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Murine models of cardiovascular comorbidity in chronic obstructive pulmonary disease


P. Padmini S. J. Khedoe¹²*, Patrick C.N. Rensen²³, Jimmy F.P. Berbée²³#, Pieter S. Hiemstra¹#

¹ Dept. of Pulmonology, ² Dept. of Medicine, Div. of Endocrinology and ³ Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center (LUMC), Leiden, the Netherlands

* Authors contributed equally
Abstract

Patients with chronic obstructive pulmonary disease (COPD) have an increased risk for cardiovascular disease (CVD). Currently, COPD patients with atherosclerosis, i.e. the most important underlying cause of CVD, receive COPD therapy complemented with standard CVD therapy. This may, however, not be the most optimal treatment. To investigate the link between COPD and atherosclerosis and to develop specific therapeutic strategies for COPD patients with atherosclerosis, a substantial number of preclinical studies using murine models have been performed. In this review, we summarize the currently used murine models of COPD and atherosclerosis, separate and combined, and discuss the relevance of these models for studying the pathogenesis and development of new treatments for COPD patients with atherosclerosis. Murine and clinical studies have provided complementary information showing a prominent role of systemic inflammation and oxidative stress in the link between COPD and atherosclerosis. These and other studies showed that murine models for COPD and atherosclerosis are very useful tools and can provide important insights relevant to understanding the link between COPD and CVD. More importantly, murine studies provide good platforms for studying the potential of promising (new) therapeutic strategies for COPD patients with CVD.

Keywords:
Chronic obstructive pulmonary disease, cardiovascular disease, mouse models
**Abbreviations**

ApoE, Apolipoprotein E  
COPD, chronic obstructive pulmonary disease  
CS, cigarette smoke  
CVD, cardiovascular disease  
ECM, extracellular matrix  
EPC, endothelial progenitor cells  
eNOS, endothelial nitric oxide  
FEV1, forced expiratory volume in 1 sec  
GOLD, global initiative for chronic obstructive lung disease  
HDL, high-density lipoprotein  
HFD, high-fat diet  
IFN-γ, interferon-γ  
IL, interleukin  
iNOS, inducible nitric oxide synthase  
LDL, low-density lipoprotein  
LDLr, low-density lipoprotein receptor  
LPS, lipopolysaccharide  
MCP-1, monocyte chemoattractant protein-1  
MMP, matrix metalloprotease  
NE, neutrophil elastase  
NO, nitric oxide  
oxLDL, oxidized LDL  
PPE, porcine pancreatic elastase  
ROS, reactive oxygen species  
TGF-β, transforming growth factor-β  
TNF-α, tumour necrosis factor-α  
VEGF, vascular endothelial growth factor  
VLDL, very-low-density lipoprotein  
WT, wild-type  
WTD, Western-type diet
Chapter 2

1. Chronic Obstructive Pulmonary Disease and Cardiovascular Disease

Patients with chronic obstructive pulmonary disease (COPD) have an increased risk for developing cardiovascular disease (CVD) (29; 163), even after correction for common risk factors (145; 193; 194). Airflow limitation and emphysema are associated with arterial stiffness (29; 233) and impaired lung function is correlated with cardiovascular morbidity (4), indicating an association between the severity of COPD and CVD. Exposure to cigarette smoke (CS) is the most important risk factor for COPD. The major risk factors for the development of atherosclerosis, the main underlying cause of CVD, are an unhealthy lifestyle including inactivity, bad eating habits (high fat intake) and smoking, which cause dyslipidemia and systemic inflammation, the main contributors to atherosclerosis (163; 228).

The exact mechanism linking COPD and atherosclerosis development is unknown, and is likely a combination of several mechanisms (260) (Figure 1). Chronic systemic inflammation is suggested to be the most important link between COPD and atherosclerosis (58; 59; 163; 228; 229). Pro-inflammatory mediators from the lung can be released into the circulation, cause systemic inflammation and contribute to atherosclerosis development (59). This may especially occur during COPD exacerbations, following e.g. bacterial and/or viral infection, resulting in increased systemic inflammation (20; 38; 163; 244) and thus a higher proatherogenic status. Changes in airway- and intestinal microbial colonization are also suggested to contribute to COPD and atherosclerosis (7; 71; 108). Colonization of the airways by specific pathogens is frequently associated with COPD exacerbations and increased systemic inflammation. However, also in a stable COPD clinical setting, airway colonization increases the risk for atherosclerotic events, due to increased systemic inflammation (71). Other important mechanisms comprise (CS-induced) endothelial dysfunction, oxidative stress and formation of reactive oxygen species (ROS) (59; 91; 151; 230), processes that aggravate both COPD and atherosclerosis. As mentioned above, CS exposure itself is also an important risk factor for several adverse events (64) including atherosclerosis initiation and progression, mainly due to its oxidative stress- and inflammation-inducing characteristics (5; 59). Hypoxia-induced pulmonary hypertension and changes in pulmonary blood flow (51) in COPD patients may further contribute to right ventricle hypertrophy and left ventricular diastolic dysfunction (138), processes that affect cardiac function.

COPD patients with atherosclerosis receive COPD treatment complemented with standard CVD treatment. However, it is unknown whether such treatment is the most optimal therapy. Because of the complexity of both diseases, mouse models are used to study the link between COPD and atherosclerosis and to develop novel treatments targeting both diseases. Several reviews provide overviews of the mouse models for COPD (21; 37; 39; 40; 69; 189; 190; 196; 197; 228-230; 240) and atherosclerosis (78; 96; 116; 236; 252) separately, and we will therefore only shortly discuss these in the current review. The aim
of this review is to provide an overview of the available murine models in which COPD and atherosclerosis development are combined, and to discuss the relevance of these models in studying the pathogenesis of both diseases and the development of novel treatment options for COPD patients with atherosclerosis.

