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General Introduction
Cardiovascular diseases (CVDs) are currently the main cause of mortality and morbidity worldwide and expected to remain the leading cause of death at least till 2030 according to the World Health Organization. CVDs are a group of disorders involving the heart and blood vessels and most people die of coronary heart disease and stroke. The root cause of most cases of CVD is atherosclerosis. Atherosclerosis affects medium and large sized arteries in the body and was primarily thought to be solely caused by accumulation of lipids in the vessel wall. Russell Ross was one of the first researchers to state that inflammatory processes play an important role in the pathogenesis of atherosclerosis. Nowadays, disturbances in lipid levels and increased inflammation are the two established contributors to atherosclerosis development. Since the liver is a key role player in both lipid metabolism and regulation of inflammatory processes, it is a potential interesting organ to study in atherosclerosis development.

In practice, in a single patient CVD is often present in combination with other diseases, such as diabetes, cancer, arthritis and chronic obstructive lung disease (COPD). Some features of these comorbidities, such as poor lung function in COPD, are found to be strong independent predictors of cardiovascular risk. Like CVD, COPD is a major cause of mortality and morbidity globally. It is the only leading cause of death that still has a rising mortality rate, and projected to be ranking as the third leading cause of death by 2020. Even though the respiratory and cardiovascular systems interact closely, not many experimental studies have been performed in which the role of both organ systems in atherosclerosis development have been investigated. Furthermore, next to the elucidation of the pathogenic process, it is important to develop new therapies to control the risk factors and inhibit the initiation and progression of CVD and its comorbidities.

This chapter provides background information on dyslipidemia and increased inflammation in atherogenesis and on a new promising anti-atherogenic drug, resveratrol. Furthermore, effects of modulations in lipid metabolism and inflammation in the liver and lungs on atherosclerosis development are highlighted.

1. Dyslipidemia

Nikolai N. Anichkov demonstrated for the first time in 1913 that cholesterol alone caused the atheromatous changes in the vascular wall. Cholesterol is essential for life, it is required to build and maintain cell membranes, involved in intracellular transport, cell signaling, nerve conduction and it is an important precursor molecule for the synthesis of bile and hormones. As it is insoluble in blood due to its lipophilic characteristics, it is transported through the circulation in lipoproteins. These particles have a lipid-rich core containing triglycerides (TGs) and cholesteryl esters (CEs) and an amphipathic surface consisting of phospholipids (PLs), unesterified cholesterol and proteins. These proteins, called apolipoproteins, bind lipids and solubilize them, and act as coenzymes and ligands for lipoprotein receptors on tissues, like the liver. Lipoproteins are classified based on their density (in the order of increasing density): chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

In the fed state, dietary lipids form chylomicrons in the intestine. Here, the products of fat digestion, TG, PL and CE are combined with apolipoprotein (apo) B48 in the enterocyte.
The lipoproteins formed in this manner are secreted into the lymph (chyle) and are termed chylomicrons. TG in chylomicrons is hydrolyzed by lipoprotein lipase (LPL) and the released fatty acids (FAs) are subsequently taken up by adipose and muscle tissue. An endogenous source for FA from TG is VLDL, which is synthesized by the liver. After processing by LPL, VLDL is converted to IDL and LDL. Remnants of apoB-containing lipoproteins can be taken up by the liver.

HDL is the smallest and only anti-atherogenic lipoprotein. Its most abundant apolipoproteins are apoA1 and apoAII. The liver synthesizes HDL as complexes of apolipoproteins and PLs, which are capable of picking up cholesterol from the periphery, e.g. atherosclerotic lesions, and transporting it back to the liver, where it can be excreted out of the body through the bile. This process is termed reverse cholesterol transport. HDL also delivers cholesterol to adrenals, ovaries, and testes for the synthesis of steroid hormones.

The process of atherogenesis was considered for many years merely to constitute of progressive accumulation of lipids within the vessel wall. Indeed at the earliest stages, accumulation and modification of LDL was observed and has been recognized as the initiating factor of atherosclerosis development. Healthy endothelium forms a smooth monolayer of elongated endothelial cells, without adhesive capacities. However, especially in areas where the blood flow is disturbed, e.g. branched or curved points, the permeability of the endothelium is increased. Endothelial dysfunction and increased permeability can also be caused by elevated levels of LDL, hypertension, hyperglycemia, increased inflammation and oxidative stress, e.g. by cigarette smoking. Remnant lipoproteins and mainly the small, dense LDL, can enter the intima of the blood vessel. LDL is retained in the intima by binding of apoB to proteoglycans of the extracellular matrix. Although disturbances in lipid metabolism have long been held responsible for atherosclerosis development, the earliest stage of atherogenesis is characterized by accumulation of (lipid-laden) inflammatory cells, and more specifically macrophages. In recent years, atherosclerosis research has turned its focus more to the inflammatory processes involved.

