The role of inflammation in lipid metabolism

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SUMMARY

Obesity is frequently accompanied by the development of metabolic disturbances such as insulin resistance that ultimately results in the onset of type 2 diabetes mellitus, and hyperlipidemia, an important risk factor for the development of atherosclerosis. Obesity is accompanied by low-grade systemic inflammation that is secondary to the onset and development of metabolic inflammation in peripheral tissues such as adipose tissue and liver. In fact, metabolic inflammation appears to be causally involved in the onset and development of metabolic disturbances such as insulin resistance and atherosclerosis.

Both hyperlipidemia and inflammation are well known risk factors for the development of atherosclerosis and have been shown to play a causal role in the progression of atherosclerotic plaques. In **chapter 2,** we discussed the interplay between lipid metabolism and inflammatory pathways, which may explain why lipid-lowering drugs and anti-inflammatory drugs reduce atherosclerosis beyond expectations as solely based on their primary mode of action. We therefore discussed the direct and indirect effects of lipid-lowering drugs (*i.e.* statin, fibrates, niacin, ezetimibe) on inflammatory processes, as well as the effect of inflammation-lowering drugs (*i.e.* salicylate, anti-TNF, IL-1ra) on lipid metabolism, both of which may be of additive value in the treatment of atherosclerosis.

Systemic inflammation induces an increase in plasma triglyceride (TG) levels, also called hypertriglyceridemia. Aspirin treatment lowers inflammation via inhibition of nuclear factor κB (NF-κB) activity, but also reduces hypertriglyceridemia in humans. The aim of **chapter 3** was to investigate the mechanism by which aspirin improves hypertriglyceridemia. Therefore, human *APOC1* mice, an animal model with elevated plasma TG levels, as well as normolipidemic wild-type (WT) mice were fed a high-fat diet (HFD) and treated with aspirin. Aspirin treatment reduced hepatic NF-κB activity in HFD-fed *APOC1* and WT mice and in addition, aspirin decreased plasma TG levels in hypertriglyceridemic *APOC1* mice. This TG-lowering effect could not be explained by enhanced VLDL-TG clearance, but aspirin selectively reduced hepatic production of VLDL-TG in both *APOC1* and WT mice without affecting VLDL-apoB production. Aspirin did not alter hepatic expression of genes involved in fatty acid (FA) oxidation, lipogenesis and VLDL production, but decreased the incorporation of plasma-derived FA by the liver into VLDL-TG, which was independent of hepatic expression of genes involved in FA uptake and transport. These data led us to conclude that aspirin improves hypertriglyceridemia by decreasing VLDL-TG production without affecting VLDL particle production. Our findings suggest that the inhibition of inflammatory pathways by aspirin could be an interesting target for the treatment of hypertriglyceridemia.

The liver is an organ that is involved in both inflammation and lipid metabolism. However, it is not known whether the liver plays a direct role in the interaction between inflammation and hypertriglyceridemia. Since NF-κB is a central regulator of inflammatory processes, **in chapter 4** we investigated the relation between chronically enhanced hepatic NF-κB activation and hypertriglyceridemia. To this end, we evaluated whether hepatocyte-specific overexpression of IκB kinase (IKK-β) would affect

VLDL-TG metabolism directly in hyperlipidemic APOE*3-Leiden (E3L) mice, a well-established model for human-like lipoprotein metabolism. We found that hepatocyte-specific IKK-β overexpression induced hypertriglyceridemia. With mechanistic *in vivo* studies we revealed that this that was caused by increased hepatic VLDL-TG production, rather than by reduced VLDL-TG clearance from plasma. Studies in primary hepatocytes showed that IKK-β overexpression also enhances TG secretion *in vitro*, indicating a direct relation between IKK-β activation and TG production within the hepatocyte. Hepatic lipid analysis and hepatic gene expression analysis of pathways involved in lipid metabolism suggested that the increased VLDL production was not caused by increased steatosis or decreased FA oxidation, but most likely by ChREBP-mediated upregulation of *Fas* expression. These findings implicate that specific activation of inflammatory pathways exclusively within hepatocytes induces hypertriglyceridemia. Furthermore, we identified the hepatocytic IKK-β pathway as another possible target to treat hypertriglyceridemia.

Inflammation is a causal factor in the development of atherosclerosis and, as mentioned above, the liver is a key organ involved in inflammation. The relation between hepatic inflammation and atherogenesis is however poorly understood. In **chapter 5** we investigated whether hepatocyte-specific IKK-β overexpression aggravates atherosclerosis development in E3L mice fed a Western-type diet for 24 weeks. We showed that hepatocyte-specific IKK-β overexpression increased the atherosclerotic lesion area in the aortic root and increased lesion severity. Hepatocyte-specific IKK-β overexpression did not affect basal levels of inflammatory parameters, but tended to increase plasma cytokine levels after administration of an inflammatory stimulus (lipopolysaccharide, LPS). In addition, hepatocyte-specific IKK-β overexpression transiently increased plasma cholesterol levels, confined to (V)LDL, which resulted in a mild increased cumulative plasma cholesterol exposure. Taken together, we showed that selective activation of IKK-β in hepatocytes considerably promotes atherosclerosis development which is (at least partly) explained by an increased sensitivity to proinflammatory triggers as well as transiently increased plasma cholesterol levels.