Figure 1: Potential mechanisms linking COPD and atherosclerosis
The main risk factor for COPD development is cigarette smoking (CS), but also recurrent respiratory infections and air pollutants are important risk factors. In the lung, pro-inflammatory mediators, such as IL-6, IL-8 and TNF-α are produced by immune and structural cells. These mediators, along with CS components, can spill-over into the circulation and contribute to systemic inflammation, which affects organs such as the liver, arteries and heart. Furthermore, CS-induced oxidative stress causes the formation of reactive oxygen species which subsequently may result in the formation of e.g. proatherogenic oxidized lipids. Endothelial dysfunction and hyperlipidemia may also contribute to the link between COPD and atherosclerosis. Finally, colonization by different pathogens (in the lung, but also in the intestine) may contribute to the link between COPD and atherosclerosis.
2. COPD

COPD is a lung disease characterized by progressive irreversible airflow limitation, chronic pulmonary inflammation and remodelling and destruction of lung tissue (80). Repeated exposure of the lung to noxious substances resulting from active and passive smoking, as well as exposure to indoor (in-house cooking and heating) and outdoor air pollution contribute to the pathogenesis of COPD (36). In addition, CS enhances the risk for recurrent respiratory infections, which further contribute to the development and progression of COPD (71). Various factors contribute to the development and progression of COPD, including number of pack years smoked, age, and genetic predisposition. COPD patients clinically present with a variety of symptoms, including dyspnea, wheezing, chest tightness, cough and sputum production, as well as symptoms resulting from a range of comorbidities (80).

2.1 COPD in humans

Development of COPD in humans occurs in response to repeated exposure to CS and recurrent respiratory infections (80). Upon exposure of immune and structural cells to CS-, bacterial- or viral components, pro-inflammatory mediators, such as monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor-α (TNF-α), interleukin-12 (IL-12) and CXCL8/interleukin-8 (IL-8), are secreted (22; 57; 260). These mediators induce recruitment of other (circulating) inflammatory cells, such as monocytes, lymphocytes and neutrophils, which contribute to the local inflammatory response (22; 230). Neutrophils and macrophages produce reactive oxygen intermediates, and a range of granule proteins including matrix metalloproteases (MMPs) and neutrophil elastase (NE), to combat pathogens. An imbalance between oxidants and anti-oxidants as well as proteases and anti-proteases is proposed to contribute to the pathogenesis of COPD (22). Whereas CS itself contains a large number of oxidants, CS exposure also increases ROS generation in the lung and the circulation, while antioxidant mechanisms such as those provided by glutathione are decreased (221). The array of cytokines, proteases, oxidants and other inflammatory mediators produced, cause mucus hypersecretion by goblet cells, induce proliferation of smooth muscle cells, and activation of fibroblasts, resulting in airway remodelling and emphysema as observed in COPD patients. Importantly, prenatal and early-life exposure to CS may predispose to COPD development as well, and several studies have addressed this predisposition (243; 259).

2.2 Mouse models of COPD

Modelling the variety of processes that contribute to COPD development and progression, and the interactions between these processes, is complicated (22; 36). A short summary of frequently used murine models to study COPD is provided below and in Table 1, but these are described in more detail elsewhere (21; 37; 39; 40; 42; 47; 56; 196; 197; 229; 230; 240).
Mouse models for COPD and CVD

**Table 1: Mouse models of COPD**

<table>
<thead>
<tr>
<th>Exposure model</th>
<th>Subtype</th>
<th>Emphysema</th>
<th>Pulmonary inflammation</th>
<th>Systemic effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term CS (several days - 6 weeks)</td>
<td>main or side stream, nose-only or whole body</td>
<td>No</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>(1; 26; 32-34; 63; 64; 109; 111; 122; 123; 128; 135; 160; 161; 169; 172; 173; 19; 225; 229; 230; 234; 239; 245; 247)</td>
</tr>
<tr>
<td>Long-term CS (4-6 months)</td>
<td>main or side stream nose-only or whole body</td>
<td>Yes</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>(2; 9; 10; 16; 27; 30; 31; 37; 39; 40; 43; 44; 46; 47; 52; 55; 56; 61; 82; 88; 92; 93; 95; 103; 109; 112; 113; 118; 121; 126; 129; 135; 153; 164; 167; 168; 174; 175; 177; 180; 181; 183; 187; 200-202; 206; 211; 220; 225; 229; 230; 234; 241; 249; 253; 254)</td>
</tr>
<tr>
<td>CS extract</td>
<td>i.p. or i.n.</td>
<td>Yes, after chronic</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>(35; 97; 98; 152; 245; 256)</td>
</tr>
<tr>
<td>PPE</td>
<td>i.t. or i.n.</td>
<td>Yes</td>
<td>Transient</td>
<td>No</td>
<td>(75; 155; 158; 191; 201; 203; 204; 209; 229; 230)</td>
</tr>
<tr>
<td>LPS/ PolyI:C</td>
<td>i.t. or i.n. acute or chronic</td>
<td>Yes, after chronic</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>(18; 54; 62; 75; 132; 133; 134; 207; 226)</td>
</tr>
<tr>
<td>VEGF blockade/ anti-endothelial antibody</td>
<td>-</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
<td>(117; 205; 217)</td>
</tr>
<tr>
<td>Combined models: CS, PPE or LPS + infection</td>
<td>Bacterial or viral</td>
<td>Yes, after chronic</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>(74; 105; 108; 144; 150; 179)</td>
</tr>
<tr>
<td>Diet-induced</td>
<td>HFD</td>
<td>Yes, after chronic</td>
<td>Yes/No</td>
<td>Increased inflammation, oxidative stress</td>
<td>(12; 66)</td>
</tr>
<tr>
<td>Genetic models</td>
<td>IL-13 overexpression</td>
<td>Yes</td>
<td>Yes/No</td>
<td>Dependent on model</td>
<td>(21; 229; 230)</td>
</tr>
</tbody>
</table>

CS exposure in mice is frequently used as model for COPD. Nose-only exposure to CS causes pulmonary inflammation and emphysema (45; 177). Whole-body exposure is performed with main- and/or side-stream CS (92). Side-stream CS induces high carbon monoxide levels, limiting inflammation, whereas main-stream CS induces strong inflammation (114).
In addition, there are differences in duration, cigarette number and brand, puffing and susceptibility to inflammation and emphysema of various mouse strains (88; 224; 247).

COPD-like features in mice can also be induced by other methods, such as administration of an aqueous extract of CS (97; 242; 256), intratracheal or intranasal administration of proteases, such as porcine pancreatic elastase (PPE), NE or papain (21), all of which induce emphysema by proteolytic degradation of the alveolar walls, accompanied by a transient acute inflammatory process (21; 120). Administration of antibodies to endothelial cells (49) as well as blockade of the receptor for vascular endothelial growth factor (VEGF) also results in alveolar septal cell apoptosis, oxidative stress and emphysema development (50; 117; 205; 217). Exposure to micro-organisms or microbial components, such as lipopolysaccharide (LPS) (156) and poly(I:C) are also frequently used to induce features of COPD in mice. The combination of CS (144), LPS or PPE (65; 74; 75) together with infection with respiratory syncytial virus, non-typable Haemophilus influenzae (74) or Chlamydia pneumoniae has been used to mimic COPD exacerbations (179). Other COPD models include the use of air pollutants (79), ozone exposure (216) and genetic models, using overexpression or deletion of genes, such as α1-anti-trypsin (8; 189).