2. Inflammation

Small, dense LDL particles easily penetrate into the endothelium, become entrapped in the intima, where they become modified by e.g. oxidation (Figure 1). This activates the endothelium and resident macrophages to produce adhesion molecules and chemokines, such as monocyte chemoattractant protein 1 (MCP-1) and vascular cell adhesion molecule (VCAM), to attract monocytes (the most numerous of the leukocytes recruited), dendritic cells (DCs), and T cells into the intima. In early atherosclerosis, vascular smooth muscle cells (VSMCs) may contribute to the development of the atheroma through the production of pro-inflammatory mediators and through the synthesis of matrix molecules required for the retention of lipoproteins. After stimulation with macrophage colony-stimulating factor (M-CSF), monocytes differentiate into macrophages in the intima, which take up the modified LDL (mLDL) through their scavenger receptors. As cholesterol accumulates, these macrophages transform into foam cells, the prototypical cell in atherosclerosis, when the LDL particles cannot be mobilized out of the cell to a sufficient extent.

Antigens of mLDL are presented by macrophages and DCs in the intima and trigger the activation of other macrophages and antigen-specific T-cells. Most of the activated
macrophages and T-cells produce cytokines, e.g. interferon (IFN)γ, interleukin (IL)-6, tumor necrosis factor (TNF)α, which attract and activate more inflammatory cells, e.g. mast cells, natural killer (NK)T-cells, leading to magnification and sustainment of the inflammatory response and the formation of a complex conglomeration of lipids, cholesterol crystals, inflammatory and necrotic cells. In addition, cytokines and growth factors secreted by macrophages and T-cells are important for VSMC migration, proliferation and production of collagen to form a fibrous cap covering the lesion. The fibrous cap maintains stability of the plaque by protecting the thrombogenic mixture of leukocytes, lipid, and debris from the blood stream. Extracellular lipid derived from dead and dying cells can accumulate in the plaque,

Fig. 1. Stages in atherogenesis. 1) When LDL becomes entrapped in the vessel wall, it becomes modified (mLDL). This mLDL induces an inflammatory response leading to 2) expression of adhesion molecules on the activated endothelium, by which leukocytes, such as monocytes are attracted to migrate into the vessel wall. 3) The monocytes mature into macrophages, take up the mLDL through scavenger receptors and 4) become foam cells. 5) In the progressing atherosclerotic lesion, smooth muscle cells (SMCs) proliferate, migrate and produce collagen to form a fibrous cap covering 6) the lipid-rich core consisting of necrotic and apoptotic foam cells, cholesterol crystals and other debris. 7) If this thrombotic content becomes exposed to the blood stream, due to weakening and rupture of the cap by e.g. proteases, a thrombus is formed which can occlude the vessel, causing an infarction.
often denoted the lipid or necrotic core. On the other hand, proteases (e.g. metalloproteinases (MMPs)), cytokines and radicals are also produced that can destabilize the lesions and break down the fibrous cap. While clinical complications, e.g. angina pectoris, can arise from growing plaques creating flow-limiting stenoses, the most severe adverse events follow the rupture of a plaque, which exposes the prothrombotic material in the plaque to coagulation factors in the blood and causes a sudden occlusion of the artery, which can result in ischemia and infarction of the supplied tissue.13

2.1. Nuclear factor-κB (NF-κB)
Nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) is a family of five protein products belonging to two classes. Class I consists of NF-κB1 (p50) and NF-κB2 (p52), which are synthesized from the precursors p105 and p100, respectively. Class II includes RelA (p65), RelB and cRel.14 The most common and best-characterized form of NF-κB is the p65/p50 heterodimer.

In unstimulated cells, the NF-κB p65/p50 dimer is kept inactive in the cytosol bound with its inhibitor, IkBα (Figure 2). IkBα keeps NF-κB inactive by masking the nuclear localization site and sequestered in the cytoplasm.15 The classical activation of the NF-κB pathway can be initiated by a wide range of extracellular stimuli, including cytokines, such as TNFα and IL-1β, viral products, bacterial components (pathogen-associated molecular patterns (PAMPs)), but also saturated FAs16 and endogenous stress signals following tissue damage, termed danger-associated molecular patterns (DAMPs)17 through different receptors, such as pattern recognition receptors (PRRs). This results in the activation of different signal transduction cascades which eventually activate the IkB kinase (IKK) complex, which consists of IKKα, -β and -γ (the latter is also called NF-κB essential modulator (NEMO)). This complex will mediate the phosphorylation of IkBα, resulting in its ubiquitination and degradation, allowing nuclear entry of the liberated NF-κB, where it can induce expression of specific target genes, encoding cytokines, growth factors, immunomodulatory molecules, apoptosis related genes and others.

Under physiological circumstances, NF-κB activation is rapid and short-acting (approximately 30-60 min), and expression of NF-κB-dependent genes is downregulated after a limited period of time. One of the target genes of NF-κB is IkBα, thereby forming a negative feedback loop. In atherosclerosis and many other chronic pathological conditions, however, NF-κB is persistently activated by a combination of numerous factors.

