

Systems biology approach to identify processes and early markers for fibrosis in a diet-induced NASH mouse model

TNO innovation for life

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Introduction

The LDLR^{-/-}.Leiden mouse is a translational, diet-induced model for metabolic syndrome which, upon high fat feeding, shows multiple obesity-related complications including non-alcoholic steatohepatitis (NASH) with associated fibrosis. Since there are no clear markers that can be used to predict progression of NASH we aim to identify early processes and pathways for NASH and liver fibrosis in a time-resolved mouse study.

Methods

LDLR^{-/-}.Leiden mice were fed a high-fat diet (HFD) for 30 weeks. Every 6 weeks, a subset of mice was sacrificed (fig 1).

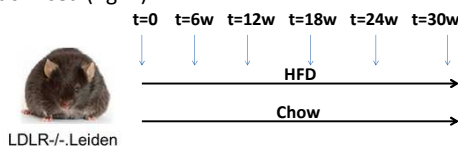


Fig 1: Study design

Liver tissue was used for time-resolved analysis of biochemical parameters, pathology, transcriptome (RNAseq) and dynamic protein profiling (by deuterated water labelling).

By using a systems biology approach, gene-expression data was linked to protein profiles and functional pathways to describe relevant processes over time.

Results: NASH induction

High fat diet feeding induced liver injury as indicated by increased liver weight, liver enzymes and development of steatosis, inflammation and fibrosis.

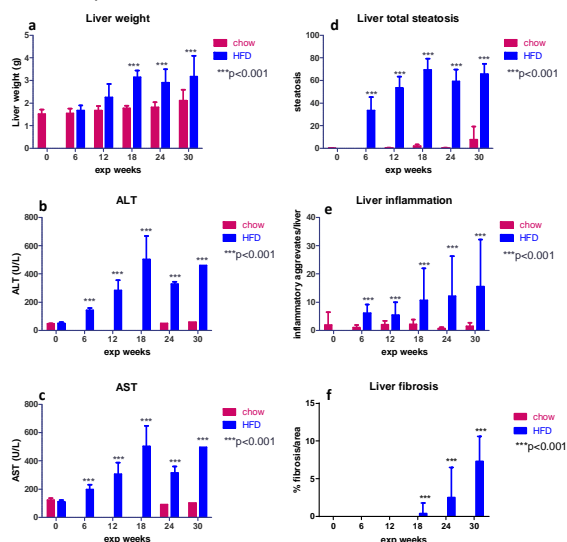
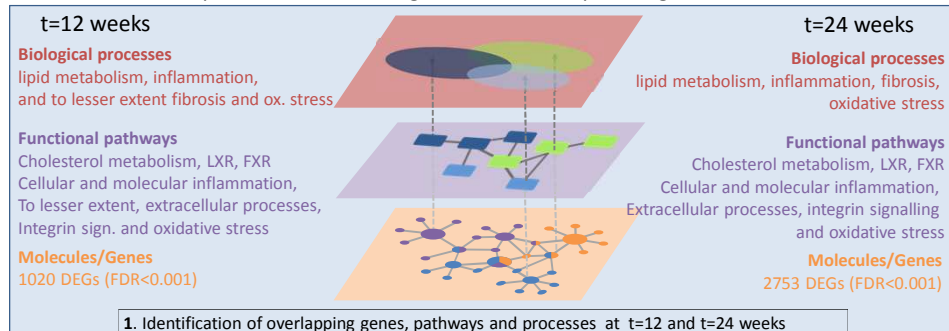


Figure 2: Effect of high fat diet feeding on liver parameters: increased liver weight (a), liver enzymes (b+c) and histopathological scoring of steatosis (d), inflammation (e) and fibrosis (f).

Results: Systems biology data integration approach

Integrative pathway analysis was used to identify which early biological processes are associated to end-point molecular changes in relation to pathological fibrosis.



1. Identification of overlapping genes, pathways and processes at t=12 and t=24 weeks

Integration of Dynamic protein profiling with RNAseq data

Dynamic protein profiling

Fatty acid beta-oxidation

Key processes

Steatosis

Inflammation

Oxidative stress

Fibrosis

RNAseq-Transcriptome

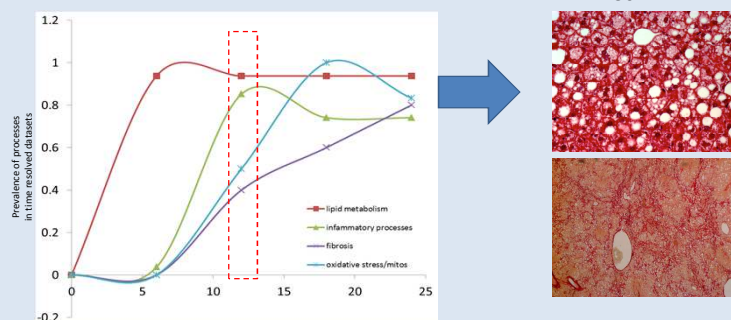
Cholesterol biosynthesis
LXR/RXR pathway
FXR/RXR pathway

DC-maturation
Leukocyte extravasation signalling
T-cell signalling
NF- κ B signalling

NRF2-mediated oxidative stress

Hepatic Fibrosis
Stellate cell activation
TNO fibrosis gene signature

2. By integrating dynamic protein profiling data with transcriptome data four key processes that are involved in NASH development were identified.



3. At t=12w, processes involved in NASH development can be detected which might predict pathological end points at t=30w.

Conclusions

- Combining transcriptome data with dynamic protein profiling using a systems biology approach elucidated the (predictive) dynamic processes involved in development of NASH
- This potentially allows early detection (t=12w) of pathological liver fibrosis (t=30w) and subsequently allows drug efficacy testing at an early stage of the disease.

