The handle http://hdl.handle.net/1887/46453 holds various files of this Leiden University dissertation

**Author:** Rampersad-Khedoe, Padmini  
**Title:** Mouse models of chronic obstructive pulmonary disease: in search of novel treatment options  
**Issue Date:** 2017-03-09
General discussion and future perspectives
Chapter 7
7.1. Mouse models to study the link between COPD and CVD

Whilst it is known that patients with chronic obstructive pulmonary disease (COPD) have an increased risk for cardiovascular disease (CVD), the mechanism underlying this increased risk is not known precisely. CVD and COPD share several risk factors including cigarette smoking, obesity, low lung function and advanced age (7), but it is difficult to identify the causes and underlying mechanisms for this link. In Chapter 2, we reviewed some of the mechanisms that may explain the link between COPD and CVD and the relevance of combined mouse models for studying the mechanisms involved in COPD and CVD.

7.1.1 The relevance of mouse models to study COPD

As cigarette smoking is the most important cause of COPD, cigarette smoke (CS) exposure in mice is the most realistic mode to induce COPD-like features in this research model. The type of smoke (main- vs. side stream), however, seems to be very important for induction of neutrophilic inflammation as an important step in COPD pathophysiology. Exposure to side-stream CS results in high carbon monoxide (CO) levels, which is toxic and may inhibit inflammation, whereas main-stream CS causes neutrophilic infiltration in the lungs (29). We exposed mice to CS using whole body smoke exposure in a Teague-TE10 machine, producing a mixture of 20% main-stream and 80% side-stream CS. Although macrophage numbers in BAL and lung tissue were increased, there was no increased neutrophil infiltration after acute or sub-chronic CS exposure (Khedoe P., Wong M. et al.; unpublished data). We made several adjustments in order to produce more main-stream CS, however, this was insufficient to induce overt pulmonary inflammation. Although several studies show development of COPD-like features after chronic side-stream CS exposure, it is important to keep in mind that COPD in humans is mainly caused by inhalation of main-stream CS (30).

Other models to induce COPD-like features, besides CS exposure, include porcine pancreatic elastase (PPE; used in Chapter 3) that causes proteolytic degradation of the alveolar walls with transient inflammation. This model not fully represents the human situation, where inflammation is an important feature of COPD, however it is well-suited to study inhibition and reversal of established emphysema and the impact of emphysema on other mechanisms. Chronic instillation of (components of) bacteria and viruses also induce pulmonary neutrophilic inflammation, which on the long-term progresses to emphysema development, and therefore mimics human COPD pathophysiology quite well. Inflammation is even increased in combined models of CS exposure and bacterial (31) or viral infection (32). Furthermore, these models mimic frequently occurring exacerbations, a sudden worsening of symptoms in COPD patients that are correlated to comorbidity and mortality. Intraperitoneal administration of cigarette smoke extract (CSE) may also lead to emphysema (33). However, as this mode of delivery is not very physiological, we administered CSE by
intranasal instillations. Intranasal CSE instillation induced a limited macrophage influx, whereas neutrophil numbers were not altered compared to instillation with vehicle (Khedoe et al., unpublished data). In addition, mice showed adverse reactions to CSE instillation most likely because of the relatively high and concentrated levels of nicotine. Finally, inflammatory responses were limited compared to intranasal LPS administration, restricting the suitability of CSE as a model for COPD.

Altogether, the mouse model that best mimics human COPD pathophysiology is chronic main-stream CS exposure without or with administration of (components of) bacteria and viruses. This model is most suited to study mechanisms and develop new treatments for COPD and COPD exacerbations and importantly can also be used for studies examining the effect of smoking on comorbidities.