In the initial stages of atherosclerosis development, NF-κB regulates the expression of cytokines, chemokines and adhesion molecules.18 Later in the progression of the lesion, NF-κB regulates gene expression of M-CSF, which is important for the formation of foam cells.19 NF-κB is an essential regulator of MMP gene expression, especially MMP-2 and MMP-9, which are critical in plaque rupture.20 Increased NF-κB activity was found especially in unstable regions of atherosclerotic plaques.21 Thus, NF-κB regulates the expression of a wide spectrum of factors influencing different stages of atherosclerosis development. Furthermore, many of these factors increase NF-κB activity which propagates a positive feedback loop. Increased NF-κB activity was not only observed locally within the lesion, but also in circulating leukocytes as
demonstrated in a study showing highest activity in circulating leukocytes from unstable angina patients when compared to stable angina patients, and low activity in control patients. 22 Nowadays, many research projects on the discovery of anti-atherogenic drugs are targeted on the inhibition of NF-κB activity. 23 NF-κB signaling and its interactions with other networks are very complex and hence the inhibition of the whole NF-κB system will be detrimental. It is therefore essential to identify and dissect the crucial signaling connections and in this way develop more specific and safer therapeutic agents. 24

3. Lipid metabolism and inflammation

The term ‘lipemia of sepsis’ was coined already in the late 1950s, when patients with cholera were noted to have lipemic blood and high serum levels of TGs. 25 The functional importance of this cytokine-induced hyperlipidemia lies not only in the mobilization of lipid stores to fuel the host immune system to combat the pathogen, but also in the capacity of TG-rich lipoproteins to bind and neutralize lipopolysaccharide (LPS), the most toxic component of the cell membrane of Gram-negative bacteria. 26 In addition, apolipoproteins, e.g. apoAI and apoE have been shown to have strong anti-inflammatory features, next to their more classical role in lipid metabolism. 27

![Diagram of NF-κB pathway]
So, there is ample evidence that lipid metabolism and inflammatory pathways interact with each other. As mentioned above, they are the two key contributors to atherosclerosis development. Thus, studying their crosstalk in atherosclerosis-prone animal models will provide more insight in the involved processes in atherogenesis. For example, hypercholesterolemic mice have a peripheral blood monocytosis that develops over time in both chow-fed and fat-fed apoe−/− mice.28 This monocytosis is mainly attributed to an increase in the pro-inflammatory population of Ly6C hi expressing monocytes compared with the ‘patrolling’ Ly6C lo expressing subset. The Ly6C hi monocytes are pro-atherogenic because they adhere more strongly to activated endothelium, accumulate in plaques and become lesional macrophages.28

4. Animal models for atherosclerosis

Analysis of human atherosclerosis has been predominantly observational, although with the advancement of non-invasive techniques, e.g. intravascular ultrasound, atherogenesis in humans in a more experimental setting is increasingly being studied. However, for mechanistic insight into the processes involved, studies in animal models remain to be a necessary resource. One of the main reasons why mice form suitable models is the possibility to generate transgenic, knock-out or knock-in strains to study the role of specific genes and their ensuing products. These advantages of murine models not only account for the field of atherosclerosis research.

Since the plasma cholesterol levels of wild-type mice are low (approximately 2 mM) and mainly confined to HDL, they do not develop atherosclerosis. Therefore, atherosclerosis-prone mouse models are developed through alteration of key components controlling circulating lipoprotein levels, causing hyperlipidemia and/or -cholesterolemia. ApoE and LDL receptor (LDLr) knockout and transgenic APOE*3-Leiden (E3L) models are most widely used.

ApoE is essential for the uptake of (remnants of) apoB-containing lipoproteins by the liver via LDLr, LDLr-related protein (LRP) and heparan-sulphate proteoglycans (HSPGs). Furthermore, apoE derived from macrophages has been shown to have an important role in cholesterol efflux from foam cells.29 ApoE−/− mice have a pronounced elevation of plasma VLDL-cholesterol level on a normal chow diet (approximately 9 mM) and, as such, develop spontaneously atherosclerotic lesions. In addition, evidence indicates that apoE has anti-atherogenic properties independent of plasma lipoprotein regulation.30 On chow as well as on a high cholesterol (1.25%) diet, apoE−/− mice have higher systemic cytokine levels than wild-type mice.31 In addition, apoE−/− mice have an increased number of circulating monocytes, and upon high fat and high cholesterol diet feeding, these monocytes are skewed to a pro-inflammatory phenotype.32 A possible mechanism for the immunomodulatory characteristics of apoE is that it can bind LPS directly, redirecting it to bile, and thereby preventing it from binding to its receptor.33, 34 Another mechanism described, involves apoE binding to cell surface receptors and HSPG and the consequential inhibition of JNK and c-Jun phosphorylation that is required for IL-6, IL-1β, and TNF-α secretion in Toll-like receptor (TLR) signaling.35 Irrespective of the involved mechanism, it is clear that not only the hyperlipidemia, but also the increased inflammatory state of apoE−/− mice accounts for their susceptibility to develop atherosclerosis.
**Ldlr**^−/− mice lack the expression of a functional LDLr due to an insertion of a neomycin resistance cassette into exon 4 of the gene, which leads to the production of an inactive truncated protein. On chow diet, the plasma cholesterol levels are not high enough (approximately 6 mM) for atherosclerosis development. To this end, these mice are fed a cholesterol-rich diet for atherosclerosis studies, resulting in plasma cholesterol levels up to 50 mM, predominantly in the LDL fraction. The elevated levels of LDL as a consequence of the lack of a functional LDLr can augment inflammation. LDL becomes immunogenic when it undergoes modifications such as oxidation and glycation in vivo. mLDL not only promotes the transformation of macrophages into foam cells but their recognition by auto-antibodies also triggers the release of pro-inflammatory cytokines by human monocyte-derived macrophages. Thus, **Ldlr**^−/− mice are likely to have an enhanced inflammatory state, at least indirectly, due to higher levels of LDL.