Whereas these models do mimic the various aspects of COPD pathology, they have some limitations. These include differences in susceptibility between mouse strains (88), differences in anatomy of the lungs between humans and mice, and the absence of substantial airway involvement in most smoking models. Furthermore, differences in tissue analysis, fixation procedure and other necropsy procedures may contribute to the differences observed between studies and groups (17). Despite such limitations, these models are considered suitable models to study COPD pathogenesis and novel COPD treatment strategies (69; 190).

3. Atherosclerosis

Atherosclerosis is the main underlying cause of CVD and affects mainly the large arteries. The development of atheromatous plaques, a process called atherogenesis, starts with endothelial injury and activation, for example by oxidative stress, inducing recruitment and migration of circulating monocytes into the intima (Figure 2). Two important factors in the development of atherosclerosis are dyslipidemia and inflammation. Dyslipidemia mainly arises from unhealthy eating habits, causing increased circulating levels of proatherogenic low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL). Lipoproteins transport triglycerides and cholesterol through the circulation. Triglyceride-derived fatty acids can be used as fuel for organs such as the heart and muscles, stored in white adipose tissue or burnt in brown adipose tissue. In addition, the remaining triglycerides can be taken up by the liver again for repackaging into nascent VLDL. Cholesterol, an important
component for membranes, vitamin D, bile fluids and steroid hormones, can be packaged into nascent VLDL and high-density lipoprotein (HDL) or cleared into bile fluid (99). The liver recognizes TG-rich lipoproteins mainly through Apolipoprotein E (ApoE) on the surface of lipoproteins, which is a ligand for receptors including the LDL-receptor (LDLr).

Figure 2: Atherosclerosis pathophysiology
A) An artery consists of endothelial cells (EC) lining the arterial lumen forming the intima. The intima is underlined by the media, containing extracellular matrix (ECM) and vascular smooth muscle cells (VSMCs), and the outer adventitial layer, which contains nerves and vessels. B) Upon injury to the EC, for example upon CS exposure or oxidative stress, LDL and VLDL can enter the intima. Either in the circulation or when entrapped in the vessel wall, these lipoproteins can be oxidized and cause activation of surrounding cells. C) This injury, together with the pro-inflammatory mediators produced by vascular macrophages and other EC, activates the EC to express adhesive surface receptors thereby enabling recruitment and infiltration of circulating monocytes into the intima. Oxidized lipoproteins can be ingested by the macrophages and cause formation of foam cells. A fatty streak is formed. D) When influx of lipoproteins is continued, the foam cells keep producing pro-inflammatory mediators, inducing further recruitment of immune cells from the circulation and proliferation of the smooth muscle cells (SMC) from the media. The SMC migrate to the luminal side of the developing plaque and form a fibrous cap by producing collagen and elastin. Due to the growth of the plaque, the core is deprived of oxygen, causing apoptosis and necrosis of (foam) cells, eventually causing formation of cholesterol clefts in the necrotic core. At the same time the lumen of the blood vessel becomes smaller.

3.1 Atherosclerosis in humans
During (CS-induced) oxidative stress, lipoproteins can be oxidized in the circulation or locally after entrapment in the vessel wall, resulting in the formation of oxidized LDL (oxLDL). OxLDL is taken up by macrophages that are infiltrated in the vessel wall which subsequently transform into foam cells, thereby initiating or further progressing atherosclerotic plaque development. During atherosclerotic lesion progression foam cells secrete pro-inflammatory
factors such as MCP-1 and TNF-α, inducing recruitment and activation of monocytes and other leukocytes from the circulation. Vascular smooth muscle cells respond to these secreted mediators by proliferation and migration to the luminal side of the plaque and by producing collagen, resulting in the formation of a fibrous cap on top of the plaque. The constant positive feedback loop of activation and cytokine production causes systemic inflammation, which further induces plaque progression and growth. During growth of the plaque, the inner core of the plaque is deprived of oxygen, which causes apoptosis and necrosis, leading to formation of extracellular cholesterol crystals. The fibrous cap prevents rupture of the plaque and subsequent spilling of the inner core into the lumen. However, upon stress, the fibrous cap becomes thinner, causing the plaque to be more prone to rupture. Plaque rupture results in spill-over of the plaque content into the lumen, formation of a blood clot, and is the most common cause of myocardial infarction and stroke.

3.2 Mouse models of atherosclerosis

As wild-type (WT) mice do not spontaneously develop atherosclerosis, genetically modified mice are used to create atherosclerosis-prone models (Table 2). The classical murine atherosclerosis models are the Apoe−/− and the Ldlr−/− mice in which the hepatic ApoE/LDLr-mediated lipoprotein clearance pathway is disrupted, resulting in high circulating VLDL/LDL levels (116). These two frequently used models show differences with respect to cholesterol distribution over lipoproteins, lipoprotein composition and oxidation susceptibility (91). Absence of ApoE also results in defective macrophage phagocytosis, which contributes to an increased systemic inflammatory phenotype in Apoe−/− mice (85).

In addition to Apoe−/− and Ldlr−/− mice, models have been developed with more human-like lipoprotein profiles, such as the APOE*3-Leiden mice which have more moderately increased circulating VLDL/LDL levels and develop diet-induced atherosclerosis (11; 223). As a result of their intact, though attenuated, hepatic ApoE-LDLr-mediated lipoprotein remnant clearance pathway, APOE*3-Leiden mice respond to lipid-lowering drugs such as statins (48; 252) and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (130) in a similar manner as humans do, while Apoe−/− and Ldlr−/− mice do not. In addition, models for disturbed shear-stress induced atherosclerosis and intimal thickening have been created, such as cuff or collar placement (3) or ferric acid-induced vascular injury. These models induce hemodynamic changes in blood flow and are sometimes combined with the genetic hyperlipidemic models (137).

