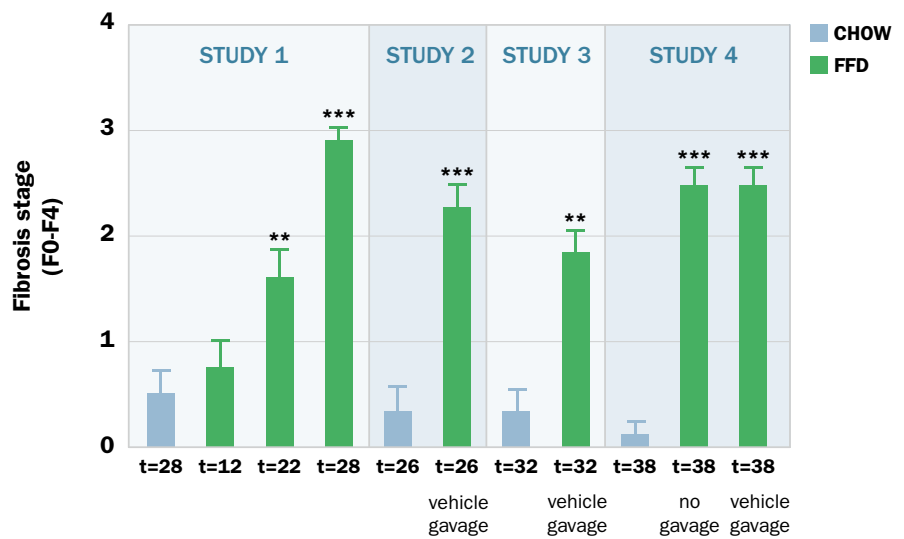
These figures demonstrate typical NASH and liver fibrosis induction in the Ldlr<sup>-/-</sup>.Leiden mouse model. Many different interventions have been applied in our Ldlr<sup>-/-</sup>.Leiden mouse model of which an overview is provided in the table on page 6.



Quantification of steatosis in Ldlr<sup>-/-</sup>.Leiden mice after different periods of HFD feeding



Quantification of hepatic inflammation in Ldlr<sup>-/-</sup>.Leiden mice after different periods of HFD feeding

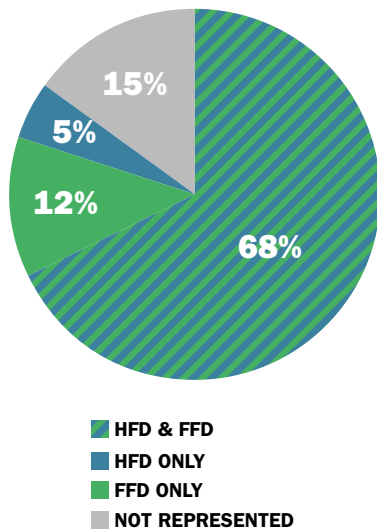


Robust induction of fibrosis in Ldlr<sup>-/-</sup>.Leiden mice

### TRANSLATIONAL UNDERLYING PATHWAYS

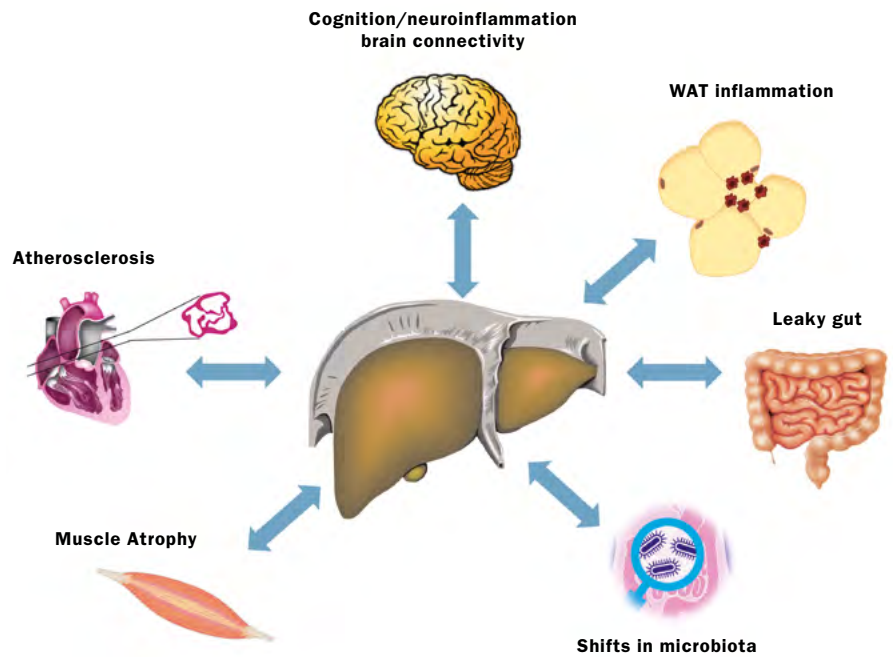
A very high percentage of differentially expressed pathways that characterize NASH patients is recapitulated in the Ldlr<sup>-/-</sup>.Leiden mouse (80% on HFD, 73% on FFD), this is in clear contrast with other diet induced and genetic mouse models (Teufel et al., Gastroenterology 2016).

### REPRESENTATION OF HUMAN NASH 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. Translationality of the model was verified on molecular level by transcriptomics and metabolomics and in head-to-head comparison with NASH patients (Morrison et al., Hepatol Commun 2018, Martinez-Arranz et al., Hepatol 2021).

### METABOLIC OVERLOAD IN Ldlr<sup>-/-</sup>.LEIDEN MICE LEADS TO MULTIPLE ORGAN DYSFUNCTION



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

At TNO we have experience with studying organ dysfunction in relation to NASH, 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<sup>-/-</sup>.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<sup>-/-</sup>.Leiden mice (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).

**THE Ldlr-/-LEIDEN NASH MODEL HAS BEEN VALIDATED WITH MANY DIFFERENT INTERVENTIONS**

Intervention	Target	Publication
Caspase-1 inhibitor	Caspase 1	Morrison et al., Int J Obes, 2016
Genicriviroc	CCR2 & CCR5	Ongoing
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	a.o. 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	Ongoing
DGAT2 inhibitor	DGAT2	In preparation
L-carnitine and nicotinamide riboside	β-oxidation, mitochondria	Salic and Gart et al., Int J Mol Sc, 2019
Casein hydrolysate	a.o. lipid modulators	Schoemaker et al., PLoS One, 2017
Branched chain amino acids; exercise	Mitochondria, TCA cycle	Gart et al., in preparation   van den Hoek et al., Metabolism 2021
Milk-fat globule membrane	Metabolism	Arnoldussen et al., Int J Obes, 2021
Natural anti-inflammatory compounds	Neutrophils	Morrison et al., Front Endocrinol, 2021

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

**SELECTED REFERENCES**

- 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).
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- Liang et al., Establishment of a general NAFLD scoring system for rodent models and comparison to human liver pathology. *PLoS ONE* (2014).

**TNO.NL**

**TNO HEALTHY LIVING**

TNO initiates technological and societal innovation for healthy living and dynamic society.

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