7.1.2. The use of mouse models that combine COPD and CVD

Mouse models that combine COPD and CVD often involve Apoe−/− or Ldlr−/− mice as described in Chapter 2. However, these mice, in addition to altered metabolic profiles (e.g. high levels of VLDL/LDL), also have impaired macrophage function (e.g. phagocytosis) (34) and higher susceptibility to (diet-induced) emphysema (35). Furthermore, since lipid moieties are components of various proteins, such as surfactant protein, which are important for proper pulmonary functioning (36), study outcomes in these hyperlipidemic models may also be affected through other mechanisms. Atherosclerosis development in these models is mainly lipid-driven (hyperlipidemia), whereas systemic inflammation is modestly increased. In humans however, the extent of hyperlipidemia compared to the mouse models is less, and systemic inflammation contributes more to atherosclerosis development, especially in many (chronic) inflammatory diseases. Therefore, when using a hyperlipidemic mouse model, addition of a pro-inflammatory stimulus may be necessary to further induce systemic inflammation and increase atherosclerosis development. A combination with a cuff or collar model may result in more inflammation-driven lesions, and may therefore better represent CVD. These pro-atherogenic models combined with CS-exposure and (a component of) bacterial or viral infection mimic COPD patients with CVD well, especially in terms of increased risk of an atherosclerotic event during COPD exacerbations. Although these models are widely accepted and are suitable for studying the mechanisms linking COPD and CVD, it would be better to use a model with more human-like characteristics with respect to lipoprotein metabolism. Therefore, more studies should be performed in the future using CS exposure with (a component of) bacterial or viral infection in pro-atherogenic mouse models with a more human-like lipoprotein profile that also respond to lipid-lowering agents, such as Western-type diet fed APOE*3-Leiden (E3L) mice. These models may represent human pathophysiology better and be more relevant to study mechanisms that may link COPD and CVD and develop new treatments for both diseases.
In addition to choosing the best available mouse model for a specific research question, it is important to measure the right parameters. A critical and defining clinical parameter to diagnose COPD in humans is the lung function measured by spirometry for example. Although in recent years, the use of lung function measurements in murine studies has increased, histological measurements (e.g. cellular infiltration and mean linear intercept, resp.) are the standard method to determine the level of inflammation and emphysema. In Chapter 3 and 4, we measured respiratory rate and amplitude using whole body plethysmography to determine the effect of PPE and/or LPS at several time points. Whole body plethysmography is non-invasive and can be measured in vivo in conscious spontaneously breathing animals. However, whole body plethysmography cannot be used to determine lung function directly and measurements may be influenced by environmental pressure changes. Using the forced oscillation technique, lung function can be determined directly by measuring lung resistance and elastance. Advantages of this method include bypassing of the upper airways, and controlled and standardized measurements. However, the forced oscillation technique is invasive, requires sedated and tracheostomized mice and can only be used as an terminal measurement. These invasive lung function measurements therefore can unfortunately not be used as to determine lung function during a (long-term) study.

COPD severity in humans can furthermore be monitored with chest X-ray, CT-scanning and measurement of several blood parameters (e.g. blood gas, white blood cell count). Blood parameters are often also measured in mouse studies as intermediate parameter. Although there are more imaging techniques (e.g. CT and MRI scanning, bioluminescence) available to determine the severity of COPD-features in mice in vivo, the use of these techniques is still limited, mostly due to the relatively high costs.

Atherosclerosis severity in mouse models is also mostly measured through histology (e.g. determination of plaque size and severity), whereas in humans electrocardiograms and radiological, MRI and CT scans are used combined with blood parameters for diagnosis of disease and to determine disease progression. Whereas inflammatory parameters in blood are already measured during mouse studies, tracking the level of emphysema and atherosclerosis in vivo seems to be limited. The application of in vivo measurements on emphysema and atherosclerosis will not only limit the number of animals used for studies, but also can provide more detailed information on the mechanisms involved in COPD and CVD and enable examination of (side) effects of new treatments. Techniques (e.g. CT and MRI scanning, bioluminescence) to measure lung function and emphysema and development of atherosclerosis in vivo are becoming available and affordable for mouse research, and therefore will be more accessible in the future. Although in the future in vivo measurements will be used more often, acquired results will still have to be verified with histological measurement at the end of a study.
Chapter 7

7.2 Treatment of COPD patients with CVD

COPD patients often receive inhaled corticosteroid therapy to control inflammation and inhaled short- or long-acting β2-adrenergic receptor agonists (LABAs) or long-acting muscarinic receptor agonists (LAMAs) as bronchodilators to improve lung function temporarily (7). CVD symptoms are targeted through lipid-lowering agents or β-adrenergic receptor blockers. Whether such therapy is the most optimal has been studied only in the last few years, in clinical trials investigating the (adverse) effect of COPD and CVD treatment on COPD and CVD outcome. Furthermore, future studies should focus more on development of a combined treatment for COPD patients with CVD, for example treatment with mesenchymal stromal cells (MSC), which we studied in Chapter 4.

7.2.1. The effect of COPD and CVD treatment on disease outcome

The bronchodilator salmeterol alone or combined with inhaled steroids in patients with moderate to severe COPD did not increase the risk for CVD events, shown in the TORCH study (37). However, inhaled or oral glucocorticoids lower circulating CRP and inflammatory cytokine levels, which may lead to a reduction in CVD as well. In an observational study, high dose oral glucocorticoid use was associated with increased heart failure and ischemic disease, which direct towards a negative effect on CVD outcome (38). These data suggest that COPD treatment with corticosteroids and bronchodilators at least do not worsen the CVD symptoms, and likely even improve lung function and lower inflammation.

Patients with CVD receive a cocktail of lipid-lowering agents, blood pressure controllers and anticoagulants. Although this may not be the most optimal treatment protocol for individual patients, in general this policy is sufficient to control parameters involved in atherosclerosis. The most well-known lipid-lowering agents are statins. They have pleiotropic effects including improvement of endothelial function (39) and lowering of inflammation. Also in COPD patients with CVD, statin administration is associated with reduced neutrophil inflammatory responses and remodelling (36) and lower mortality, especially after exacerbations (40). Therefore, addition of statins to conventional COPD therapy is likely beneficial for both COPD and CVD outcome. COPD patients with CVD often also receive cardioselective β-adrenergic receptor blockers, mostly selective for the β1-adrenergic receptors to reduce hypertension, ischemic heart disease and heart failure. Treatment with β-adrenergic receptor blockers of COPD patients, reduces exacerbations and mortality, even when they were used combined with inhaled corticosteroids, LABAs or LAMAs (41). Most of these studies however, have been performed in observational studies, in which treatments are often adjusted during studies. Therefore, it would be better to study the effects of drugs that target COPD features and the effects of lipid-lowering drugs in a randomized placebo-controlled trial, where patients are matched at baseline after which they receive various
treatments. Although this design would be most optimal from a scientific perspective, there may be clinical and ethical issues which impede such a study.