Despite the fact that these models show features of human atherosclerosis, there are several limitations. For example, the knockout models are whole body knockouts, and the absence of for example ApoE affects multiple processes, such as macrophage phagocytosis, cholesterol efflux from macrophages and inflammation (48; 86). Furthermore, high plasma lipid levels are the main determinant for atherosclerosis formation in these knockout,
whereas risk factors may be more heterogeneous in humans. Despite these limitation, these murine models are considered suitable models to study atherosclerosis pathogenesis as well as for studies on the anti-atherogenic effects of drugs in development.

Table 2: Mouse models of atherosclerosis

<table>
<thead>
<tr>
<th>Exposure model</th>
<th>Diet</th>
<th>Systemic inflammation</th>
<th>Atherosclerosis</th>
<th>Drug sensitivity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow, WTD, HFD</td>
<td>Yes/No</td>
<td>Yes</td>
<td>Low</td>
<td>(77; 78; 85; 86; 96; 104; 116; 125; 139; 141; 143; 156; 185; 186; 199; 231; 236; 252)</td>
</tr>
<tr>
<td>Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>WTD</td>
<td>Yes/No</td>
<td>Yes</td>
<td>Low</td>
<td>(78; 96; 116; 156; 236; 252)</td>
</tr>
<tr>
<td>APOE*3-Leiden</td>
<td>WTD</td>
<td>Yes/No</td>
<td>Yes</td>
<td>High</td>
<td>(96; 127; 130; 232; 251; 252)</td>
</tr>
<tr>
<td>APOE*3-Leiden.CETP</td>
<td>WTD</td>
<td>Yes/No</td>
<td>Yes</td>
<td>High</td>
<td>(96; 251; 252)</td>
</tr>
</tbody>
</table>

4. Combined mouse models of COPD and CVD

Several mechanisms are proposed to contribute to the link between COPD and atherosclerosis, including systemic inflammation, diet, hyperlipidemia, oxidative stress and endothelial dysfunction (Figure 1). Although CS and air pollution are suggested to contribute significantly to the development of both COPD and atherosclerosis (41), it is surprising that the lung is often considered an inert transport organ in studies investigating the effect of CS or other air pollutants on atherosclerosis, rather than an organ that may be actively involved in atherogenesis. Not many combined studies of air-pollutant-induced COPD and CVD have been described and are therefore not reviewed here. Most of the combined murine studies of COPD and atherosclerosis (Table 3) have been performed using CS. Therefore combined models which explore the potential mechanisms that link CS-induced COPD-like features and atherosclerosis will be described and discussed in the following sections.

4.1 Chronic systemic inflammation linking COPD and atherosclerosis

Systemic inflammation is suggested to be one of the most important mechanisms to link COPD and atherosclerosis. Increased systemic levels of the acute phase C-reactive protein (CRP), TNF-α and IL-6 in COPD patients are associated with an increased risk of atherosclerosis development (163; 228). Especially COPD exacerbations increase circulating levels of pro-inflammatory mediators and are associated with atherosclerotic events and death. There are several factors which may contribute to systemic inflammation in COPD, including CS exposure and recurrent infections. Several murine studies on systemic inflammation linking COPD and atherosclerosis are described below.
Table 3: Combined mouse models of COPD and atherosclerosis

<table>
<thead>
<tr>
<th>COPD model</th>
<th>CVD model</th>
<th>Emphysema</th>
<th>Inflammation</th>
<th>Atherosclerosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term CS</td>
<td>Apoe&lt;sup&gt;e&lt;/sup&gt;, Ldlr&lt;sup&gt;e&lt;/sup&gt;</td>
<td>No</td>
<td>Systemic Pulmonary</td>
<td>Yes/No</td>
<td>(4; 72; 91)</td>
</tr>
<tr>
<td>Long-term CS</td>
<td>Apoe&lt;sup&gt;e&lt;/sup&gt;, Ldlr&lt;sup&gt;e&lt;/sup&gt;, human transgenic ApoB100</td>
<td>Yes</td>
<td>Systemic Pulmonary</td>
<td>Yes</td>
<td>(4; 6; 14; 14; 15; 15; 28; 52; 72; 72; 73; 73; 81; 89-91; 91; 102; 102; 110; 115; 124; 125; 128; 131; 131; 131; 140; 146; 148; 186; 250; 250)</td>
</tr>
<tr>
<td>Caloric restriction/</td>
<td>Apoe&lt;sup&gt;e&lt;/sup&gt;, Ldlr&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Yes</td>
<td>Systemic Pulmonary</td>
<td>Yes/No</td>
<td>(4; 6; 14; 14; 15; 15; 28; 52; 72; 72; 73; 73; 81; 89-91; 91; 102; 102; 110; 115; 124; 125; 128; 131; 131; 131; 140; 146; 148; 186; 250; 250)</td>
</tr>
<tr>
<td>High cholesterol/</td>
<td>Apoe&lt;sup&gt;e&lt;/sup&gt;, Ldlr&lt;sup&gt;e&lt;/sup&gt;, human transgenic ApoB100</td>
<td>Yes</td>
<td>Systemic Pulmonary</td>
<td>Yes</td>
<td>(12; 30; 81; 146; 146; 147; 147; 159; 159)</td>
</tr>
<tr>
<td>High fat diet</td>
<td>Combined models: Apoe&lt;sup&gt;e&lt;/sup&gt;, Ldlr&lt;sup&gt;e&lt;/sup&gt;, human transgenic ApoB100</td>
<td>Yes</td>
<td>Systemic Pulmonary</td>
<td>Yes</td>
<td>(3; 23; 23; 89; 104; 110; 120; 125; 154; 157; 257; 257)</td>
</tr>
<tr>
<td>Combined models:</td>
<td>Other effects: Metabolic organs</td>
<td>Yes/No</td>
<td>Systemic Pulmonary</td>
<td>Yes/No</td>
<td>(9; 10; 27; 30-34; 52; 97; 98; 103; 135; 181; 182; 249; 250; 256)</td>
</tr>
</tbody>
</table>

The role of CS in systemic inflammation

Most murine studies aimed at the interaction between COPD and CVD are performed with sub-chronic or long-term CS exposure. CS exposure alone generally results in increased pulmonary leukocyte recruitment and pulmonary endothelial cells adhesion (178), inflammation and oxidative stress in mice (21; 111). Long-term CS exposure in WT mice alone not only induced pulmonary inflammation and emphysema, but also increased systemic pro-inflammatory cytokines including TNF-α and IL-1β (82). Although this study did not combine COPD and CVD, the increased systemic cytokines were indicative of a pro-inflammatory systemic response after CS (82) already in a normolipidemic WT model. In two other studies, exposure to side-stream CS in WT mice not only increased systemic pro-inflammatory cytokine levels, but also decreased heart stroke volume, decreased antioxidant levels in the heart and increased vascular resistance (253; 254), suggesting that CS exposure directly affects the cardiovascular system. Long-term CS exposure of human APOB100-transgenic mice, which develop moderate hyperlipidemia when fed an atherogenic diet, increased levels of MCP-1 in the circulation, heart and aorta and enhanced atherosclerotic lesion area and macrophage content in the atherosclerotic plaque (250). These data suggest that CS exposure leads to systemic inflammation, possibly in part through spill-over of inflammatory mediators from the lung.

