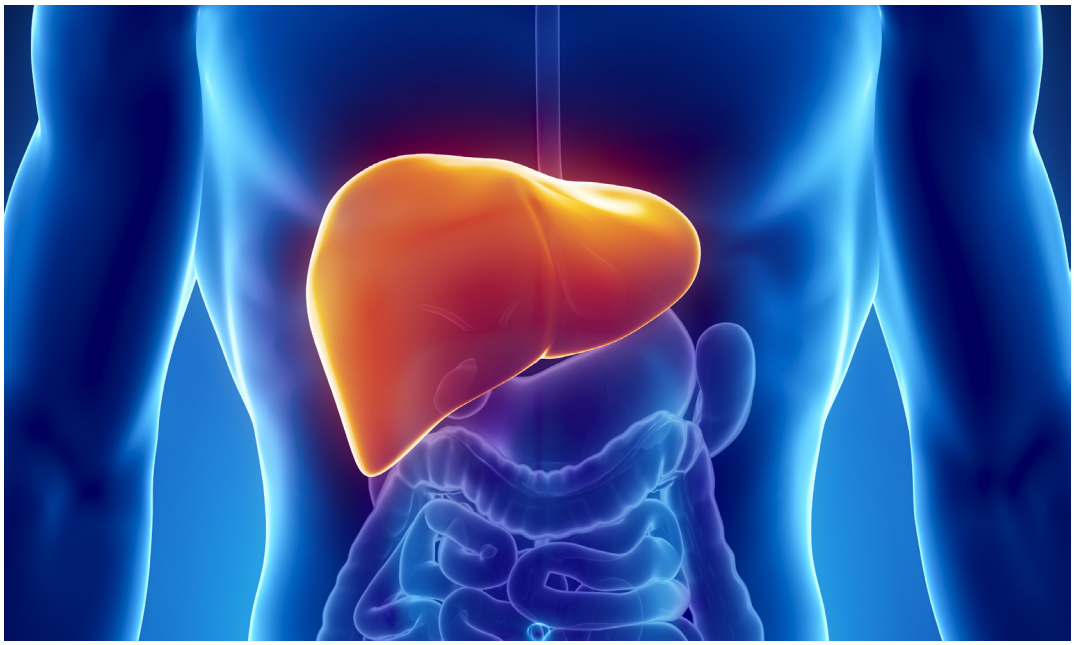
PRECLINICAL NASH MODELS



TNO innovation for life

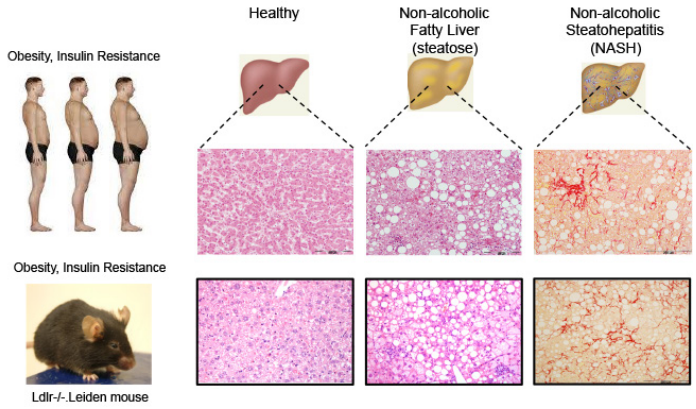
Nonalcoholic fatty liver disease (NAFLD) is emerging as one of the most common liver disorders in modern societies. Its prevalence in the general population is strongly increasing together with obesity, insulin resistance and the metabolic syndrome. The most benign form of NAFLD, bland steatosis, can further progress to nonalcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis with high rates of morbidity and mortality. Preclinical model systems that accurately mimic the etiology of the disease in humans are of great value for target identification, efficacy testing and the establishment of translational biomarkers.

UNIQUE AND VERSATILE PORTFOLIO

TNO has developed several diet-inducible models of NAFLD/NASH and liver fibrosis in which the disease process can be studied in the context of obesity, insulin resistance and dyslipidemia. Animals sequentially develop steatosis, NASH and liver fibrosis including hallmarks of human disease such as infiltration of specific inflammatory cells, activation of distinct proinflammatory transcription factors, formation of cholesterol crystals, and collagen deposition in macrovesicular rich areas. Notably, this can be achieved under experimental conditions that are translational to the situation in humans. Importantly, the TNO models for NASH are sensitive to nutritional and pharmacological interventions (e.g. drugs; nutrients; anti-microRNAs). Interventions are typically started at later time points, i.e. in the ongoing disease processes, to mimic therapeutic regimens in clinical practice. Experimental lesions resemble those of human disease and are characterized by microvesicular and macrovesicular steatosis, mixed-type inflammatory cells aggregates, and liver fibrosis. Features of experimental disease have been validated in human tissue and are scored according to a human grading system.

