Chapter 1 provides a general overview about the pathogenesis of atherosclerosis and NASH. Furthermore, the central hypothesis regarding the role of disturbed cholesterol trafficking in macrophages in the development of chronic inflammation is briefly introduced. Finally, the general aim and the outline of the thesis are described.

In chapter 2 the central hypothesis of this thesis is discussed in more details and recent evidence that shows the involvement of lysosomal cholesterol accumulation in driving inflammation during atherosclerosis and NASH are described. In addition, this chapter discusses the challenges in improving cholesterol trafficking in macrophages and recent successful research directions.

One potential mechanism by which lysosomal cholesterol accumulation can lead to inflammation is via the activation of inflammasomes. In chapter 3, we hypothesized that activated inflammasomes in Kupffer cells (KCs) stimulate cholesterol crystallization, thereby leading to hepatic inflammation during NASH. In order to test this hypothesis, Ldlr−/− mice were transplanted with bone marrow from caspase-1/11−/− mice and fed a HFC diet for 12 weeks. As expected, less severe hepatic inflammation was present in caspase-1/11−/−-tp mice compared to Wt-tp mice. Furthermore, KCs from caspase-1/11−/−-tp mice showed less cholesterol crystals, enhanced cholesterol efflux and increased autophagy. We concluded that hepatic inflammation during NASH is maintained by a vicious cycle whereby disturbed autophagy and reduced cholesterol efflux leads to newly formed cholesterol crystals, thereby further activating inflammasomes.

As lysosomal cholesterol accumulation is also observed during atherosclerosis, inflammasome activation may be involved in the progression of atherosclerosis. Therefore, in chapter 4, we hypothesized that hematopoietic caspase-1/11−/− deficiency leads to reduced atherosclerosis development. For this purpose, caspase-1/11−/− bone marrow cells were transplanted into Ldlr−/− mice and fed a HFC diet for 12 weeks. Hematopoietic deletion of caspase-1/11 resulted in a strong reduction in atherosclerotic plaque size and decreased necrotic core content. The data in this chapter indicate that hematopoietic caspase-1/11 activation plays an important role in vascular inflammation and atherosclerosis development.

Previously, 27-hydroxycholesterol (27HC), a derivative of cholesterol formed by Cyp27a1, has been shown to mobilize cholesterol from the lysosomes to the cytoplasm in vitro.
In chapter 5, we hypothesized that 27HC can redirect the intracellular cholesterol distribution in vivo, thereby influencing hepatic inflammation. To test this hypothesis, Ldlr-/- mice were depleted from Cyp27a1 in their hematopoietic system by bone marrow transplantation and fed a HFC diet for 12 weeks. Furthermore, 27HC was administered subcutaneously to Ldlr-/- mice that received regular chow or an HFC diet for 3 weeks. We demonstrated that Cyp27a1-/-tp mice show increased hepatic inflammation and lysosomal cholesterol accumulation, while 27HC administration led to reduced hepatic inflammation and lysosomal cholesterol trapping. Therefore, we conclude that 27HC may be a potential target to reduce lysosomal cholesterol and to treat NASH.

In chapter 6, we hypothesized that Cyp27a1 overexpression (the enzyme involved in the production of 27HC) in KCs reduces hepatic inflammation. To test our hypothesis, Ldlr-/- mice were transplanted with bone marrow from mice overexpressing Cyp27a1 and given a HFC diet for 12 weeks. In line with our expectations, hepatic inflammation was reduced in mice with hematopoietic overexpression of Cyp27a1. These changes occurred even though 27HC levels in plasma and liver were not different from Wt-tp mice. Therefore, we concluded that hematopoietic Cyp27a1 reduces hepatic inflammation independently of 27-hydroxycholesterol levels in plasma and liver.

In chapter 7, we hypothesized that dietary plant sterol and stanol esters, which are similar to 27HC, will also reduce hepatic inflammation. In order to test our hypothesis, Ldlr-/- mice were given HFC diet with or without supplementation of plant sterols or stanols. We demonstrated that adding plant sterol/stanol esters reduced hepatic inflammation dramatically. This protective effect of plant sterol and stanol esters on hepatic inflammation is expected to open new venues in the treatment or prevention of NASH.

Finally, in chapter 8 we discuss the major findings of this thesis in the context of the current status in the field.