Temporal dynamics of metabolic dysfunctions in liver, adipose tissue and the gut during diet-induced NASH development in Ldlr-/-.Leiden mice

Eveline Gart[1,2*], Wim van Duyvenvoorde[1], Jessica M. Snabel[1], Christa de Ruiter[1], Joline Attema[1], Martien P.M. Caspers[3], Serene Lek[4], Bertie Joan van Heuven[5], Arjen G.C.L. Speksnijder[5], Martin Giera[6], Aswin Menke[1], Kanita Salic[1], Kendra K. Bence[7], Gregory J. Tesz[7], Jaap Keijer[2], Robert Kleemann[1], Martine C. Morrison[1]

1 Department of Metabolic Health Research, The Netherlands Organisation for Applied Scientific Research (TNO). 2 Human and Animal Physiology, Wageningen University. 3 Department of Microbiology and Systems Biology, TNO. 4 Clinnovate Health UK Ltd, Glasgow. 5 Naturalis Biodiversity Center, Leiden. 6 Center for Proteomics and Metabolomics, Leiden University Medical Center. 7 Pfizer Worldwide Research, Development & Medical, Internal Medicine Research Unit, Cambridge.

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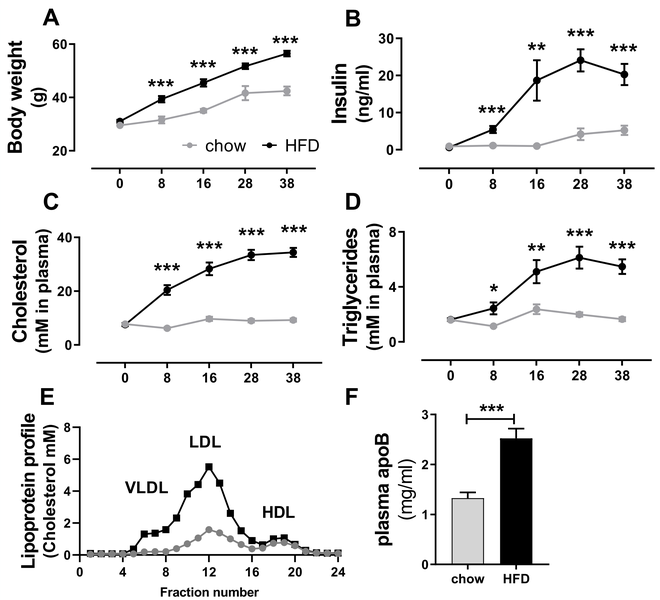
1. Introduction

NAFLD progression, from steatosis to inflammation and fibrosis, results from an interplay of intra- and extrahepatic mechanisms. Disease drivers likely include signals from white adipose tissue (WAT) and gut. However, the temporal dynamics of disease development remain poorly understood.

2. Study aims and design

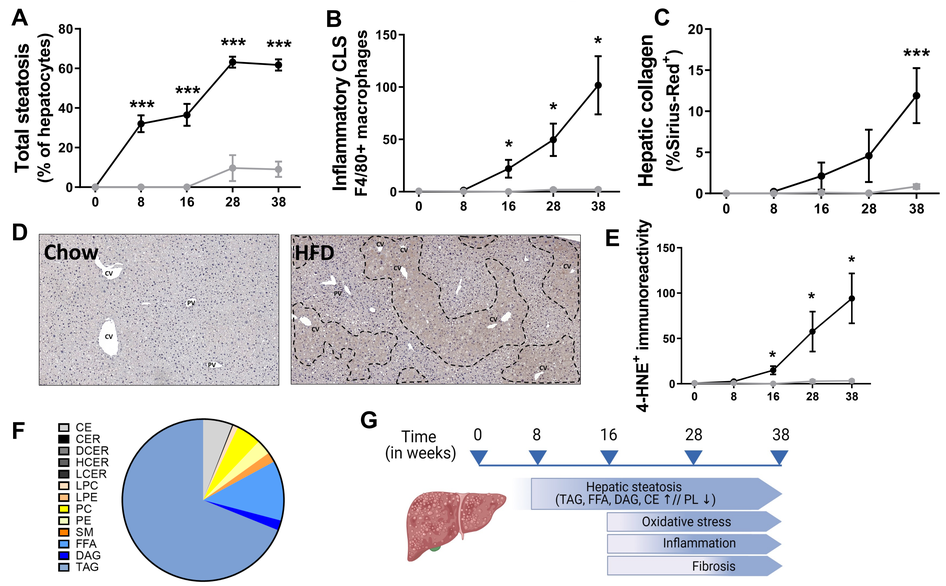
The aim of this study was to investigate the sequence of pathogenic events and organ cross-talk during NAFLD development. For this high-fat diet (HFD)-fed Ldlr-/-.Leiden mice were compared with chow-fed controls. At t=0, 8, 16, 28 and 38w mice were euthanized, and liver, WAT depots and gut were analyzed biochemically, histologically and by lipidomics and transcriptomics together with circulating factors.