**E3L** mice carry a construct containing the human APOE^*3-Leiden* gene, a dominant negative mutation of apoE that causes hyperlipidemia, together with apoCI that elevates plasma TG by inhibiting LPL activity. In addition, apoCI also interferes with apoE-mediated uptake of apoB-containing lipoproteins. This results in moderately raised plasma cholesterol and TG levels compared to the **apoE**^−/− and **Ldlr**^−/− models, which in addition can be modulated by varying the dose of cholesterol in the diet. Furthermore, **E3L** mice are more sensitive to lipid modulating drugs, such as statins, fibrates and nicotinic acids, than **apoE**^−/− and **Ldlr**^−/− mice.

To further humanize the lipoprotein profile of **E3L** mice, **E3L** mice have been crossbred to mice expressing human cholesteryl ester transfer protein (CETP). This protein is naturally missing in wild-type mice and transfers cholesteryl esters from HDL to (V)LDL in exchange for triglycerides, thereby lowering HDL and increasing VLDL. These **E3L.CETP** mice respond to HDL-modifying drugs similar to humans and are therefore used extensively in studies in which potential anti-atherogenic therapies are investigated, such as described in chapter 5.

Crossbreeding of these atherosclerosis-prone mice with mice that carry deletions in genes encoding crucial components of the immune system or bone marrow transplantation has provided important information on the role of the immune system in atherogenesis. Organ-, tissue- or cell-restricted genetic altering of mice is another technique to gain more insight in the role of certain cell populations or organs in the pathogenesis of atherosclerosis, which will be further elaborated in the next section.

### 5. Extravascular lipid metabolism and inflammation

To study how the interaction of different organ systems plays a role in atherosclerosis development, experimental animals form a suitable model. CVD has been associated with other chronic inflammatory autoimmune diseases, e.g. systemic lupus erythematosus and rheumatoid arthritis. Chronic inflammation can also derive from exogenous and/or infectious agents, when they are not eradicated effectively by the acute inflammatory response, as has been shown for *Porphyromonas gingivalis*, one of most important bacteria implicated in periodontitis and *Chlamydophila pneumoniae*, a gram-negative bacterium that is a frequent cause of low-grade respiratory infection.
Most pathogens enter their host through the mucosa of the lungs and gut by the route of inhalation or ingestion, respectively. Following the gut, the liver is the second line of defense for incoming pathogens. The lungs and liver are therefore equipped with critical immune defense mechanisms. Apart from their role in immunity, the liver is known to be a key regulator of lipid metabolism. In the framework of the two main contributors to atherosclerosis, dyslipidemia and especially, enhanced inflammation, the role of the liver and the lungs in atherosclerosis development are described in the following paragraphs.

5.1. Liver

The liver is the largest internal organ in the human body comprising about 1/50 of the adult body weight. It is the only organ in the body that receives blood from both the systemic circulation (via the hepatic artery, ~20%) and the gastrointestinal tract (via the portal vein, ~80%). In the liver, blood from the portal vein and arterioles derived from the hepatic artery flows to the central vein, while passing the hepatic sinusoids. The hepatic sinusoids are fenestrated and lack a basement membrane, which facilitates the interaction between the contents of both blood supplies with liver sinusoidal endothelial cells, hepatic immune cells in the space of Disse, and hepatocytes, arranged in cords (Figure 3). This blood passing the hepatic sinusoids contains products of digestion, including carbohydrates, peptides, FAs, along with antigens and microbial products that originate from the bacteria in the small and large intestine. Apart from its role in energy metabolism and immunity, which will be discussed more extensively below, the liver, or more specifically, the hepatocytes, carry out various vital functions, such as the production of coagulation factors, break down or modification of toxic substances and drugs, storage of vitamins, production of albumin and synthesis of angiotensinogen.

The liver is composed of many different cell types which are divided into parenchymal cells (hepatocytes) and non-parenchymal cells, i.e. sinusoidal endothelial cells (SECs), lymphocytes, Kupffer cells, biliary epithelial cells, hepatic stellate cells (HSCs) and DCs (Figure 3). The hepatocyte population accounts for approximately 80% of all cells in the liver, while non-parenchymal cells constitute 20%, of which about 50% are endothelial cells, 25% lymphocytes and 20% Kupffer cells. Compared with peripheral blood, the liver is enriched with NK and NKT cells.

5.1.1. Lipids

The liver plays a major role in energy metabolism of the three nutrients, carbohydrates, proteins and fat. It synthesizes, stores and releases carbohydrates through gluconeogenesis, glycogenolysis and glycogenesis. In addition, it is responsible for the synthesis and the degradation of proteins and removal of ammonia from the body by synthesis of urea. Excess carbohydrates and proteins are converted into FAs and TGs to be exported to the rest of the body. Because of its prominent role in atherogenesis, the role of the liver in lipid metabolism is described more in detail.