ADDITIONAL ENDPOINTS

Depending on the diets employed, the models also develop other complications associated with the metabolic syndrome, insulin resistance and type 2 diabetes such as atherosclerosis, sterile adipose tissue inflammation, reduced heart function as well as microvascular complications in kidney and eyes. These additional complications are currently further developed in pre-competitive shared research programs (see under Partnerships).



Similarity of liver pathology in human and mice

OUTCOME PARAMETERS

- **Biochemistry:** plasma lipids, lipoprotein composition, glucose, insulin, profiling of cytokines/adipokines, plasma inflammation markers (systemic inflammation, vascular inflammation markers.)
- **Histology:** liver pathology is graded according to a human grading system which was adapted for assessment of rodent pathology including the early stages of the disease. Furthermore, analysis of infiltrating inflammatory cells, collagen content and modifications, advanced immunohistochemistry etc.
- **Advanced technologies:** lipidomics/metabolomics; microRNA profiling; gene expression analysis (e.g. fibrosis fingerprints; targeted or microarray-based); proteolytic enzymes; profiling of inflammatory transcription factor activation; disease networks and signatures; dynamic protein labelling using deuterated water.

ADVANTAGES

- Validation in human tissue: TNO validates experimental models together with clinicians using human tissue biobanks.
- Tailor-made studies (>25 years of experience in preclinical studies; large preclinical tissue biobanks for pilots; >200 preclinical studies; successful FDA registration trajectories).
- Therapeutic protocols: Intervention at specific stages of the disease process.
- In-house breeding to ensure high quality (AAALAC certified), guarantee timelines and to reduce variability.
- 3R principles: TNO leads programs on replacement, reduction and refinement of animal experiments and provides tools for optimal design.
- One-stop shop for translational research (*in vitro*; *in vivo* models); PK/PD; toxicology; microdosing studies in humans.

PARTNERSHIPS

- **Collaborations:** TNO provides access to in-depth knowledge and special funds for pre-competitive research. We are continuously setting up new shared research programs, in case of NAFLD/NASH on:
  - etiology of NAFLD (taxonomy, events over time from steatosis to liver fibrosis);
  - reversibility of liver fibrosis;
  - novel intervention strategies for NASH/liver fibrosis;
  - biomarkers reflecting specific liver disease stages and processes incl. microRNA as markers;
  - key disease processes and targets in human tissue.
- **Research partnerships:** TNO provides access to in-depth knowledge and special funds for pre-competitive research. We are continuously setting up new shared research programs (e.g. on NAFLD/NASH, diabetic complications). Companies with interest in this area are invited to contact TNO.

TNO invites you to join our 'Open Innovation Shared Research Projects' the development of translational models and biomarkers.

References

150 publications in peer-reviewed scientific journals in the field of cardiometabolic and liver disease. A full list is available on request. Selected references:

- *Liang et al., Lab. Invest. 2014 (in press) (Characterization of inflammation in human and rodent NAFLD/NASH; comparison of human and experimental pathology)*
- *Radonjic et al. PLoS One 2013;8(2):e56122 (Differential effects of drug and lifestyle interventions in developing type 2 diabetes and organ complications; systems biology)*
- *Kleemann et al., PLoS One, 2010 (Time-resolved and tissue-specific analysis of the development of insulin resistance in liver, adipose tissue and muscle)*
- *Zadelaar et al., Arterioscler.Thromb.Vasc.Biol. 2007, 27:1706–1721 (review on models of atherosclerosis and differences in sensitivity to drugs)*

TNO.NL

TNO HEALTHY LIVING

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

TNO
Zernikedreef 9
P.O. Box 2215, 2301 CE Leiden
The Netherlands

Robert Ostendorf
P +31 (0)88 866 61 42
E robert.ostendorf@tno.nl

North America (Sales Office)
Tineke Meijers
P +1 416 837 75 00
E tmeijers@tnopharma.com

Japan (Sales Office)
Kaz Ariga
P +81-(0)3-6234-4195
E ariga@tno-pharma.com