6

General Discussion and Future Perspectives

(Extra)vascular inflammation and lipid metabolism in atherogenesis

Dyslipidemia has been mainly associated with metabolic diseases, and classically regarded as the most prominent underlying cause of atherosclerosis. As a consequence, modulation of lipids gained most interest in the treatment of cardiovascular diseases (CVDs). Although lipid-modulating drugs have been studied extensively and applied on large scale in the clinic, two thirds of cardiovascular events cannot be prevented by current lipid-modifying strategies, and CVD remains to be the leading cause of mortality in the Westernized world.[1] While in recent decades inflammatory processes have been recognized to contribute largely to atherosclerosis development as well, the therapeutic potential of anti-inflammatory drugs in atherosclerosis development is less studied and well-defined.

This disparity can be partly explained by the large role of inflammation in many physiological and pathophysiological processes other than CVD. Interference with physiological inflammatory processes can impair human host defense and result in life-threatening infections. While a healthy immune response includes sporadic bouts of acute inflammation to fight harmful stimuli, chronic low-grade inflammation is associated with the development of major diseases such as cancer, diabetes, asthma, rheumatoid arthritis, and atherosclerosis.[2]

So far, many traditional anti-inflammatory therapies do not improve cardiovascular outcomes, and some may even aggravate cardiovascular events. These observations are usually derived from post-hoc analyses of clinical studies which may reflect off-target actions of the drugs studied, *e.g.* glucocorticoids, non-steroidal anti-inflammatory drugs, or tumor necrosis factor (TNF) inhibitors.[3] While general suppression of inflammation is undesirable, selective regulators of inflammation that are able to normalize the enhanced inflammation or skew the inflammatory response towards the anti-inflammatory side or resolution phase may be beneficial.

In the past two decades atherosclerosis research has focused primarily on local vascular inflammation.[4] The crosstalk between dyslipidemia and inflammatory processes within and close to the arterial wall has been shown to be the primary cause of atherogenesis. Not only in the vessel wall, but also within other organs or tissues there is interaction between lipid metabolism and inflammation. This is for example demonstrated in **chapter 2**, in which increased activity of hepatocyte-specific nuclear factor-κB (NF-κB), primarily known as a central regulator of inflammatory processes, was shown to increase VLDL production by these hepatocytes. In addition, we found that enhanced activation of NF-κB in hepatocytes results in aggravated atherosclerosis development in **chapter 3**. This latter study exemplifies the increasing interest in the interaction between different organs and tissues with the vascular wall in the elucidation of the underlying processes involved in atherosclerosis development (Figure 1). While changes in certain tissues or organs can affect atherogenesis, it is also conceivable that the enhanced arterial inflammation and lipid accumulation which is coupled to atherosclerosis development, can act on other organs, *e.g.* by increase of systemic inflammatory mediators.

This thesis addresses the interaction between lipid metabolism and inflammation and the role of two extravascular organs, *i.e.* the liver and lungs, in atherosclerosis development. In this chapter, implications of our findings and future perspectives are discussed, with special

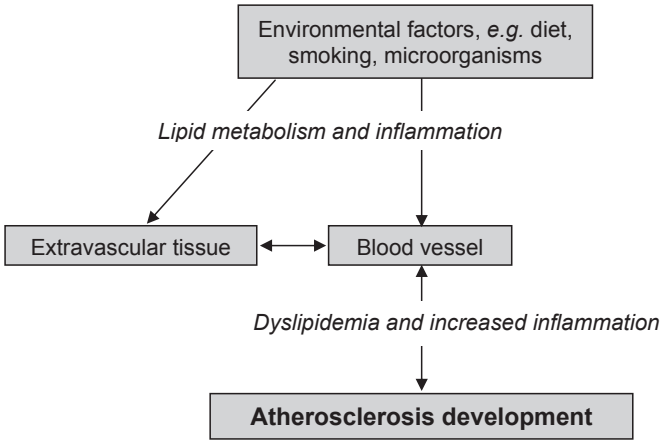

Fig. 1. The relationship between dysregulation of lipid metabolism and inflammatory processes in extravascular tissue and atherosclerosis. In addition to endogenous, *e.g.* hereditary characteristics, environmental factors can affect lipid metabolism and the inflammatory state of different organs and tissues. Disturbances in lipid metabolism and inflammation in these organs can 'spill over' to the systemic compartment and result in lipid accumulation and increased inflammation in the arterial wall, contributing to atherosclerosis development. The inflamed artery in itself also constitutes a source of inflammatory mediators for the systemic circulation.

emphasis on inflammation, since the possibilities of anti-inflammatory therapy in atherosclerosis development have been explored to a lesser extent than lipid-modulating drugs. Furthermore, the role of the lungs in atherosclerosis is discussed. Worldwide, chronic obstructive pulmonary disease (COPD) is the only major cause of death that still has a rising mortality, and it has been estimated that by the year 2020, COPD will be the third leading cause of death. Given the fact that COPD patients are at 2-3 times greater risk for CVD,[5] a better understanding of the interaction between COPD and CVD may help to decrease the burden of these two major killers.