Chylomicrons from the intestine travel through the lacteals to join lymph from other parts of the body, and enter the blood circulation via the thoracic duct. LPL releases TG from the core of the chylomicron by hydrolyzing them to FA and monoglycerides, which are taken up by the
Anatomical organization of the liver. **Hepatic lobule.** When blood drained from the gastrointestinal system flows from the portal vein to the central vein, it passes cords of hepatocytes through sinusoids. **Hepatic sinusoid.** Sinusoidal endothelial cells (SECs) form the specialized, fenestrated endothelium lining the sinusoids. Hepatic stellate cells (HSCs) are found in the space of Disse, while Kupffer cells, lymphocytes and dendritic cells (DCs) can be found both in the sinusoids and space of Disse.

Remaining chylomicron remnants are removed from the circulation largely by the liver. VLDL is synthesized by the liver and hydrolyzed by LPL resulting in the formation of IDL, which is partly taken up by the liver by apoE. The remainder is further processed by LPL and hepatic lipase to become LDL, which is recognized by the receptors on liver and peripheral tissues. VLDL, IDL and LDL are apoB100-containing particles. Within the hepatocyte, lipidation
of apoB100 requires an adequate supply of lipids and microsomal triglyceride transfer protein (MTTP). The lipids added to apoB100 can originate from the diet or be synthesized de novo by hepatocytes. De novo lipogenesis is upregulated via insulin-mediated stimulation of sterol response element-binding protein 1c (SREBP-1c), a major lipogenic transcription factor. Fatty acid synthase (FAS) is one of the most important genes involved in de novo synthesis of TG, while carnitine palmitoyltransferase 1A (CPT1A) is an enzyme essential for fatty acid oxidation.

High circulating levels of LDL can cause retention of LDL within the vessel wall, which is the first step of atherosclerosis development, as described above. Hepatic synthesis of bile acids accounts for the majority of cholesterol removal out of the body. Bile is excreted in the duodenum and facilitates the digestion and transport of lipids. The largest amount (~95%) of bile acids excreted in the duodenum, are absorbed back into blood within the ileum, where it returns to the sinusoids of the liver through the portal vein (enterohepatic recirculation). Bile acids are then transported across the hepatocytes to be resecreted into bile canaliculi.

5.1.2. Inflammation

Facing the continuous exposure to gut-derived antigens present in portal venous blood, the liver’s immune cells exist in a state of tolerance which is also termed ‘liver tolerance’. On the one hand, a reasonable speculation is that most of these antigens originate from harmless material in food, on the other hand, however, this makes the liver also susceptible to invasion by pathogens that breach the intestinal mucosa and invade the circulation. Therefore, the liver is an organ with a specialized immune system. Kupffer cells and lymphocytes, including a relative high number of NK cells and NKT-cells, make up the majority of immune cells in the liver. Kupffer cells are specialized macrophages located in the liver and form the body’s largest compartment of macrophages. They reside within the lumen of the liver sinusoids; therefore, they are the first cells to be exposed to materials absorbed from the gastrointestinal tract. In addition to their phagocytic capacities, Kupffer cells process and present antigens. Cytokines produced by Kupffer cells induce the expression of acute phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA), α1-antitrypsin, ceruloplasmin or haptoglobin, partly through NF-κB signaling, in hepatocytes.

NK cells make up as many as 50% of liver lymphocytes and respond both to cytokine activation and to engagement of an excess of activating receptors over inhibitory receptors, i.e. recognizing major histocompatibility complex (MHC) class I alleles. NKT cells recognize glycolipid antigens that are conserved features of bacterial walls, thus respond to those derived from the intestinal bacteria. From this perspective, NKT cells can be considered to represent a bridge between lipids and inflammation. Given that lipid accumulation is a hallmark of atherosclerosis and the fact that NKT cells are activated by glycolipid antigens, it is not surprising that NKT cells were hypothesized to play a role in atherogenesis. ApoE on circulating lipoproteins can enhance NKT cell responses to glycolipid antigen presented by human DCs and activation of NKT cells has been demonstrated to accelerate atherogenesis.

The other non-parenchymal cells in the liver, i.e. SECs, DCs and biliary epithelial cells all express TLRs and play an immunomodulatory role, but are not further discussed in this introduction.
5.1.3. Lipids and inflammation
Hypercholesterolemic apoe\(^{-/-}\) mice on chow diet display not only inflammation in atherosclerotic lesions, but also in periadventitial and visceral adipose tissue, liver and pancreatic islets. Dietary cholesterol can induce hepatic inflammation in \(ldlr^{-/-}\) and \(E3L\) mice. Like atherosclerosis, insulin resistance is commonly associated with both chronic inflammation and a metabolic dyslipidemic profile with increased levels of atherogenic apoB-containing lipoproteins. In an insulin resistant experimental animal model, it was shown that inhibition of NF-\(\kappa\)B activity resulted in reduced apoB100 synthesis by primary hamster hepatocytes. This implies that hepatic inflammation is an important factor underlying hepatic atherogenic lipoprotein production observed in insulin resistance and atherosclerosis. Moreover, Luchtefeld et al. have shown that hepatocyte-specific deficiency of gp130, a key component of the IL-6 signaling pathway, reduces atherosclerosis development in apoe\(^{-/-}\) mice. The liver is a prototypical organ in which lipid metabolism and inflammation converge, thus it is not surprising that it plays a central role in atherosclerosis.