Leukocyte recruitment towards the lung during local pulmonary or systemic inflammation may also be different. Systemic intravenous LPS administration for example induced leukocyte...
trapping in pulmonary capillaries, whereas intratracheal LPS administration induced leukocyte accumulation in venules. Furthermore, local intratracheal LPS administration induced alveolar space leukocyte recruitment, which was not observed after intravenous administration of LPS. The difference in recruitment towards the lung after local or systemic LPS administration may be due to leukocyte priming after systemic inflammation (235).

The role of respiratory infections in systemic inflammation

Smokers with normal lung function and COPD patients have a high risk for recurrent respiratory infections, which may contribute to systemic inflammation and atherosclerosis. Especially COPD exacerbations associated with respiratory infections cause a rise in circulating inflammatory mediators and are associated with CVD morbidity and mortality (20; 58; 163). Respiratory infections alone can aggravate atherosclerosis, through induction of systemic pro-inflammatory mediators (165; 215). Combining Chlamydia pneumoniae infection with side-stream CS exposure in Apoe−/− mice exacerbated atherosclerosis due to enhanced pulmonary inflammation, apoptosis and defective pulmonary phagocytosis of pathogens, whereas systemic lipid levels and inflammation were not altered (257). Respiratory viral infections are also positively associated with atherosclerosis development (188). Upon exposure of Apoe−/−, Ldlr−/− and WT mice to intranasal influenza virus, live virus was measured in lungs, heart and aorta, and Apoe−/− mice showed increased atherosclerotic plaque area and inflammatory cell content in the plaque compared to controls (89; 157).

CS exposure and respiratory infections may have direct effects on atherosclerosis development, due to leakage of CS components or pathogens to the vessel wall. Therefore, several studies also examined the effect of emphysema alone on atherosclerosis development, thus in absence of CS or infection. We showed, for example, that PPE-induced emphysema in WTD-fed ApoE*3-Leiden mice did not affect pulmonary inflammation, systemic inflammatory or lipid parameters, and did not affect atherosclerosis development, suggesting that emphysema alone does not aggravate atherosclerosis (120). Addition of biweekly chronic intranasal LPS administration, to mimic chronic pulmonary inflammation, did induce low-grade systemic and pulmonary inflammation as well as increased atherosclerosis development. This proatherogenic effect is likely caused by leakage of inflammatory mediators into the circulation, but this remains to be elucidated.

These studies collectively show that CS and respiratory infections induce systemic inflammation in mice, similar to COPD patients with atherosclerosis. Systemic inflammation arises most likely in part from leakage of pro-inflammatory mediators from the lung to the circulation (194; 207). It needs to be noted that development of features of atherosclerosis and COPD requires long term exposures, and therefore aging of the mice during the experiment may affect outcomes. Although most studies have an age-matched control group, aging is associated with low-grade systemic inflammation (‘inflammaging’) and
may affect the lungs, immune responses and response towards CS exposure and infections (238). Furthermore, there is evidence that in the absence of systemic inflammation, the extent of atherosclerosis development is limited. In addition, models of diet-induced atherosclerosis may be more lipid- and less inflammation-driven, suggesting that further research is necessary with more inflammation-driven atherosclerosis models, such as the cuff- or collar models. However, other CS-induced mechanisms linking COPD and CVD could also be involved and are described below.

4.2 Hyperlipidemia linking COPD and atherosclerosis

Another proposed mechanism that links COPD and atherosclerosis is hyperlipidemia, a common feature in COPD patients, which may arise from unhealthy eating habits, and other lifestyle factors including smoking. Smokers and COPD patients show increased circulating levels of VLDL, LDL and triglycerides and low levels of HDL (5). Dyslipidemia not only affects circulating lipids and lipoproteins, but lipids are also important in normal physiology and homeostasis of organs and tissues, including the lung (84). Furthermore, diet-induced hyperlipidemia may contribute to systemic inflammation and pulmonary pathophysiology. Murine studies in which the role of hyperlipidemia was examined as a link between COPD and atherosclerosis are described below.

The role of hyperlipidemia in systemic inflammation

Apart from spill-over of pro-inflammatory mediators from the lungs, systemic inflammation may also arise from hyperlipidemia. For example, intraperitoneal LPS injection increased plasma levels of IFN-γ and TNF-α in Apoe\(^{-/-}\) and WT mice fed an atherogenic diet and aggravated atherosclerosis development in Apoe\(^{-/-}\) mice. Interestingly, it also induced leukocyte infiltration around pulmonary vessels in WT mice, and even more pronounced in hyperlipidemic Apoe\(^{-/-}\) mice (162), suggesting that systemic inflammation, especially in combination with hyperlipidemia, increases pulmonary inflammation. Also diets rich in cholesterol and fat increase pulmonary inflammation (214) and emphysema development (81). Feeding Apoe\(^{-/-}\) mice the highly atherogenic Paigen diet, for example, increased plasma IL-6 and IL-1β levels and induced pulmonary arterial hypertension and right ventricular hypertrophy (139). This is further supported by the observation that 12 weeks of HFD feeding in Apoe\(^{-/-}\) mice also increased circulating inflammatory cytokines and pulmonary inflammation (159). Furthermore, pulmonary inflammation was associated with collagen deposition and MMP-9 activity, suggesting that HFD also induced remodelling in the lung. CS exposure increased pulmonary inflammation and emphysema associated with decreased lung function in chow-fed Apoe\(^{-/-}\) mice, which still experience mild hyperlipidemia compared to chow-fed WT mice (4). Apoe\(^{-/-}\) mice fed a WTD for 10 weeks also developed emphysema, which was not observed in WT or Ldlr\(^{-/-}\) mice (81). Since macrophages from these Apoe\(^{-/-}\)
mice have a reduced cholesterol efflux and increased expression of pro-inflammatory genes, a link between abnormal cholesterol efflux and lung inflammation was suggested.