Extravascular inflammation I: liver

Since NF-κB is one of the most important regulators of inflammation, it is an interesting target for the development of new anti-atherogenic agents. However, just like inhibiting all inflammatory processes would have adverse effects, *e.g.* on host defense, full suppression of NF-κB would be harmful. Almost all danger-sensing receptors of the innate and adaptive immune system activate NF-κB to mediate effector function. In addition, complete NF-κB inhibition is undesirable, as NF-κB is involved in many more processes than inflammation, including cell proliferation, differentiation, survival and death, and as we show in **chapter 2**, lipid metabolism. This raised the interest for tissue- or cell-specific interference of NF-κB activity in atherogenesis.[6] The tissue, *i.e.* endothelium,[7] or cells, *i.e.* macrophages[8, 9] that were firstly being investigated were obvious choices because of their prominent role in atherosclerosis development. These studies demonstrate that dependent on the level and tissue, NF-κB activation is not only pro-atherogenic,[7, 9] but can also be anti-atherogenic.[6, 8]

Going more distant from the endothelium and bearing in mind the two key processes involved in atherogenesis, *i.e.* lipid metabolism and inflammation, our interest was drawn towards the role of the liver in atherosclerosis development. Different factors, *e.g.* dietary cholesterol and saturated fatty acids (FAs) can activate the NF-κB pathway in the liver.[10] By utilizing a hepatocyte-specific transgenic murine model, we found that increased hepatocyte-specific NF-κB activity increases very-low-density lipoprotein (VLDL) production (**chapter 2**) and aggravates atherosclerosis development (**chapter 3**) (Figure 2). High concentrations of apolipoprotein (apo) B-containing lipoproteins result in elevated levels of triglycerides (TGs), which can accumulate in the liver, leading to nonalcoholic fatty liver disease (NAFLD).[11] NAFLD affects 20-30% of the general population and is associated with an increased risk for CVD. The term NAFLD spans a spectrum of conditions ranging from accumulation of fat in the liver, *i.e.* steatosis, to progressive nonalcoholic steatohepatitis (NASH), when the liver also exhibits increased inflammation. Patients with NASH display increased hepatic NF-κB activation[12] and are more prone to develop CVD than patients with simple hepatosteatosis.[13] In line with this, our findings that an increased activity of hepatocyte-specific NF-κB aggravates atherosclerosis (**chapter 3**), provide a pathophysiological explanation for the observed association between NASH and CVD. Apart from giving molecular insight into the role of hepatic inflammation in atherosclerosis development, these results also implicate that targeting the NF-κB in the liver would be an interesting anti-atherogenic therapeutic approach avoiding the major obstacle of adverse effects that may arise with broad-spectrum anti-NF-κB therapy. In addition, it is likely that not only patients with liver steatosis, but also hepatitis, due to endogenous or exogenous agents, are at higher risk for increased atherosclerosis. Treatment of these hepatic disorders may therefore not only improve the condition of the liver, but may also slow down the development of CVD.

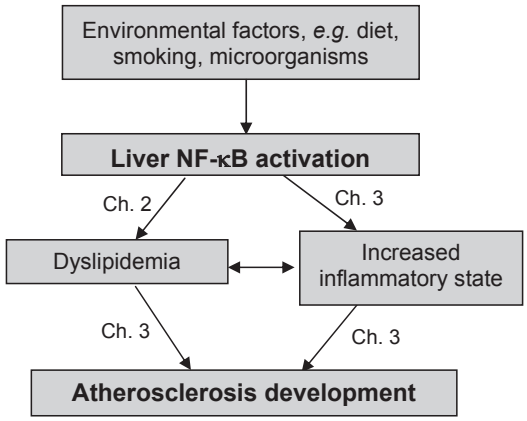

Fig. 2. The role of hepatic NF-kB activation in atherosclerosis. Various environmental factors such as a high dietary intake of cholesterol and saturated fatty acids can activate the NF-κB pathway in the liver. Increased activation of this pathway in hepatocytes enhances VLDL production (**chapter 2**) and aggravates atherosclerosis development (**chapter 3**) in *APOE*3-Leiden* (*E3L*) mice.

Interestingly, smoking increases the risk of developing NAFLD through oxidative stress and the unfavorable metabolic action of tobacco, *e.g.* causing dyslipidemia.[14, 15] It also directly promotes insulin resistance.[16] It is thus likely that smoking increases the hepatic NF-κB activity (Figure 2). In view of our results of **chapter 3**, in which we found that an increased hepatic NF-κB activity aggravates atherosclerosis development, another way by which smoking promotes atherogenesis may be through the liver. Alternatively, smoking may be a contributor to NAFLD and atherosclerosis development independently. In COPD, the NF-κB pathway is activated and cigarette smoking is a strong activator of the pathway.[17] Since an exaggerated inflammatory response to inhaled stimuli (in the Westernized world mainly cigarette smoking) is thought to be central to the pathogenesis of COPD, this may also provide clues to help understand why COPD patients are at increased risk for developing CVD compared to smokers without COPD. In the following part, we will focus on the role of COPD in atherosclerosis development.

