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

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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. As there are no clear markers which 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 and every 6 weeks, a subset of mice was sacrificed (fig 1).

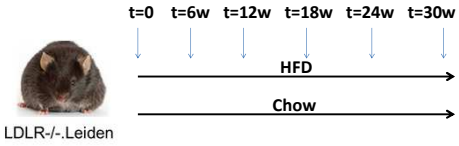


Fig 1: *Study design*

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

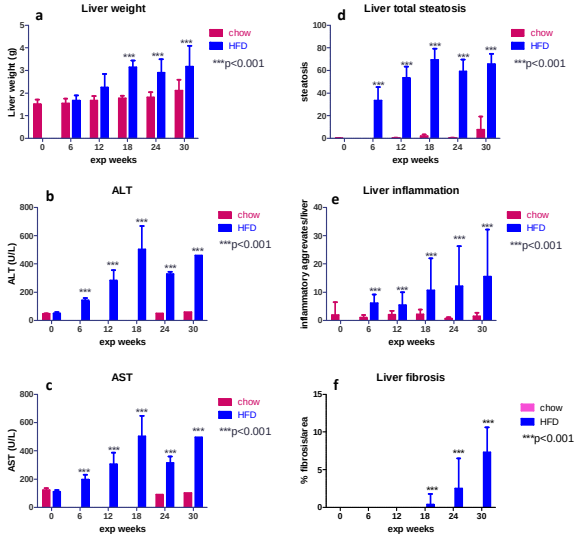
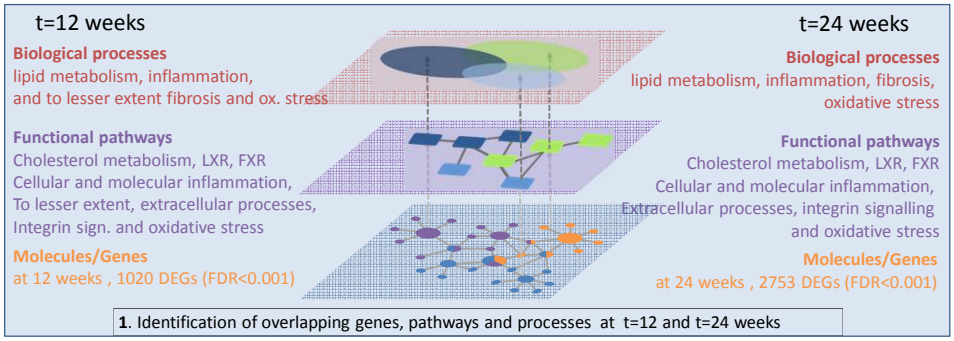


Figure 2: Effect of high fat diet feeding on liver parameters: *increased liver weight (**a**), liver enzymes (**b+c**) and histopathological scoring of steatosis (**d**), inflammation (**e**) and fibrosis (**f**).*

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.

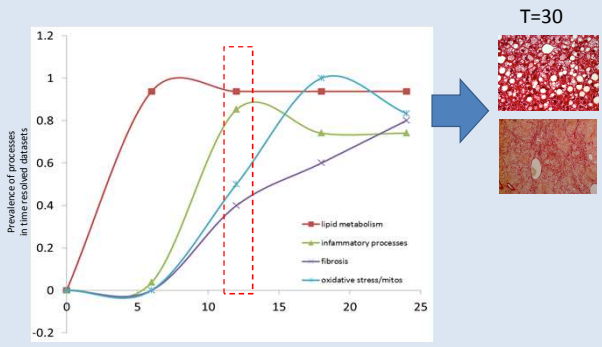


1. Identification of overlapping genes, pathways and processes at t=12 and t=24 weeks

Integration of Dynamic protein profiling with RNAseq data

Dynamic protein profiling		*RNAseq-Transcriptome*
Fatty acid beta-oxidation	Steatosis	Cholesterol biosynthesis LXR/RXR pathway FXR/RXR pathway
	Inflammation	DC-maturation Leukocyte extravasation signalling T-cell signalling NF-kB signalling
Oxidative phosphorylation Mitochondrial dysfunction ER-stress	Oxidative stress	NRF2-mediated oxidative stress
Hepatic Fibrosis Stellate cell activation TNO fibrosis gene signature	Fibrosis	Hepatic Fibrosis Stellate cell activation TNO fibrosis gene signature

2. By integrating dynamic protein profiling with transcriptome data, common pathways involved in disease development were identified.



3. At t=12w, processes involved in disease development can be detected which can be associated with 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.**