These data show that diet-induced hyperlipidemia contributes to systemic inflammation in mice and may vice versa also induce pulmonary inflammation, tissue destruction and emphysema through MMP activation and other mechanisms. These findings may be relevant for COPD patients with dyslipidemia, due to a Western lifestyle with high fat intake, high cholesterol levels and insufficient exercise (222). These factors may thus, in addition to smoking, affect pulmonary inflammation, emphysema and systemic inflammation, which enhance the risk for atherosclerosis development.

The effect of CS on metabolic organs
CS exposure is also known to affect metabolic organs including liver, muscle, and white and brown adipose tissue, organs important in lipid and lipoprotein metabolism. These organs are also implicated in the metabolic syndrome, which is more prevalent amongst smokers and contributes to CVD development (107).

The liver is a key organ in regulation of lipid metabolism and (systemic) inflammation. Hepatic steatosis (fatty liver disease) has been linked to atherosclerosis, although causation is unclear. CS exposure may increase the risk of hepatic steatosis, through inflammation, lipid accumulation and hypoxia, which even worsens on a hypercholesterolemic background (6; 249). Low body weight and muscle wasting are commonly observed in patients with severe COPD and contribute to overall morbidity and mortality. Muscle wasting in patients with severe COPD (69) may contribute to atherosclerosis development, most likely by induction of systemic inflammation involving cytokines such as TNF-α (129; 136). Chronic CS exposure lowers body weight and white adipose tissue mass in WT mice (167), at least in part exerted via CS-mediated reduction of the neurotransmitter neuropeptide Y, which decreases appetite (30-34). There are also indications that caloric restriction increases pulmonary resistance and decreases lung volume (12). CS-induced reduction of body weight is driven by cytokines, such as IL-6 and the adipokines leptin and adiponectin (93; 195). Adiponectin is considered protective in several diseases, such as in metabolic syndrome and COPD (94). Adiponectin-deficient mice develop alveolar enlargement and other COPD-like characteristics in addition to systemic inflammation, wasting and endothelial dysfunction similar to COPD patients (158). Also, mice with PPE-induced emphysema have low levels of adiponectin (153). Moreover, leptin expression in bronchial epithelial cells and alveolar macrophages was higher in WT mice exposed to CS as compared to air-exposure (227). In spite of these findings, the physiological role of these adipokines in lung pathology and inflammation is not clear (195).

Several murine models showed that exposure to CS activates brown adipose tissue, which in contrast to white adipose tissue, burns triglyceride-derived fatty acids and produces
heat (101; 119). CS exposure induces expression of genes related to brown adipose tissue activation and thus may contribute to the lower body weight observed in smokers (31; 34). Moreover, brown adipose tissue activation reduces hypercholesterolemia and atherosclerosis development in APOE*3-Leiden.CETP mice (11; 101). As this cholesterol-reducing effect of brown adipose tissue activation is dependent on an intact ApoE-LDLr-mediated hepatic lipoprotein remnant clearance pathway, the cholesterol- and atherosclerosis-reducing effect was not seen in ApoE<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice (11; 52). Although brown adipose tissue activation in APOE*3-Leiden.CETP mice appears atheroprotective, the relation between brown adipose tissue activation and the relatively unfavourable lipid profile in smokers and COPD patients is unclear. And in contrast to murine studies, the effect of CS exposure on brown adipose tissue in humans is not known yet, although resting energy expenditure in smoking individuals was increased compared to non-smoking individuals (13; 48), suggesting that brown adipose tissue may be activated. Furthermore, there are no reports so far that have investigated the role of brown adipose tissue activation in the link between COPD and CVD.

As described above, murine models and human studies have shown that CS has various effects on metabolic organs, such as the liver, brown and white adipose tissue and muscle. Whether CS-induced metabolic changes in these organs contribute to atherosclerosis development remains to be elucidated.

**The role of CS in hyperlipidemia**

In addition to contributing to systemic inflammation and dysfunction of metabolic organs, as described in the previous sections, CS may also contribute to atherosclerosis development by inducing hyperlipidemia. However, the effects of CS exposure on lipid levels in murine COPD and CVD models are inconclusive. Long-term exposure to both main- (102) and side-stream (72; 73) CS alone or in combination with WTD-feeding in Apoe<sup>−/−</sup> aggravates atherosclerosis development, but this could not be explained by increased plasma cholesterol levels. However, in CS-exposed Ldlr<sup>−/−</sup> mice fed the same saturated-fat diet, both systemic cholesterol levels and atherosclerotic lesion area were increased, also without affecting pulmonary inflammation (91). Importantly, pulmonary inflammation was determined only in the latter study, and was not changed in both Apoe<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice (91).

In other studies in Apoe<sup>−/−</sup> mice, CS exposure did result in increased circulating lipid levels (15; 140). Exposure to main-stream CS in Apoe<sup>−/−</sup> mice enhanced levels of cholesterol, triglycerides and phospholipids in the circulation, liver and aorta. Furthermore aortic plaque size and cholesterol content were increased, whereas atheroprotective polyunsaturated fatty acids levels were decreased. The liver showed proatherogenic transcription profiles for genes involved in lipid metabolism, suggesting that exposure to main-stream CS directly affects lipid profiles in blood, liver and aorta (15). Finally, chronic mainstream CS exposure for 10 weeks in Apoe<sup>−/−</sup> mice increased plasma cholesterol levels, arterial thrombosis and
neointima formation upon ferric acid-induced vascular injury (186). In line with these findings, smoking cessation in Apoe−/− mice decreased CS-induced atherosclerosis and hyperlipidemia, despite an increased body weight upon cessation (140). Furthermore, smoking cessation after exposure to main-stream CS in Apoe−/− mice resulted in reduced inflammatory cell recruitment, decreased expression of genes related to tissue remodelling in the lungs and reduced emphysema (14).