Extravascular inflammation II: lungs

The respiratory system is anatomically as well as functionally closely related to the cardiovascular system. Although many epidemiological studies demonstrate that COPD is a strong risk factor for the development of CVD, the causal link and underlying mechanisms are unclear.[18] These two diseases share a couple of risk factors, such as smoking, aging and increased inflammation, which can explain the observed relation. However, studies also indicate that COPD is associated with CVD, independent of these risk factors.[19, 20]

As alveolar destruction is a prevalent manifestation in COPD and the major characteristic of emphysema, the effects of alveolar destruction without the presence of pulmonary inflammation in atherosclerosis development was studied in **chapter 4**. We found that elastase-induced emphysema did not enhance atherosclerosis, and even reduced atherosclerosis severity. This implies that other aspects of COPD than alveolar destruction are involved in the increased risk of atherosclerosis. Notably, consequences of COPD, such as hypoxia and physical inactivity also predispose to atherosclerosis development (Figure 3). It has been shown that after correction for physical activity, COPD remains an independent risk factor for CVD.[5] In experimental animal models, hypoxia, which occurs in COPD, is likely to contribute to atherosclerosis development.[21] In our study, hypoxia and physical inactivity resulting from elastase-induced emphysema were not likely to have played a significant role on atherosclerosis development. The duration and level of hypoxia was at least partly compensated by an increase in respiration amplitude, right ventricular hypertrophy and an increase in number of erythrocytes.

Numerous mechanisms have been proposed to explain the observed link between COPD and CVD,[22] including systemic oxidative stress, hypoxia, physical (in)activity, activation of the sympathetic nervous system, vascular dysfunction, accelerated aging, microbial airway colonization and infections, and probably the most advocated one: increased (low-grade) systemic inflammation (Figure 3). COPD is characterized by pulmonary inflammation, and systemic inflammation has been established as a major pathophysiological factor not only for CVD, but also for COPD.[23] It is thus likely that the presence of increased inflammation explains (part of) the pathophysiological link between COPD and CVD. As mentioned before, increased inflammation in any organ can 'spill over' to the systemic compartment, thereby affecting the

vasculature. To dissect the contributions of two main features of COPD, alveolar destruction and pulmonary inflammation on atherosclerosis development, these two aspects have to be investigated in one study. To address this research question, an appropriate COPD model has to be chosen. In the following section, three commonly used COPD models and their advantages and limitations are described.

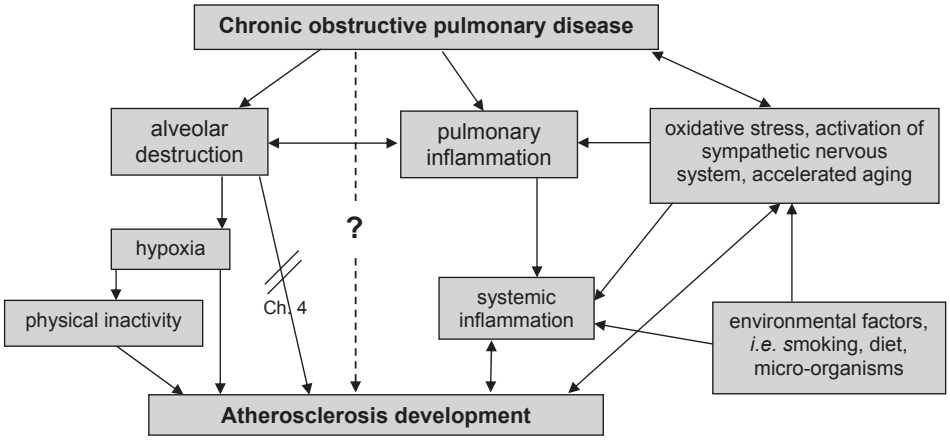

Fig. 3. Potential pathophysiological mechanisms for increased atherosclerosis development in patients with COPD. COPD is characterized by alveolar destruction and increased airway and parenchymal inflammation in the lungs. Possible mechanisms linking the increased CVD risk in COPD are sequelae of several of these features, *e.g.* systemic inflammation, hypoxia. It is hypothesized that the enhanced production of inflammatory mediators in the lungs spills over into the systemic compartment and thereby contributes to atherosclerosis development. The hallmark of emphysema, a major hallmark of COPD, is alveolar destruction and we found that this factor in itself does not explain the increased risk of atherosclerosis development observed in COPD (**chapter 4**).