5.2. Lungs
The lungs are not only the organ system that is localized nearest to the heart, but also work in close collaboration with the cardiovascular system. Lungs are filled with about 90% air and 10% tissue, which reflect their most important function, i.e. gas exchange of oxygen and carbon dioxide. Inhaled air travels from the nose to the trachea, bronchi, bronchioles, terminal bronchioles, respiratory bronchioles and finally the alveoli, which are the functional units of lungs, where most of gas exchange takes place (Figure 4). Oxygen and carbon dioxide are transported to and from the rest of the body respectively, in erythrocytes and dissolved in plasma.

The pulmonary epithelium in the bronchi and bronchioles consists of different cell types: ciliated cells, goblet cells, basal cells, brush cells and Clara cells. The cilia of the ciliated cells beat in concert cranially, effectively moving secreted mucus containing trapped foreign particles to the oropharynx for either expectoration or swallowing to the stomach where the acidic pH helps to neutralize foreign material and micro-organisms. Goblet cells secrete mucus, which acts as a barrier and traps particulate material and pathogens moving through the airway. Basal cells serve as a reserve population by maintaining individual cell replacement in the epithelium. Brush cells have a synaptic contact with an afferent nerve ending at the basal surface and are therefore regarded as receptor cells with microvilli. Clara cells increase in number as the ciliated cells decrease along the length of the bronchiole. They secrete surfactant-related proteins, which are needed to prevent luminal adhesion in case the wall of the airway folds on itself, particularly during expiration.

The most important types of cells surrounding the alveoli are type I pneumocytes, type II pneumocytes and alveolar macrophages (Figure 4). Type I pneumocytes are the major cell type lining the alveolar surfaces, through which gas is exchanged. The type II cells serve as stem cells for themselves and the type I cells. They secrete a fluid which acts as a surfactant by reducing surface tension, and thereby increase pulmonary compliance, prevent atelectasis (collapse of the lung) at the end of expiration and facilitate recruitment of collapsed airways. The alveolar
Fig. 4. Epithelium of the bronchial tree and alveoli. Inhaled air first passes the nasal cavities, naso-, oropharynx, larynx, trachea, primary bronchi, before it enters the lungs, where it sequentially travels through the internal bronchi, bronchioles, alveolar ducts, - sacs, and alveoli. **Bronchial epithelium.** Basal cells, ciliated columnar cells, and goblet cells are the principal cell types in the bronchial epithelium. The basal cells, located at the base of the epithelial layer, are the stem cells from which the other cell types arise. The ciliated columnar cells are the most numerous and provide sweeping of the mucus toward the pharynx, thus serving as an important protective mechanism for removing small inhaled particles from the lungs. Subepithelial glands are present in the larger bronchi and are a main source of mucus in the central airways. **Bronchiolar epithelium.** The composition of the epithelium changes as the ducts narrows from bronchi, the larger diameter bronchioles towards smaller ones: cartilage plates and subepithelial glands are not present in bronchioles, the ciliated columnar epithelium transforms to simple cuboidal epithelium, the number of goblet cells decreases, while Clara cells increase in number. Clara cells are nonciliated cells that secrete surfactant-related proteins. **Alveoli.** Alveoli are surrounded and separated from each other by a thin connective tissue layer that contains numerous blood capillaries. The tissue between adjacent alveolar air spaces is called the alveolar septum. The alveolar epithelium is composed of two types of pneumocytes: type I which line most of the surface of the alveoli, and type II that secrete surfactant and are important for host defense. Alveolar macrophages are present both in the connective tissue of the septum and in the air space of alveoli.
macrophages phagocytose the bacteria, dust particles or other debris and move towards the bronchioles, where phagocytosed materials are eliminated by coughing.

5.2.1. Lipids

The role of lipids in the lungs has not been a subject of extensive study in current literature. Lipids are naturally secreted in the pulmonary surfactant in the lungs by type II pneumocytes and play an important role in the maintenance of airway patency by lowering the surface tension during respiration. Surfactant consists for 90% of lipids (mainly saturated phospholipids) and 10% of protein. It has been demonstrated that deletion of the ATP-binding cassette (ABC) transporter, ATP-binding cassette sub-family G member 1 (ABCG1) in macrophages causes lipid accumulation in the alveolar macrophages.59 Traditionally, ABCG1 is known for its role in facilitating cellular cholesterol and phospholipid efflux from macrophages to mature HDL. Another ABC transporter, ATP-binding cassette sub-family A member 1 (ABCA1) mediates the efflux of cholesterol and PLs from macrophages. In addition, ABCA1 has been shown to be expressed in the lung and highest expressed in type II pneumocytes,60 implying a role in surfactant regulation.61 It controls transport of cholesterol and phospholipids to apoA1 in type II pneumocytes. Defective ABCA1 manifests as Tangier’s disease, characterized by severely reduced levels of HDL, and abca1/- mice have pulmonary lesions consisting of foamy type II pneumocytes, lipid-laden alveolar macrophages and cholesterol clefts and display pathophysiologic hallmarks similar to human Tangier’s disease.62 Abca1-/-/abcg-/- mice exhibit extreme lipid accumulation in tissue macrophages of the lung amongst other organs (liver, spleen, Peyer’s patches, and lymph nodes).63 Whether the accumulation of lipid-laden cells in these murine models is also accompanied with increased pulmonary inflammation is likely, but not reported. Together, these data demonstrate that lipids do play an important role in the lungs, which becomes evident when ABC transporters involved in lipid trafficking are affected.

