Chapter 8

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Obesity, a state of increase in energy intake over energy expenditure, is the current health dilemma worldwide. Parallel with increase in obesity, there is an increase in obesity-associated diseases such as type 2 diabetes (T2D) and nonalcoholic fatty liver disease (NAFLD). NAFLD has become the most common cause of liver disease worldwide. The early stage of NAFLD starts with lipid accumulation in the liver also known as hepatic steatosis. This benign and reversible state of NAFLD may, however, evolve into non-alcoholic steatohepatitis (NASH), a condition of inflamed liver, which can further progress to liver fibrosis, cirrhosis and ultimately hepatocellular carcinoma (HCC). In chapter 1, an overview of mechanisms involved in obesity, type 2 diabetes and NAFLD is discussed. Moreover, NASH progression and its underlying triggers, which are arising from genetic and environmental factors, are described. Candidate mechanisms promoting the onset and the progression of NAFLD; of which, aging, fatty acid transporter CD36, and epigenetics are described in chapter 1.

Aging is a risk factor for the development of NAFLD. Nevertheless, the relationship between the progression of NAFLD/NASH/HCC and old age is obscure. Chapter 2 discusses the evidence both supporting and confuting the interrelationship between aging and NAFLD, NASH and HCC. We question whether aging is an actual risk factor for liver diseases, or an innocent bystander. Furthermore, we argue that there might be an age window in which the liver becomes resistant to the development of injury; this needs to be studied to understand fully the interaction between age and liver diseases from a therapeutic perspective.

Obesity is frequently accompanied by low-grade systemic inflammation that is secondary to the development of metabolic inflammation in peripheral tissues such as adipose tissue and liver. While this inflammation is associated with the development of insulin resistance, the extent to which inflammation in the adipose tissue and the liver contributes to insulin resistance remains unknown. In chapter 3, we aim to unravel the origin of metabolic inflammation in obesity, and study the temporal relationship between adipose tissue and liver inflammation in the development of insulin resistance in C57BL/6J male mice fed a low-fat diet (LFD; 10% kcal fat) or high-fat diet (HFD; 45% kcal fat) for either 24, 40 or 52 weeks. Our data show that obesity-induced metabolic inflammation in the adipose tissue precedes hepatic inflammation, suggesting that hepatic inflammation is not a cause but a consequence of insulin resistance.

In chapter 4, we studied the role of aging as a risk factor in the development
of NAFLD. We assessed the link between aging and NAFLD development in mice fed a chow diet and following the consumption of a high-fat diet (HFD, 60% kcal fat). We observed that the aging process by itself did not accelerate NAFLD development in the livers of mice fed a chow diet. However, aging did greatly promote the development of hepatic steatosis and inflammation in combination with diet-induced obesity. In chapter 4 we also explore the role of CD36, a long-chain fatty acid uptake protein, in age-associated nonalcoholic fatty liver disease (NAFLD). We aimed to identify whether increased CD36 expression may underlie the increased susceptibility to the development of NAFLD with age. Chapter 4 shows that aging increases CD36 membrane expression in the livers of both mice and humans. Furthermore, our data show that aging, in combination with HF-feeding in mice, triggers the presence of CD36 at the cell surface of hepatocytes, which may contribute to enhanced fat uptake in NAFLD and drive the progression of simple steatosis towards NASH. Therefore, our data suggest that therapies to prevent the increase in CD36 expression and CD36 from anchoring at the membrane may prevent the development of NAFLD.

In chapter 5 the role of CD36 in the development and progression of NAFLD is examined. CD36 deficiency in mice was found to be associated with an increase in hepatic steatosis, mediated by a reduction in VLDL-TG production and secretion into the circulation. In CD36 deficient mice, the level of inflammation did not change, suggesting that CD36 deficiency affects lipid metabolism pathways in the liver, without altering the level of hepatic inflammation. Therefore, CD36 is likely to play an important role in the maintenance of lipid homeostasis in the liver and CD36 may thus protect against early NAFLD development. In addition, chapter 5 also shows that the type of mice used as experimental controls can explain some of the discrepancies in data related to lipid accumulation in CD36 KO mice. Therefore, for a correct outcome, care should be taken in the experimental design and choice of controls.

In the context of obesity, epigenetic mechanisms regulate cell-specific chromatin plasticity, perpetuating gene expression responses to nutrient excess. MacroH2A1, a variant of histone H2A, emerged as a key chromatin regulator sensing small nutrients during cell proliferation and differentiation. However, despite compelling in vitro evidence that macroH2A1 modulates gene expression programs involved in cell metabolism, proliferation and differentiation, its role at the organism level under nutritional stress conditions, especially during obesity, is not understood. In chapter 6, we challenged macroH2A1 KO mice with an obesogenic diet for 12 weeks. Our data show that genetic ablation of macrohistone H2A1 leads to increased leanness, glucose tolerance and energy...
expenditure in mice fed a high-fat diet. Thus, genetic ablation of this histone confers protection against diet-induced obesity and metabolic derangements in mice, suggesting that inhibition of macroH2A1 might be a helpful strategy for epigenetic therapy of obesity.

In chapter 7, the major findings of this thesis are discussed in the context of the current state of this field and addresses future perspectives of the results. In conclusion, this thesis shows the influence of parameters such as chronic obesity, aging, fatty acid transporter CD36 and macroH2A1 in the development of NAFLD. Our data clearly suggest that a broad spectrum of genetically and environmentally originated factors are involved in the development of NAFLD. These factors however according to the current knowledge of the field, is not yet well understood and require more research and understanding to pave a path to treatment of NAFLD. Existing puzzles in NAFLD progression, might explain why no direct medication has yet been approved for the treatment of NAFLD.