Experimental COPD models

As smoking is the predominant cause of COPD, smoke exposure systems offer the ability to use the primary disease-causing agent to model several key features of the disease in small animals. Although these models mimic the human COPD development more closely than any other *in vivo* model, an operational smoke exposure system is more laborious to set-up and perform experiments with than most other COPD models. Many aspects have to be kept in mind and can be crucial in succeeding in setting up a smoke exposure model for animals. Factors concerning the choice of smoke exposure machine, set up, dose, frequency, duration, differences in branch and batch of cigarettes used, suppliers, strains and gender of mice, techniques to quantify emphysema and pulmonary inflammation, can all contribute to variations not only between different laboratories, but even within the same laboratory. In addition, because these models require a long time, *i.e.* ~4–6 months, to generate pathologies and functional changes consistent to those observed in merely mild COPD, practical application of these smoking models for assessing potential therapeutic interventions is more time- and labor-consuming

than other COPD models. Smoke exposure induces emphysema in combination with increased pulmonary inflammation. It also affects lipid metabolism unfavorably[15] and increases systemic inflammation,[24] which are known to contribute to atherosclerosis development. These concurrent effects of smoke hinder the differentiation of the impact of the two major features of COPD, *i.e.* alveolar destruction and pulmonary inflammation, independently on atherosclerosis development. However, as smoke exposure models best resemble the natural development of COPD, these models are essential, when the pathophysiological processes involved in the development of COPD are the subject of investigation.

Instillation of elastase in the lungs initially causes edema, hemorrhage and inflammation, consisting of infiltration by mainly neutrophils and monocytes.[25] Once this acute inflammatory response has disappeared, destruction of alveolar walls becomes evident which is consistent with the anatomical lesions in the lung observed in patients with emphysema.[26] One of the major drawbacks of this model to use for studying pathophysiology of COPD is the fact that inflammation is transient, resolves within a week of elastase administration, and does not reflect the progressive, slowly resolving inflammation associated with human COPD. However, the main advantages of the elastase models over other *in vivo* models for COPD are the possibility to easily titrate the severity of emphysema development with the dosage of elastase instilled, and the rapid onset of the emphysematous destruction of the lung. For this reason, the elastase model is ideal for testing therapeutic approaches aimed at reversing or repairing emphysematous damage to the lung. Another advantage of this model is that it is suitable to study the effects of alveolar destruction without the presence of chronic pulmonary inflammation on the development of other diseases, such as comorbidities of COPD (**chapter 4**).

Lipopolysaccharide (LPS) is a bacterial endotoxin that is present in cigarette smoke and, when instilled into the lung, can elicit a pronounced neutrophilic inflammatory response.[27] Because neutrophils are the largest source of neutrophil elastase, it was regarded as the central player driving the pathologies associated with COPD for many years. Similar to elastase instillation, the dosage of intrapulmonary LPS is more easily controlled than *e.g.* cigarette smoke exposure. In consideration of the research question whether pulmonary inflammation can affect atherosclerosis development, intrapulmonary LPS instillation can be adjusted in such way that the amount of systemic inflammation is minimized and the effects of pulmonary inflammation *per se* on atherosclerosis development can be investigated. However, the dosage of LPS needs to be carefully chosen, as a sufficient high dosage and duration of administration, can induce emphysema.[28] This may interfere with the distinction of the role of alveolar destruction and pulmonary inflammation in atherosclerosis development. In addition, if the LPS dose instilled is too high, systemic inflammation may be induced, which directly affects atherosclerosis development.

Taken together, the best approach to investigate the effects of alveolar destruction and pulmonary inflammation on atherosclerosis development, independently and together, seems to be the combination of the elastase-induced emphysema model (chapter 4) with chronic administration of a low dose of LPS in the lungs. Since smoking by itself induces various pro-atherogenic triggers, *e.g.* dyslipidemia, pulmonary and systemic inflammation and oxidative stress, a smoke exposure model is less suitable to discern the role of alveolar destruction and pulmonary inflammation on atherosclerosis development without the interference by confounding factors.

Therapeutical agents for COPD and CVD

COPD is increasingly being recognized as a complex disorder, characterized not only by local pulmonary inflammation, but also by systemic inflammation that may have an adverse impact on various extrapulmonary organs, such as the blood vessels and the heart among others.[29] CVD is one of the most important causes of death in COPD patients. Although smoking cessation is the cheapest, safest and most effective strategy to treat COPD and CVD, it is a hard task to accomplish for the patient and the inflammatory response in many COPD patients persists after smoking cessation.[30] Therefore, new and more effective therapies that deal with not only COPD, but preferably also CVD are needed. Many existing therapeutic options used to treat COPD and CVD appear to have other beneficial properties apart from their classical actions. This raises the idea whether drugs currently applied for COPD could also be beneficial to treat CVD (Table 1), and *vice versa* (Table 2).