5.2.2. Inflammation

The obstructive lung diseases COPD and asthma are the most frequent causes of respiratory ill health, covering all ages.64 Both conditions are associated with many comorbidities, including CVD,65, 66 albeit asthma to a lesser extent than COPD. One of the most obvious reasons for this is that most COPD patients are aging individuals, and therefore are at higher risk to have more comorbidities. It has been proposed that COPD and CVD are entities of the same systemic inflammatory syndrome, of which other diseases, such as Alzheimer’s disease, osteoporosis also take part. This syndrome has also been called ‘inflamm-aging’ by some,67 with a reduction in adaptive immunity and an increase in innate immunity driven by NF-κB activation.68 There is abundant evidence of increased systemic inflammation in COPD and CVD, as demonstrated by the presence of activated circulating leukocytes and increased levels of circulating inflammatory mediators.69 CRP for example, known as a biomarker of systemic inflammation, is not only a marker of increased mortality in COPD,70 but also a marker of increased cardiovascular risk.71 Although, it is often hypothesized that inflammation in the systemic compartment is the result of ‘spill over’ of the inflammatory process locally, in this case from the lungs, evidence from
cross-sectional studies did not point out a correlation between pulmonary and circulatory inflammatory markers in stable COPD. As mentioned before, a poor lung function has been shown to be a strong independent risk factor for CVD-related morbidity and mortality in many epidemiological studies. It is not clear however whether COPD also plays a causative role in this increased risk, or COPD and CVD are part of a ‘syndrome’ where systemic inflammation is a common denominator.

5.2.3. Lipids and inflammation

In clinical COPD studies, the prevalence of obesity is higher with a lower GOLD stage, i.e. less severe COPD, in line with the intriguing finding that a higher body mass index (BMI) (and higher fat-free mass) is associated with a lower mortality rate in COPD patients. On the other hand, obesity can cause low-grade systemic inflammation and as high CRP levels in young healthy adults are associated with a faster decline in lung function, obesity may be a risk factor for COPD. This obesity paradox in COPD can be ascribed to several possible causes. Firstly, the different phenotypes of COPD partly contributed to this paradox. Obesity in COPD is more prevalent in the chronic obstructive phenotype, whereas underweight is more prevalent in the emphysematous phenotype. A reduced respiratory function in COPD impairs physical activity and increases the risk of developing obesity. In addition, there is no plausible reason why adiposity should protect against mortality in COPD. In fact, one study indicates that lean mass index is a better predictor of mortality than BMI in moderate to severe COPD, while fat mass index is not a significant prognostic indicator. Another study demonstrated that excessive visceral fat (independent of total fat mass) contributes to increased plasma IL-6, which, in turn, is strongly associated with all-cause and cardiovascular mortality in patients with obstructive lung disease. These data suggest that the overall physical condition as reflected by a larger lean mass index and/or the fat composition rather than obesity per se are important determinants of the severity and inflammatory state of COPD.

Interestingly, feeding apoe/− mice a Western-type diet (high fat 21% and high cholesterol 0.15% content) also induces increased inflammation in the lung. These mice had higher levels of pro-inflammatory cytokines than the control wild-type mice on the same diet. Furthermore, Goldklang et al. demonstrated that apoe/− mice fed a Western-type diet (21% fat and 0.21% cholesterol) for 10 weeks compared to chow-fed apoe/− mice exhibited elevated number of macrophages and lymphocytes in the lung, accompanied by an increase in MMP-9 and -12 activity and more importantly, air space enlargement, i.e. emphysema, a major feature of COPD. These changes did not occur in ldlr/− mice fed the same diet. In addition, macrophages from the apoe/− mice treated with a TLR4 ligand showed enhanced MMP-9 expression, which was further augmented with the addition of oxidized LDL. These studies provide a possible mechanism linking emphysema and atherosclerosis, where persistent systemic inflammation, from multiple different sources, act through e.g. the TLR4 signaling pathway with the resultant inflammatory and protease cascade contributing to the development and disease progression of both COPD and atherosclerosis. Moreover, they support the hypothesis that COPD and CVD may be entities of a shared systemic inflammatory disease, which may be triggered by a Western-type diet.
6. Pharmacological therapies

Among all available anti-atherogenic drugs, e.g. fibrates, nicotinic acid, peroxisome proliferator-activated receptor (PPAR)α and -γ agonists, and statins are the most well-studied and proven to be effective in preventing CVD events.\textsuperscript{80} Although powerful drugs for atherosclerosis, like statins, are abundantly used in developing countries, CVDs remain to be the leading cause of morbidity and mortality in the Western world. Next to statins, a new multi-target therapeutic agent, resveratrol is discussed below. Resveratrol is a moderate activator of sirtuin (silent mating type information regulation 2 homolog 1, (SIRT1), a nicotinamide adenine dinucleotide-dependent histone deacetylase with anti-aging and anti-inflammatory properties. The potential benefit of the use of statins and SIRT1 activation has been studied in CVD, but notably, also in COPD.\textsuperscript{81, 82}