Since most combined studies for COPD and atherosclerosis have been performed in Apoe−/− mice, one should keep in mind that lungs of these mice are congenitally different from lungs of WT mice. Apoe−/− mice have impaired developmental alveologenesis, increased airway resistance and a rapid loss of lung elastic recoil compared to WT mice, predisposing Apoe−/− mice to pulmonary problems (146). In addition, ApoE itself has anti-inflammatory, anti-oxidative, anti-proliferative (for example on smooth muscle cells) and anti-thrombotic properties, which may all contribute to its anti-atherosclerotic effects. The immunomodulatory effects of ApoE may substantially contribute to immune activation and inflammation during atherosclerosis (252). Upon exposure to main-stream CS, chow-fed Apoe−/− mice showed an increased pulmonary inflammatory response, MMP activity and emphysema development compared to chow-fed WT mice, suggesting that the lungs of Apoe−/− mice are more sensitive to CS (4). Deficiencies in other proteins, such as scavenger receptors involved in intracellular and membrane cholesterol metabolism and trafficking in the lung, can result in altered immune responses in the lung (84). Furthermore, exposure to CS affects phagocytic activity of alveolar macrophages, which in combination with an abnormal cholesterol efflux in Apoe−/− mice, worsens pulmonary inflammation and contributes to atherosclerosis development (166). Mutations in ApoE thus not only affect atherosclerosis development, but can also affect both systemic and pulmonary inflammatory responses that are observed in models of COPD and airway hyperresponsiveness (248). Next to hyperlipidemia in Apoe−/− mice, obesity and diabetes may also affect lung function, structure, immune cell function (66), as well as the pulmonary circulation and hypertension (67).

In conclusion, findings in murine studies with regard to the contribution of hyperlipidemia to the link between COPD and atherosclerosis are inconclusive so far. This difference in outcome is most likely explained by differences in duration of the studies, diet, outcome parameters and smoking protocols.

4.3 Oxidative stress linking COPD and atherosclerosis

Another proposed mechanism to link COPD and CVD is CS-induced oxidative stress and formation of ROS (26; 132). Oxidative stress causes dysregulation of athero-protective mediators and induces endothelial injury and atherosclerosis development (151). Acute CS exposure already causes oxidative (DNA) damage in lungs, heart and liver (103; 113; 161). Mitochondria are susceptible to oxidative stress, and CS-induced alterations in mitochondrial
function can thereby affect energy production and metabolism (1), which may contribute to vessel wall damage and atherogenesis. This is supported by studies in hypercholesterolemic Apoe<sup>−/−</sup> mice exposed to side-stream CS, in which oxidative damage to mitochondrial DNA and protein damage in the heart was observed together with a lowering in antioxidants such as vitamin C and E (124). Increased systemic lipid peroxidation also caused depletion of vitamin E in liver and heart (254). CS-induced oxidative stress also causes lipid modification, such as formation of proatherogenic ox(V)LDL (208). In the lung, CS can oxidize lipids such as phospholipids, which impairs the phagocytic capacity of alveolar macrophage and thus may contribute to defective immune responses to invading pathogens (211).

Arginase is an enzyme which competes with endothelial nitric oxide synthase (eNOS) for the precursor of nitric oxide (NO), and is involved in many pathologies. Side-stream CS exposure in WT mice increased arginase activity and ROS levels, whereas NO levels were decreased, causing endothelial dysfunction and vascular stiffness (192). NO was not lowered in arginase-2-deficient mice, indicating that CS lowered eNOS activity. Furthermore, CSE also inhibits eNOS activity in pulmonary artery endothelial cells, which may contribute to CVD in cigarette smokers (198). Whereas eNOS is considered atheroprotective, inducible nitric oxide (iNOS) contributes to oxidative stress. CS exposure induces iNOS, causing ROS formation and cuff-induced intimal thickening in WT mice (3).

After removal or lowering of atherogenic components such as nicotine and tar from cigarettes, exposure to CS still resulted in increased biomarkers of systemic oxidative stress, such as oxLDL. Other parameters, including plasma lipid levels (28) blood pressure and blood leukocyte count, were not different, although lesion area was still higher as compared to non-smoking mice (28). Antioxidant therapy using vitamin E lowered these effects, indicating that atherosclerosis was accelerated in response to CS-induced systemic oxidative stress (131). Collectively, these data show that CS-induced oxidative stress in mice is an important contributor in the link between COPD and CVD.

4.4 Endothelial dysfunction linking COPD and atherosclerosis

Injury to the endothelium induces endothelial dysfunction, an important step in atherosclerosis development (5; 76; 151). CS exposure causes endothelial damage and may thereby contribute to the link between COPD and atherosclerosis. CS is associated with endothelial apoptosis and reduced vascular barrier function, enabling leakage of inflammatory mediators to the circulation and vice versa, thereby contributing to inflammation (142; 180).

Chronic CS exposure of WT mice caused high blood pressure, high blood cell counts, oxidative stress, systemic inflammation, low NO levels and lowered cardiac function (206), all of which may contribute to injury and activation of the endothelium. Also, endothelial progenitor cells (EPC) recruitment from bone marrow and function may be hampered by
Mouse models for COPD and CVD

CS. Intraperitoneal injection of CS extract, used to induce emphysema in mice, interrupted EPC recruitment from bone marrow to the lung (97). Antioxidant therapy reduced CS-induced systemic oxidative stress, EPC dysfunction and neovascularization development in WT mice (218), indicating that CS-induced EPC dysfunction is at least in part mediated via oxidative stress. CS exposure may also lead to enhanced platelet reactivity and thrombosis in Apoe⁻/⁻ mice, further contributing to vascular injury (52). For example, circulating levels and pro-coagulant activity of tissue factor, which is involved in thrombus formation, were increased after CS exposure in Apoe⁻/⁻ mice (148). In addition, thrombin generated upon CS exposure is involved in thrombus formation, but can also induce pulmonary endothelial hyperpermeability and decrease barrier protection (258) Interestingly, this process could be inhibited by bone marrow-derived EPC. Finally, CS affects vascular endothelial growth factor (VEGF), which is important in the maintenance of lung structure and endothelial function. CS exposure reduced both VEGF and VEGF receptor in the lung, which was also observed in smoking COPD patients (202).

Although human studies on the effect of endothelial dysfunction in the link between COPD and CVD are scarce (19), data suggest that patients with mild COPD have increased levels of endothelial microparticles suggesting endothelial activation and/or injury (212). Furthermore, patients with severe COPD show elevated markers of endothelial activation. Despite the limited data for humans, murine studies seem to show an important role for the endothelium and EPCs in the link between COPD and CVD.