Application of COPD therapies in CVD

As inhaled corticosteroids (ICSs) are widely prescribed in COPD patients and CVD is a prevalent comorbidity of COPD, the effects of ICS on CVD are an interesting subject to explore. Glucocorticoids are potent inhibitors of NF-κB activation,[31] and thus may be a promising anti-inflammatory agent for both COPD and CVD. However, not all studies with COPD patients have demonstrated a clear anti-inflammatory and beneficial effect of ICS[32], and such effects may be restricted to subgroups of COPD patients.[33] In a retrospective study it was demonstrated that very low doses of ICSs (50-200 μg/day) were associated with a reduced risk of acute myocardial infarction.[34] However, with higher doses of ICSs, the risk returned to baseline. This lack of benefit at higher doses might be due to counterbalancing adverse effects of other risk factors, or the fact that patients with more severe disease, which in itself is linked to CVD morbidity, were prescribed the higher doses. In line with the latter finding, randomized controlled trials also fail to show any significant effect of ICSs on myocardial infarction and cardiovascular mortality.[35, 36] A controlled trial of high-dose ICS with or without a long acting β-agonist showed no reduction in systemic inflammation in COPD patients, as measured by circulating interleukin (IL)-6 and C-reactive protein (CRP) concentrations, indicating corticosteroid resistance of systemic, as well as local inflammation in patients with COPD.[37] Overall, these data suggest that ICSs do not have a significant beneficial effect on CVD.

Table 1. Possible application of COPD therapies for CVD.

Therapy	Main mechanism in COPD	Beneficial effect on CVD?
Inhaled corticosteroids	Anti-inflammatory?	Probably not
β_2-agonists	Bronchodilatory	Probably not
Anti-cholinergics	Bronchodilatory	Caution in patients with high risk for cardiovascular event
Theophylline	Anti-inflammatory	Possibly
Phosphodiesterase 4 inhibitors	Anti-inflammatory	Probably yes
Supplemental oxygen	Restore normal oxygen levels	Probably yes
Lung volume reduction surgery	Improved lung breathing mechanics	If successful probably yes Surgical risk

Bronchodilators, *i.e.* long-acting β_2-agonists and anticholinergics are useful in COPD, but are not known to have marked anti-inflammatory or other anti-atherogenic effects. One trial demonstrated that tiotropium, an anticholinergic drug, had no effect on inflammatory markers in sputum or in the circulation of COPD patients.[38] Whether inhaled or oral β_2-agonists by themselves have any beneficial effects on the systemic inflammatory state of COPD patients has not yet been clarified. There has been concern that long-term use of inhaled bronchodilators may increase the risk of cardiovascular complications.[39] However, in the large Towards a Revolution in COPD Health (TORCH) trial, the three-year risk of cardiovascular adverse events of the use of salmeterol (a β_2-agonist), fluticasone (an ICS), both medications combined, or placebo in COPD patients was similar in all groups.[36] In addition, results from a meta-analysis on the occurrence of cardiovascular events and the use of anticholinergic agents to treat COPD in trials show that there is no increased risk.[40] It is not clear, however, whether this also accounts for patients with an increased risk for cardiovascular events, such as those with coronary artery disease, heart failure and cardiac arrhythmia, because they are excluded from participation for obvious ethical reasons. Furthermore, a poor lung function which is inherent to COPD patients, is a marked risk factor for CVD.[41] Taken together, although bronchodilators are valuable in the treatment of COPD, care must be taken for cardiovascular complications, especially in high-risk patients.

Theophylline seems to be a more promising candidate as a concurrent treatment for inflammation in COPD and CVD. It has been shown to reduce neutrophilic inflammation in patients with COPD[42] and also has the potential to reverse corticosteroid resistance in COPD.[43] However, the molecular mechanism for the anti-inflammatory action of theophylline is currently unknown and deserves at least the same priority of exploring its potential as therapeutic agent for CVD in COPD patients.

Roflumilast, a phosphodiesterase 4 (PDE4) inhibitor, has recently been registered as a novel therapy for COPD and thought to be effective through its anti-inflammatory properties.[44, 45] One of the major anti-inflammatory effects of PDE4 inhibitors is their ability to reduce TNFα release,[46] which supports the potential of these agents for treating systemic inflammation. PDE4 inhibitors increase levels of cyclic adenosine monophosphate (cAMP) through inhibition of its metabolism. The resulting increase in protein kinase A activation stimulates increased protein phosphorylation, with subsequent inhibition of pro-inflammatory cells and mediators. Because of their anti-inflammatory properties, PDE4 inhibitors may be beneficial in treating CVD. This has not been investigated thus far, but several reports support a protective role of cAMP in atherosclerosis.[47] Therefore, based on their anti-inflammatory effects, PDE4 inhibitors would be an interesting subject for future studies on CVD.

