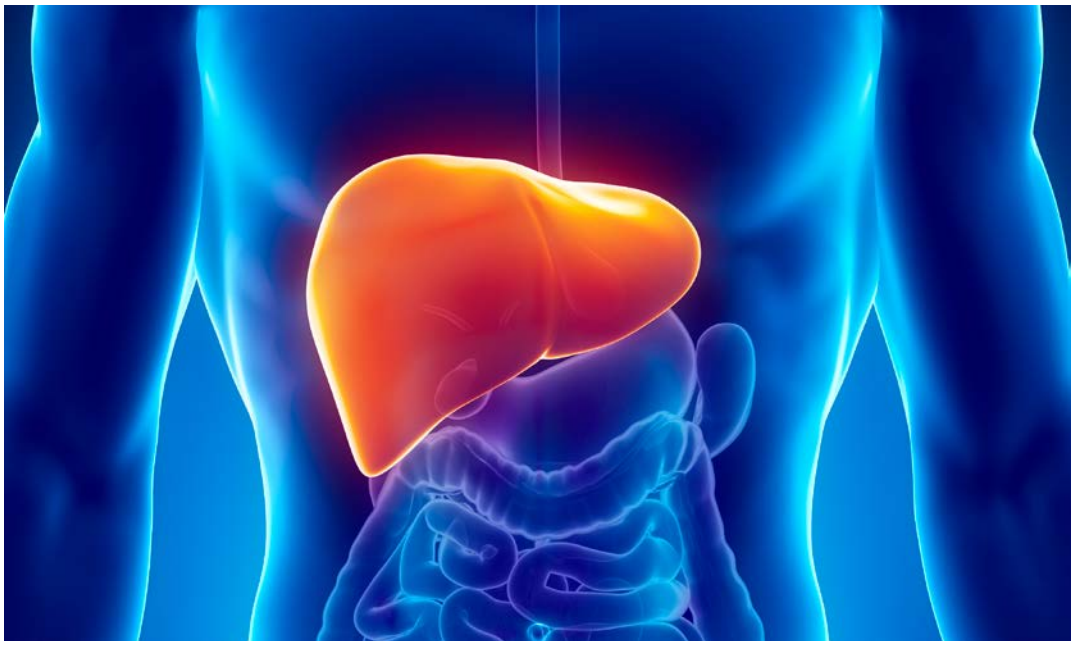
PRECLINICAL NASH MODELS



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.

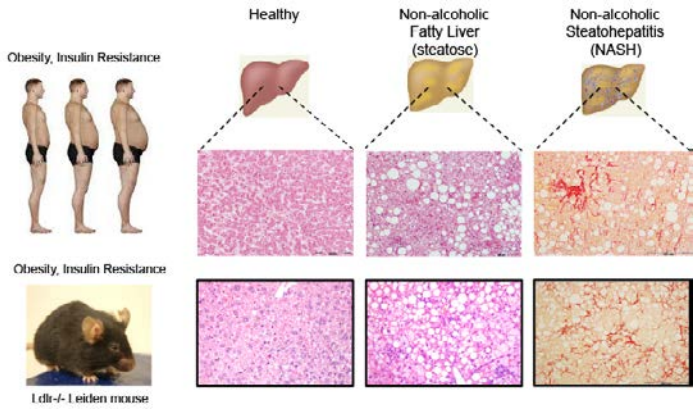
UNIQUE PRECLINICAL MODELS

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. PPARgamma activators and FXR activators; nutrients, such as polyunsaturated fatty acids; anti-inflammatory interventions).

Experimental lesions resemble those of human disease and are characterized by microvascular and macrovesicular steatosis, mixed-type inflammatory cells aggregates, and liver fibrosis.

YOUR ADVANTAGE

- Disease development and features are similar to human disease development: TNO validates experimental models together with clinicians using human tissue biobanks.
- Histopathology is scored on basis of the human grading system.
- All stages of disease development are present in the model, including fibrosis, therefore intervention at specific stages of the disease process is possible.
- Tailor-made study designs.
- In-house breeding to ensure high quality (AAALAC certified), guarantee timelines and to reduce variability.



Similarity of liver pathology in human and mice (Liang et al., PLoS ONE, 2014)

- One-stop shop for other translational research (*in vitro*; *in vivo* models); PK/PD; toxicology; microdosing studies in humans.

OUTCOME PARAMETERS

For this we use the following 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.

ADDITIONAL DISEASE ENDPOINTS

Currently, we have additional endpoints under development. 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, adipose tissue inflammation, reduced heart function as well as microvascular complications in kidney and eyes.

PARTNERSHIPS

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;
- biomarkers reflecting specific liver disease stages and processes;
- key disease processes and targets in human tissue.

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TNO HEALTHY LIVING

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