6.1. Statins

Numerous clinical trials have established that statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, can reduce various atherosclerotic complications.\textsuperscript{80} As more than two thirds of the body’s total cholesterol is synthesized by the body itself (mainly in the liver) and not consumed through diet, blocking this pathway to decrease serum cholesterol is a logical step to take in an effort to lower cholesterol levels. By blocking HMG-CoA reductase, the rate limiting enzyme in cholesterol synthesis, statins lower the endogenous VLDL production and thereby reduce plasma LDL levels. Some classes of statins also induce an increase in HDL levels by an enhancing apoA1 production up to approximately 15%, primarily in the liver.\textsuperscript{83} Statin treatment not only results in a more favorable lipoprotein profile by reducing LDL and increasing HDL levels, but there is evidence that part of the clinical benefit of statins can be ascribed to other effects, e.g. anti-inflammatory, antithrombotic, and improvement of endothelial function.\textsuperscript{84} Part of the pleiotropic actions of statins is through NF-κB suppression. It has been reported that statins stabilize IκB by inhibiting kinases in monocytes and VSMCs.\textsuperscript{85} These off-target beneficial side-effects of statins have expanded the attention of these drugs to non-cardiovascular fields. Simvastatin, for instance, has been demonstrated to be able to reverse or inhibit pulmonary emphysema, a common manifestation of COPD in animal models.\textsuperscript{86, 87} Trials are being undertaken to evaluate the potential benefit of statins on morbidity and mortality in COPD.\textsuperscript{81} Given that the majority of COPD patients die due to a cardiovascular event, it is also important, but difficult to delineate the beneficial effects of statins on COPD independently from their favorable effects on the cardiovascular system.

6.2. Resveratrol

NF-κB appears to be a tempting therapeutic target, because it is a point of intersection of multiple pathways in atherogenesis. A number of compounds, especially anti-oxidants, proteasome- and IKK inhibitors, have been shown to suppress NF-κB. NF-κB modulation is often part of the pleiotropic actions from pharmaceutical drugs, e.g. glucocorticoids and statins.\textsuperscript{88, 89} In addition, NF-κB inhibiting compounds have been present in our diet from ancient times, such as curcumin,\textsuperscript{90} and resveratrol, a polyphenol found in red wine.\textsuperscript{91} Resveratrol is primarily
present in the skins of grapes and thus in red wine. In addition, resveratrol is present in Chinese herbs, peanuts and a large variety of fruits including various berries and jackfruit.

The mechanism of action of resveratrol is not completely understood. As mentioned before, resveratrol is also a moderate activator of SIRT1, which has been shown to physically interact with the p65 subunit of NF-κB and to inhibit its activity by deacetylating p65 at lysine 310.92 Resveratrol has been shown to potentiate the SIRT1-mediated anti-inflammatory response.93 Atheroprotective effects of resveratrol are numerous, such as suppression of IL-6 and M-CSF secretion,94, 95 prevention of oxidation of LDL and uptake into the vascular wall,96 lowering of blood pressure and glucose levels.97 Similar to statins, resveratrol is reported to have many pleiotropic effects. Thus, resveratrol is considered a promising candidate for the new generation of anti-atherosclerotic drugs, and as mentioned previously, its therapeutic possibilities are currently also being explored in lung diseases. Whether resveratrol can beat the current leading anti-atherogenic drug statin, is a challenging question which remains to be answered.

7. Outline of thesis

Increased inflammation is a main contributor to atherogenesis. Inflammatory processes within the vessel wall are studied extensively. Less is known about the effects of more distal organs. The studies in this thesis focus on the role of two organs, i.e. the liver and lungs, in atherosclerosis development.

The liver is an important organ in the regulation of both lipid metabolism and inflammation, the two key role players in atherosclerosis. In chapter 2, we studied the interaction between liver inflammation and lipid metabolism. We investigated whether hepatocyte-specific NF-κB activation by transgenic expression of IKKβ affected VLDL production in E3L mice. Increased levels of VLDL are an important risk factor for CVD. So, it is reasonable that any effect of hepatocyte-specific NF-κB activation on lipid metabolism found in chapter 2 would influence atherosclerosis development. By using the same transgenic mouse model the role of transgenic NF-κB activation in hepatocytes on both lipid metabolism and inflammation, with atherosclerosis development as primary outcome was examined in chapter 3.

Apart from the classical risk factors such as dyslipidemia and inflammation, poor lung function, most commonly caused by COPD, has been identified as another predictor for CVD.99 Pulmonary emphysema is a major component of COPD and is characterized by loss of alveolar integrity leading to an alveolar space enlargement. We studied whether alveolar destruction per se would have an effect on atherosclerosis development in chapter 4.

New therapies are continuously being developed to reduce evolving and existing CVD. One of the most promising therapeutic components which currently receives a lot of attention, not only in the field of CVD, is resveratrol. In chapter 5 we evaluated the potential anti-atherogenic capacity of resveratrol alone or combined with atorvastatin.

Finally, the major findings of the studies described in this thesis, the clinical implications and the future perspectives are discussed in chapter 6.
References


20. Berbee JF, Havekes LM, Rensen PC. Apolipoproteins modulate the inflammatory


44. Symmons DP, Gabriel SE. Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE. *Nat Rev Rheumatol*. 2011.


68. Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T. Activation of