COPD patients are subject to intermittent hypoxia and at a more severe stage of disease to sustained hypoxia. Hypoxia can induce increased inflammation and oxidative stress, which contribute to atherosclerosis development. Mice subjected to chronic intermittent hypoxia have increased atherosclerosis development.[21] Long-term use of supplemental oxygen improves survival in patients with COPD and severe resting hypoxemia.[48] Whether oxygen therapy is beneficial in patients suffering from atherosclerosis with a normal lung function has

never been investigated, but based on the findings above, oxygen therapy is likely to reduce the risk of atherosclerosis in COPD patients, in addition to the relieve of pulmonary symptoms.

Lung volume reduction surgery in the treatment of properly selected patients with COPD, *i.e.* with severe and predominantly upper-lobe emphysema and low-exercise capacity, improves survival and quality of life, including exercise tolerance, dyspnea, oxygen requirement and functional status.[49] However, this invasive procedure is mostly offered to severely impaired emphysema patients as one of the last resorts and accompanied with a high risk on cardiopulmonary morbidity (up to 58.7% in the National Emphysema Treatment Trial (NETT)). In the same study, cardiovascular morbidity, *i.e.* myocardial infarction, pulmonary embolus, or cardiac arrhythmia requiring treatment within 30 days of surgery occurred in 20% of patients.[50] Although this kind of therapy has proven to be effective in a subgroup of severe emphysema, the surgical risk of the procedure is a major barrier withstanding broad application for patients with CVD.

Application of CVD therapies in COPD

The discovery of novel effective treatments for COPD, other than bronchodilators and ICS has proven difficult.[51] Thus, it is worthwhile to explore whether drugs commonly used in CVD might also have beneficial effects in COPD.

Promising candidates are statins which are primarily prescribed for patients with CVD, but nowadays seem to have potential additional benefits in many other diseases, including COPD.[52] It was shown in a retrospective study that the use of statins was associated with a reduced mortality in COPD patients, independent of sex, age, smoking, pulmonary function and comorbidities.[53] In addition, ICS appeared to increase the survival benefit associated with statin use. Several possible mechanisms for the beneficial effects of statins have been proposed, including their anti-inflammatory property. This feature of statins makes them an attractive candidate in the treatment of COPD in which inflammation has a fundamental pathophysiological role. In experimental COPD animal models, simvastatin inhibited cigarette smoke-induced emphysema, which was associated with a decrease in pulmonary inflammation.[54, 55] Moreover, in a murine model in which emphysema was already established by elastase instillation in the lungs and the acute inflammation after elastase instillation has resolved, simvastatin was even able to reverse emphysema.[56] Interestingly, in this study the therapeutic effect of simvastatin was not ascribed to anti-inflammatory effects, but to a tendency towards an increase of vascular endothelial growth factor (VEGF) in bronchoalveolar lavage fluid. A study in *apoe*$^{-/-}$ mice demonstrated that a Western-type diet high in fat and cholesterol content not only induced increased systemic, but also pulmonary inflammation in these mice.[57] As an enhanced pulmonary inflammation is one of the most important pathophysiological causes of COPD, these data imply that a Western-type diet can stimulate the development with COPD. The lipid-lowering action of statins thus may be another mechanism through which they can be useful in the treatment of COPD. Taken together, statins seem to be effective in both COPD and CVD, which also indicates that COPD and CVD may have a common pathophysiological cause.

Next to statins, angiotensin converting enzyme (ACE) inhibitors and the more specifically acting angiotensin II receptor blockers (ARBs), classically prescribed to treat hypertension,

have been shown to reduce the risk of COPD hospitalization, and cardiovascular events and death in COPD patients.[58] ACE inhibitors reduce pulmonary hypertension, but may have other beneficial effects in COPD, *e.g.* by inhibiting angiotensin II that has pro-inflammatory properties.[59] Angiotensin II receptors are shown to be expressed in the lung and more highly in lungs of COPD patients.[60] In mice, treatment with the ARB irbesertan after induction of emphysema with elastase, significantly improved the exercise capacity and reduced the development of morphological emphysema.[61] Therefore, the main action of ACE inhibitors and ARBs, *i.e.* inhibition of the pro-inflammatory angiotensin II, makes them potential valuable therapeutic agents in COPD.

Both activators of peroxisome proliferator-activated receptor (PPAR)α and -γ have demonstrated anti-inflammatory properties and other anti-atherogenic effects in human and mice.[62] In fact, part of the anti-inflammatory effects of statins may be explained by activation of PPARα and -γ.[63] PPARα and -γ agonists have been shown to exert beneficial effects mostly in experimental asthma or acute pulmonary inflammation models.[64] So far, no studies are published on the role of PPARs in animal models of COPD. However, PPARα and -γ inhibit airway neutrophil and macrophage influx, as well as cytokine and chemokine production induced by LPS in the mouse,[65, 66] suggesting that activators of these PPAR subtypes may have a beneficial effect on the inflammatory response associated with COPD.

