A systems-biological perspective of non-alcoholic fatty liver disease in mice

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Non-Alcoholic Fatty Liver Disease (NAFLD) is a progressive disease and the most common form of chronic liver disorders in western countries [1]. The increased prevalence in the past decades is mainly due to changes in life-style and eating habits. NAFLD is strongly associated with hepatic and systemic insulin resistance and the metabolic syndrome. In the early stages of this disease, there is an aberrant accumulation of triglycerides (TG) in the liver as lipid droplets. This eventually leads to an inflammatory response which can progress to more severe disease forms like Nonalcoholic Steatohepatitis (NASH) and even cirrhosis or liver cancer [2–4] . Even though it is well-known that the accumulation is caused by an imbalance between the TG uptake and removal, it remains a challenging task to discover the precise factors and mechanisms that promote the storage of TG in the liver. Mouse models can recapitulate several features of the human disease pattern and have already provided valuable insights into possible pathological mechanisms.

The aim of our study was to further investigate the pathogenesis of this disorder in three different fatty liver mouse models: C3Heb/FeJ (C3H), C57BL/6J (B6J) and C57BL/6N (B6N). In all three studies the fatty liver phenotype was introduced by a prolonged safflower oil rich high fat diet (SAFF). Littermates fed on a standard diet (Chow) served as controls. Measurements including liver specific messenger-RNA (mRNA) transcriptomics (Affymetrix MoGene 1.0 ST microarrays) as well as clinical parameters (e.g. plasma insulin concentrations, liver TG levels) were assessed during the study at different points of time.

In the case of the C3H study we modified a constraint-based modeling technique [11] to predict the metabolic fluxes of the mice based on the measured transcript levels. In this manner we collected “metabolic flux profiles” for each group (SAFF and Chow) at the different stages of the challenge. Based on the comparison of these qualitative “metabolic flux profiles” we identified potential pathways and targets which we suggest to be involved in the progression of the NAFLD phenotype. Integration of additional data about regulatory actions and data from previous studies led to new hypotheses about the molecular actions of the disease. Our results show strong evidence that the TG accumulation is based on an imbalance in the metabolic flux of the TG synthesis pathway. Furthermore we identified a potential target which links this anabolic pathway to a reduced hepatic insulin sensitivity. It is discussed, how constraint-based modeling techniques can be powerful tools in the analysis of complex diseases like NAFLD.

To study the metabolic responses of the B6J and B6N strains we utilized the currently largest and most comprehensive genome-scale metabolic model (GSMM) published by Sigurdsson et al. [8] comprising a total of 1,415 enzyme-coding genes. In analogy to Nielsen et al. [9] we mapped calculated z-scores from the differential data onto the enzyme nodes of the network. We then utilized a scoring algorithm [9] as well as simulated annealing [10] to explore the organization of these transcriptional changes in the metabolic network (highly correlated subnetworks and reporter metabolites). Our results showed that NNT has a global impact on the transcriptional regulation and the flux through the corresponding metabolic network under the studied conditions. The deletion variant of NNT led to significant changes in the expression of numerous genes located in different compartments (e.g. mitochondrion, peroxisome) involved in various cellular processes and seems to have a beneficial effect on the metabolism of hepatocytes in context of NAFLD.

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