5. Treatment of COPD patients with atherosclerosis

Although smoking cessation is the most effective measure to reduce COPD symptoms (5; 260), it is difficult in practice due to the addictive nature of tobacco. Smoking cessation does result in an increase in atheroprotective HDL and can be beneficial for reducing endothelial injury (5). However, even after smoking cessation, a considerable risk for both COPD and CVD remains, which was also shown in murine studies (140). COPD patients are treated with bronchodilators such as long-acting beta-2 agonists and anticholinergics and anti-inflammatory agents such as inhaled corticosteroids, which are especially indicated for patients with frequent exacerbations (260) (Figure 3). Treatment with inhaled steroids reduced all-cause mortality in COPD in a case-control study (25). However, in the TORCH study no significant reduction was observed in all-cause mortality (including cardiovascular cause) after a combination therapy of a bronchodilator and an inhaled steroid. Nevertheless, treatment did reduce the frequency of exacerbations and improve overall health status (24). Most studies on the effect of bronchodilatory and anti-inflammatory agents on COPD and CVD parameters are performed in human trials, and unfortunately murine COPD models are not often used.
COPD patients with CVD receive additional, lipid-lowering drugs such as statins which inhibit the rate-controlling enzyme of cholesterol synthesis, 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. Various murine studies showed beneficial effects of statin therapy on the lungs, which is most likely mediated by its anti-inflammatory actions (237) in both acute and chronic disease states, for example during sepsis after Streptococcus-induced acute lung injury (255), but also air-pollution-induced pulmonary inflammation (210). Statins also reduced acute CS-induced inflammation and oxidative stress (63) and elastase-induced emphysema are reduced upon statin treatment in WT mice (204). However, the importance
of these findings for assessing the clinical benefit of statin treatment on COPD is a matter of ongoing debate.

There are several potential new drug targets (Figure 3) for the treatment of COPD patients with CVD. Recent findings suggest that also other lipid-lowering (i.e. liver x receptor agonists, ApoE-mimetic peptides) and HDL-raising strategies (i.e. ApoAI-mimetic peptides) may be beneficial for various lung disorders (237), including COPD (84), and are therefore attractive treatment targets and subject of further research for both COPD and CVD. Injection of HDL, which is also a transporter for α-antitrypsin, injected together with α-antitrypsin reduces CS-induced pulmonary inflammation and elastase-induced emphysema (155), and may be an attractive therapeutic option for both COPD and CVD.

Other treatment approaches include vitamin D therapy, microbiome alteration and administration of EPC or mesenchymal stromal cells (MSC). Vitamin D-deficiency is associated with COPD and CVD (100; 149; 184), and COPD patients with low vitamin D levels have an increased exacerbation risk (170; 171). Mice exposed to CS whilst being fed a vitamin D-deficient diet developed more severe emphysema compared to CS exposure alone (43; 171). Vitamin D also contributes to antimicrobial defence and to control of inflammation, and thus may contribute to the control of the development of both COPD and CVD. Thus, vitamin D may be an attractive target in combined therapy for COPD and CVD. Another potential new treatment target is the microbiome, the collection of micro-organisms that live inside and on the body. Diet-induced changes, such as hyperlipidemia, but also high fiber intake, have marked effects on the (intestinal) microbiome (53). Especially diet-induced changes may affect the intestinal microbiome and hyperlipidemia and contribute to COPD and CVD (176). More research on this subject is necessary to gain more insight on modulation of the microbiome and its effect on COPD and CVD. Finally, treatment with MSC or EPC is a promising new target (83) in combined treatment for COPD and CVD. MSC have anti-inflammatory and regenerative properties. Several studies have shown that MSC reduce pulmonary inflammation and emphysema (87; 106; 213). Also in the context of CVD research, MSC therapy is promising (70; 246). However, no studies have been performed yet, to study the potential beneficial effect of MSC treatment on COPD and CVD combined.

Murine COPD and CVD models are well suited to study these new potential therapies. For example, studying the microbiome in humans is difficult because of differences in genetic background and environmental factors such as lifestyle. Laboratory mice are identical and environmental factors can be controlled well. Furthermore, the microbial status of animals can be tracked during studies. Also for cell-therapy (MSC and EPC administration), murine studies can provide much information. Furthermore, because of the heterogeneity of symptoms and (environmental) background of COPD patients with CVD, studying the effect of new treatments in mice provides clear results, without interfering factors.
6. Concluding remarks

CVD is one of the most important comorbidities of COPD (59; 68) and in this review we have provided an overview of the available murine models that combine COPD and CVD, and discussed the relevance of these models in explaining the link between COPD and CVD and the development of new treatments for COPD patients with atherosclerosis.

Currently, only a few murine studies assessed parameters of both atherosclerosis and COPD. Despite the different protocols (e.g. CS-exposure, diet, duration and genetic background) and readouts, both these murine studies as well clinical studies have revealed a prominent contribution of systemic inflammation and oxidative stress to the link between COPD and CVD. However, these murine studies also clearly indicate that other shared mechanisms such as increased pulmonary inflammation, hyperlipidemia and endothelial dysfunction, may contribute to this link. As outlined in this review, several murine models are characterized by human-like features of both COPD and atherosclerosis. It needs to be noted however that the readouts of the murine studies may differ from those in clinical studies. Whereas lung function measurements and imaging are important tools in clinical diagnosis, the use of these parameters in murine studies is often limited. Although murine studies do include readouts such as atherosclerosis and emphysema at the end of a study, measuring lung function or imaging atherosclerosis in alive animals could provide important additional information for comparison with human studies. Therefore, the increased use of in vivo lung function measurements and live CT- and MRI-scanning may provide relevant information in future studies.

Only in the last few years the effects of COPD drug treatments on CVD outcomes and CVD treatments on COPD progression have been studied. In general, the effect of COPD treatment on CVD outcome and vice versa is currently largely unknown. Furthermore, because of the variety of drugs that COPD patients with CVD receive, it is difficult to determine the effect of a single drug, and of drug interactions. This issue limits the interpretation of study outcomes on the combined effects of COPD and CVD drugs in clinical trials and illustrates the necessity of new experimental models and studies. In such (murine) models, disease pathology and the effect of current and novel drug strategies for COPD with CVD can be assessed in a more controlled way.

In conclusion, combinations of models for COPD and atherosclerosis are very useful tools and can provide important insights relevant to understanding the link between COPD and CVD. More importantly, murine studies provide good platforms for studying the potential of promising (new) therapeutic strategies for COPD patients with CVD.
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References


Chapter 2


Mouse models for COPD and CVD


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