Accelerated aging may be a characteristic common to COPD and CVD.[67] The concept of 'inflamm-aging' is now gaining attention, with a reduction in adaptive immunity and an increase in innate immunity driven by NF-κB activation.[68] This suggests that anti-aging drugs may be beneficial in CVD and COPD. One of the key players that has gained much interest in this field is sirtuin 1 (SIRT1). SIRT1 is an enzyme which deacetylates proteins that contribute to cellular regulation, thereby playing an important role in determining lifespan of all organisms.[69] We demonstrated that resveratrol, a moderate SIRT1 activator, protects against atherosclerosis (**chapter 5**). While resveratrol is known to have many pleiotropic effects, we did not find many other systemic anti-atherogenic effects, *e.g.* anti-inflammatory, anti-oxidative, than lipid lowering. This can be attributed to the use of a relatively low dose of resveratrol (0.01% (w/w) in the diet, compared to other studies.[70, 71] Nevertheless, the reported pleiotropic effects of resveratol may have therapeutic potential in COPD.[72] In fact, resveratrol was found to inhibit cigarette smoke extract-mediated pro-inflammatory cytokine release in a human monocyte-macrophage cell line.[73] Furthermore, resveratrol was shown to protect against cigarette smoke-mediated oxidative stress in human lung epithelial cells by inducing glutathione synthesis.[74] Additional studies are required to be able to extrapolate the dosages and effects of resveratrol of these *in vitro* studies and our *in vivo* study with mice to (clinical) human use for CVD and COPD. To address this, experimental studies are needed in which the effects of resveratrol on pulmonary damage induced by cigarette smoke are studied in hyperlipidemic atherosclerosis models and compared to studies in which pulmonary damage is induced by other agents, *e.g.* intrapulmonary elastase-instillation, which do not have the diverse direct effects of smoke on atherosclerosis. Moreover, as resveratrol is just a moderate SIRT1 activator, other more potent SIRT1 activators may have even more beneficial effects, and are possibly more effective than resveratrol in the treatment of COPD. In addition, there is clinical evidence

that SIRT1 may be an interesting therapeutic target, as its expression is decreased in lungs of patients with COPD.[72]

Table 2. Possible application of CVD therapies for COPD.

Therapy	Mechanism in CVD	Beneficial effect on COPD?
Statins	Hypolipidemic Anti-inflammatory Anti-oxidative	Probably yes
Angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs)	Anti-hypertensive Anti-inflammatory	Probably yes
PPARα agonists	Hypolipidemic Anti-inflammatory	Possibly; in experimental phase
PPARγ agonists	Hypoglycemic Anti-inflammatory	Possibly; in experimental phase
Resveratrol	Hypolipidemic Anti-inflammatory Anti-oxidative	Possibly; in experimental phase

In summary, COPD has been demonstrated to be an independent predictor of cardiovascular death.[75] The value of existing drugs that treat both CVD and COPD simultaneously is currently extensively being studied. Despite some promising findings, we still do not know whether treatment of lung inflammation decreases, for example the progression of atherosclerosis, or the risk of acute cardiac events. Alternatively, it is also unclear whether treatment of heart disease can reduce the progression of lung disease. There are data suggesting that a Western-type diet can induce pulmonary inflammation, which is the most important causal factor in COPD development.[57] Minimizing dyslipidemia thus may be another target to inhibit not only CVD but also COPD. In addition, initial data seem to indicate that drugs, originally prescribed for CVD such as statins, ARBs and PPAR agonists, also have the potential to benefit COPD patients. It can even be hypothesized that one of the ways by which these drugs can inhibit CVD is by slowing down progression of the lung disease in COPD patients. Furthermore, since CVD is a major cause of mortality in COPD patients and the presence of a poor lung function is proven to be an important risk factor for CVD, screening for the presence and treatment of CVD in COPD patients is recommended.

Treating infections in CVD and COPD

The clinical course of COPD is punctuated by recurrent episodes of acute increase in both airway and systemic inflammation, otherwise known as exacerbations, with a major negative impact on the patient's quality of life, hospital admission and lung function. The increase in systemic inflammation and oxidative stress during exacerbations are likely to contribute to the inflammatory process underlying atherosclerosis. Furthermore, the peaks of inflammatory

activity accompanying COPD exacerbations could precipitate acute exacerbations of the atherosclerotic process with increased risk of plaque rupture and thrombotic occlusion.[76] Infection is considered the main cause of acute COPD exacerbations and the standard treatment for exacerbations is usually corticosteroids and/or antibiotics.[77]