3.1 Diet-induced obesity, hyperinsulinemia and dyslipidemia



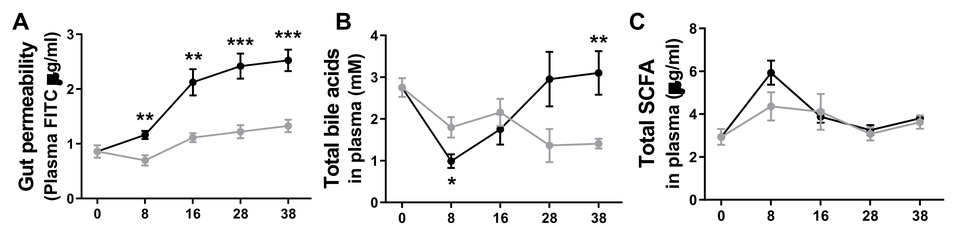
Ldlr-/-.Leiden mice on a HFD develop (**A**) obesity, (**B**) hyperinsulinemia as indicator of insulin resistance, and plasma dyslipidemia (e.g., increased (**C**) cholesterol and (**D**) triglycerides, mainly confined to the (**E-F**) atherogenic apo-B containing lipoproteins) as observed in NASH patients.

3.2 NASH and liver fibrosis development



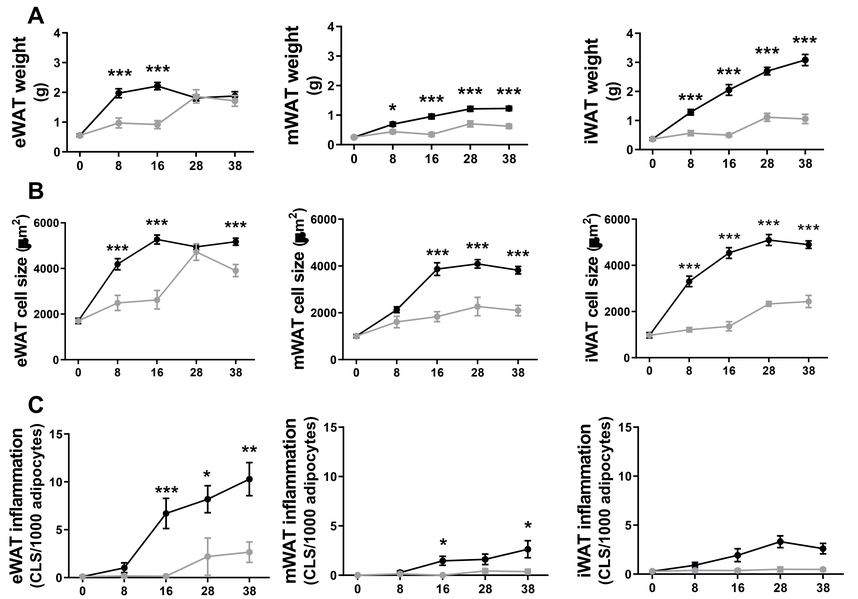
In the liver, early metabolic dysfunction manifests as an increase in lipid accumulation (**A**; steatosis), followed by an increase in (**B**) inflammation and (**C**) fibrosis development. Intrahepatic hits from oxidative stress-induced 4-hydroxynonenal (4HNE) (**D**) representative 4HNE-stained liver sections with portal veins (PV) and central veins (CV) indicated, (**E**) quantification of 4HNE staining. Accumulation of specific lipids (**F**) such as free cholesterol and FFA can promote hepatic inflammation and fibrosis. These hepatic temporal dynamics are summarized in **G**.

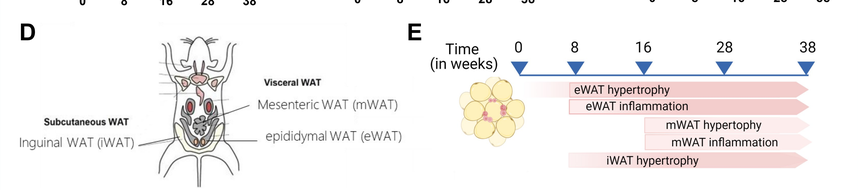
3.4 Increased gut permeability, altered microbiota composition and gut-derived metabolites precede NASH and liver fibrosis



In the gut, HFD significantly increased permeability (**A**), induced a shift in microbiota composition associated with changes in circulating gut-derived metabolites: (**B**) bile acids and (**C**) short-chain fatty acids (SCFA). Temporal gut dysfunctions are summarized in (**D**).

3.3 Visceral white adipose tissue hypertrophy and inflammation precede NASH and liver fibrosis





HFD feeding increases white adipose tissue (**A**) weight, (**B**) cell size and (**C**) inflammation of different depots (**D**). More specifically, epididymal WAT (eWAT) was maximally hypertrophic from t=8w, which coincided with inflammation development. Mesenteric (mWAT) and subcutaneous WAT (iWAT) hypertrophy developed slower and did not appear to reach a maximum, with minimal inflammation. These temporal dynamics are summarized in **E**.

4. Conclusions

HFD-fed Ldlr-/-.Leiden mice develop obesity, dyslipidemia and insulin resistance, essentially as observed in obese NAFLD patients, underlining their translational value. We demonstrate that marked epididymal-WAT inflammation, and gut permeability and dysbiosis precede the development of NAFLD stressing the importance of a multiple-organ approach in the prevention and treatment of NAFLD.

contact: eveline.gart@tno.nl