HFD feeding increases hepatic inflammation, as evidenced by increased hepatic Toll-like receptor 4 (TLR4)/NF-κB signaling. In lean animal models, administration of LPS, a well-known ligand for TLR4, as well as activation of hepatic NF-κB both directly increase VLDL-TG production, pointing towards an important link between TLR4/NF-κB signaling and hepatic VLDL-TG production. Furthermore, TLR4 deficiency has been shown to protect against HFD-induced hepatic inflammation. In **chapter 6**, we therefore aimed to investigate 1) whether FA composition of HFD based on lard (HFD-L) and palm oil (HFD-P) differentially affect hepatic NF-κB signaling and VLDL-TG production and 2) whether TLR4 deficiency reduces the hepatic VLDL-TG production in HFD-fed mice. We demonstrated that FA composition of the HFD strongly affects hepatic inflammation, whereby HFD-P, but not HFD-L, markedly increased hepatic NF-κB signaling. However, the increase in hepatic NF-κB signaling was not accompanied by an increased VLDL-TG production. Furthermore, in contrast

to our hypothesis, TLR4 deficiency did not reduce hepatic VLDL-TG production in mice fed HFD-L and even increased VLDL-TG production in mice fed HFD-P. Based on these data, we therefore concluded that 1) FA composition determines the ability of HFD to induce hepatic inflammation, 2) HFD-P-induced hepatic inflammation does not increase VLDL-TG production, and 3) TLR4 deficiency does not reduce VLDL-TG production in HFD-fed mice.

Besides hepatic inflammation, HFD feeding induces adipose tissue inflammation, characterized by macrophage infiltration and production of proinflammatory cytokines that play a causal role in the development of insulin resistance and type 2 diabetes mellitus. The proinflammatory cytokines interleukin (IL)-1β and IL-18 require cleavage by caspase-1 to become activated. This enzyme is part of an intracellular multi-protein complex called "the inflammasome" that besides caspase-1 consists of NLRP3 and adaptor protein ASC. In **chapter 7**, we aimed to investigated the role of these components of the inflammasome in HFD-induced obesity, adipose tissue inflammation and insulin resistance. We showed that mice deficient for NLRP3, ASC or caspase-1 were resistant to the development of HFD-induced obesity and had lower plasma leptin and resistin levels. Furthermore, absence of components of the inflammasome reduced hepatic TG content, adipocyte size, and macrophage infiltration in adipose tissue, as well as adipose tissue expression of monocyte chemoattractant protein (MCP)-1, a key molecule that mediates macrophage infiltration. The reduction in HFD-induced obesity and adipose tissue inflammation in mice deficient for ASC and caspase-1 was paralleled by increased insulin sensitivity. Detailed metabolic and molecular phenotyping demonstrated that the inflammasome is involved in energy expenditure and adipogenic gene expression during chronic overfeeding, although the exact mechanisms are still unclear. These findings revealed a critical function of the inflammasome in HFD-induced obesity and insulin resistance.

Since the mechanism underlying the observation that absence of caspase-1 strongly reduced HFD-induced weight gain remained unclear, we aimed in **chapter 8** to elucidate the mechanism by which caspase-1 deficiency modulates resistance to HFD-feeding by focusing on the role of caspase-1 in the regulation of TG-rich lipoprotein metabolism. To this end, we used caspase-1 deficient and wild-type control mice to determine postprandial TG kinetics, intestinal TG absorption, VLDL-TG production as well as TG clearance, all of which strongly contribute to the supply of TG for storage in adipose tissue. We showed that caspase-1 deficiency reduced the postprandial response to an oral lipid load. The tissue-specific clearance of TG-rich lipoproteins was not changed, evidenced by unaltered kinetics of i.v. administered VLDL-like emulsion particles. An oral gavage of radiolabeled TG-containing olive oil revealed that caspase-1 deficient mice had decreased intestinal chylomicron-TG production and reduced uptake of TG-derived FA in liver, muscle, and adipose tissue. Similarly, despite an elevated hepatic TG content, caspase-1 deficiency reduced the hepatic VLDL-TG production without reducing VLDL-apoB production. Pathway analysis

of microarray data revealed that caspase-1 deficiency reduces intestinal and hepatic expression of genes involved in lipogenesis. From these data we concluded that the resistance to HFD-induced obesity in caspase-1 deficient mice is caused by a hampered assembly and secretion of TG-rich lipoproteins, which decreases the availability of TG-derived FA for uptake by peripheral organs including adipose tissue. These studies revealed that that caspase-1 represents a novel link between innate immunity and the regulation of TG-rich lipoprotein metabolism. Based on **chapter 7** and **chapter 8**, we thus anticipated that inhibition of the inflammasome could be a potential therapeutic strategy in the treatment of obesity and insulin resistance.

Taken together, the studies described in this thesis show that inflammation plays an important role in TG-rich lipoprotein metabolism both in the intestine, liver and adipose tissue. We show that inhibition of inflammation by aspirin reduces the hepatic VLDL-TG production, while hepatocyte-specific NF-κB activation enhances the hepatic VLDL-TG production. Our studies reveal a central role of the liver in the inflammation-induced hypertriglyceridemia and atherosclerosis. In addition, we identified caspase-1 as a novel link between the innate immune system and TG-rich lipoprotein assembly and secretion and as a new therapeutic target in the treatment of obesity and insulin resistance.