Chlamydophila pneumoniae (*C. pneumoniae*) has been implicated as an infectious trigger for acute exacerbations of COPD[78] and postulated to contribute to inflammation in atherogenesis,[79] making it an interesting candidate in linking the pathophysiology of CVD and COPD. Several infectious agents have been associated with an increased risk CVD, but for the Gram-negative bacteria *C. pneumoniae* and *Porphorymonas gingivalis* (*P. gingivalis*) most compelling evidence is found.[80] Many studies demonstrated both an associative and causal relation for *C. pneumoniae* with regard to atherosclerosis formation. *C. pneumoniae* is an obligate intracellular pathogen that infects both epithelial cells and macrophages within the lungs and may disseminate outside the lungs through infected monocytes and macrophages.[81] It was the first infectious organism to be found in macrophages and smooth muscle cells of human atherosclerotic plaques but rarely within normal (adjacent) arterial cells.[82] In addition, a number of studies in experimental models showed an acceleration of atherosclerotic lesion development following respiratory infection with *C. pneumoniae*.[79] Macrolides are one of the first choice antibiotics to treat infections with *C. pneumoniae* and reported to have anti-inflammatory properties apart from their antimicrobial activities, making them interesting agents to treat sustained infection and low-grade inflammatory states as encountered in COPD and CVD.[83] Recently, it was demonstrated that erythromycin ameliorates cigarette-smoke-induced pulmonary inflammation and emphysema in rats.[84] Similar to *C. pneumoniae*, *P. gingivalis*, which colonizes the gingival plaque where it can cause periodontitis, has been shown to increase systemic cytokines and acute-phase proteins and increase atherosclerotic lesion development in experimental models.[85] Although a number of studies have shown that treatment of chronic periodontitis results in a reduction in systemic inflammation,[86] a direct causal link between *P. gingivalis* with CVD has not been demonstrated so far.

As mentioned before, although a large body of evidence for the role of infection as risk factor for atherosclerosis exists, intervention trials with antibiotics until now have ended up with disappointing results.[80] A few considerations should be taken into account in the interpretation of the failed trials. First, it is not clear whether pathogens were effectively cleared by antibiotic treatment or chronic (low-grade) infection persisted. This accounts especially for *C. pneumoniae* that replicates intracellularly and exists in a metabolically inactive form, which is not susceptible for antibiotics. Second, the participants of the trials had advanced atherosclerosis, and events being measured were likely due to plaque destabilization and rupture rather than progression of occlusive disease, while the pathophysiological and experimental studies performed so far were focused on the initiating process of atherogenesis. Studies focusing on the effects of antibiotics in patients with early stage atherosclerosis have not been carried out, and are intuitively difficult to design. Furthermore, the beneficial effects of antibiotics in experimental studies with *C. pneumoniae* were only observed when given shortly, *i.e.* within days rather than weeks, after infection,[87] and the acute *C. pneumoniae* infection in humans frequently passes clinically unnoticed. Third, there is an emerging concept that not one organism but an

aggregate of multiple organisms, the infectious or pathogen burden as a whole, is responsible for the effects of infection on atherosclerosis development. This is for example supported by a study in which it was found that an increased pathogen burden was significantly associated with increased coronary artery disease, even after adjustment for traditional cardiovascular risk factors.[88] The antibiotic treatment in the secondary prevention trials performed thus might be ineffective due to the infectious burden of unsusceptible pathogens, allowing these organisms to still contribute to the progression of atherosclerosis.

The failure of antibiotic trials in CVD should therefore not lead to dismissal of the potential role of infectious agents in the pathogenesis of atherosclerosis, although considerations to be taken into account, such as persistent infection despite antibiotic treatment, emergence of antibiotic resistance and requirement of multiple antimicrobial therapies to treat the pathogen burden, are not easily overcome. In conclusion, microbial pathogens may form a bridge between COPD and CVD in acute exacerbations as well as chronic infections.

Concluding remarks

The understanding of the pathophysiological processes involved in the local environment where atherogenesis takes place has resulted in effective treatment strategies to fight CVD. However, the endothelium does not stand by itself, as it is part of a complicated network with intricate connections and interactions with other cells, organs and tissues. Therefore, apart from investigating the local processes, we have to keep in mind that more distal effectors and reactors are present and should broaden our attention when studying the atherosclerotic process.

In this thesis, the role of inflammation in the liver and lungs in atherosclerosis development was addressed. Models combining various conditions that are often observed concomitantly in clinical practice, such as NASH and CVD or COPD and CVD, represent an exciting new approach in order to understand the mechanisms underlying the pathophysiology of these diseases. Investigating the interaction between vascular and extravascular changes may expand the number of therapeutic options and lead to novel approaches that can be used to help manage CVD and associated diseases.

When considering a role of extravascular inflammation in atherosclerosis, conventional therapies used in certain diseases which also seem to be linked to each other, such as CVD and COPD, may be applicable for more domains and may even have synergistic beneficial effects. Given the complex nature of both COPD and CVD, it seems likely that no single cause of the association will be found. However, further studies on the interaction between COPD and CVD are expected to improve early identification of patients likely to develop both diseases to prompt early intervention, as well as promote stratified medicine and tailored therapy in which patients achieve the best possible medicinal care dependent on their individual needs.

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