7.2.2. Treatment of COPD and CVD with mesenchymal stromal cells

Inflammation, lung tissue destruction leading to emphysema, and impaired lung function are progressive and irreversible in COPD. Current treatment is aimed towards lowering inflammation and limiting obstruction, but is not targeted towards improving or curing the disease because of the lack of appropriate interventions that can achieve this. In the last decades more research groups are focused on at least reducing and ideally curing the disease. This can be accomplished by lung regeneration combined with inhibition of inflammation. Lung regeneration may be achieved through induction of endogenous repair mechanisms or cellular therapy with for example MSC.

MSC treatment in several acute and chronic inflammatory disease models limits not only inflammation, but can also reduce tissue destruction through activation of regeneration and repair mechanisms. Therefore, MSC administration may also be used to limit pulmonary inflammation and even repair emphysema (42-44). An additional advantage is that MSC can be derived from patients, expanded in vitro, and then used for autologous treatment. Whether there are differences in MSC function between healthy subjects and patients, is not known. However, in a study from our group no differences were observed in morphology, proliferation and migration, except for increased adipocyte differentiation potential in the bone-marrow derived MSC from COPD patients compared to MSC from healthy controls (45). Although there are not many clinical trials studying the effect of MSC therapy on either COPD or CVD, MSC treatment seems to be feasible in COPD patients. In the LUMC, a safety and feasibility phase I study showed that MSC can be cultured from COPD patient bone marrow, expanded ex vivo and safely given back to the patient. There was no improved lung function after 12 months follow-up, however, CD31+ cells were increased in lung tissue following MSC administration, suggesting increased angiogenesis (46), which is important in repair and recovery of tissues (47).

Conditioned medium from bone marrow cells or MSC also reduced CS-induced emphysema in mice (48), suggesting that these cells act most likely through paracrine mechanisms. More research is necessary to identify the secreted factors which contribute to inhibition of inflammation and induce repair and regeneration. Candidate factors that may mediate the therapeutic effect include angiogenic and growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), anti-inflammatory cytokines and chemokines, and factors that promote cell proliferation and inhibit cell death. Furthermore, mediators important for embryonic development, such as members of the Wnt-, Notch, Hedgehog- and TGF-β family, may be important for induction of tissue regeneration upon injury. These secreted factors can be measured in supernatant of control
vs. stimulated MSC in *in vitro* experiments. To better understand the *in vivo* mechanism of MSC, tracking studies using GFP-labelled MSC can be performed. Neutralizing antibodies or MSC from mice deficient in selected mediators can be administered, to determine the contribution of the individual factors. Another option to determine whether MSC treatment is relevant for COPD patients with CVD, may be local administration of MSC. MSC home to sites of injury and inflammation, suggesting the presence of certain danger- or damage associated molecules on MSC. Furthermore, because of this homing-property, MSC may be used in the future also as carrier for drugs, to even further limit inflammation and induce repair. Finally, autologous MSC may be administered, in addition to regular treatment, to COPD patients with CVD in a randomized control trial, to determine whether MSC further improve COPD and CVD symptoms.

In the future MSC treatment may be applied with different subpopulations of MSC. MSC can have pro-inflammatory (MSC1) or anti-inflammatory (MSC2) properties, which may be important for treatment of acute versus chronic inflammation. MSC polarize towards anti-inflammatory MSC2 in a pro-inflammatory environment, whereas polarization towards pro-inflammatory MSC1 occurs in absence of inflammatory cytokines and in response the LPS-TLR4 ligation (49). This difference in MSC polarization may explain the difference between our acute and chronic study in Chapter 4 after MSC treatment. In the acute study, LPS instillations induced a pro-inflammatory environment, resulting in an anti-inflammatory MSC phenotype, which lowers inflammation. However, in our chronic study, repeated LPS instillation may have resulted in tolerance and low levels of inflammatory cytokines, driving the administered MSC towards a pro-inflammatory phenotype. Although the injected MSC were not labelled and therefore could not be traced back to confirm this hypothesis, this is a possible explanation for the difference between the effect of MSC administration in the acute and chronic study.

Furthermore, recently a subpopulation of MSC, the Muse cells, have been described (50, 51). These cells reside within the MSC population, however, they are larger and express the pluripotency marker stage-specific embryonic antigen 3 (SSEA-3). Administration of Muse cells induced repair of injury to various tissues including skin, liver and lung (50). Therefore, it is worthwhile to study the efficacy of Muse cell treatment in a combined model of COPD and CVD in future studies.

### 7.2.3. Potential therapies for COPD patients with CVD

In additional to conventional therapies to prevent and treat CVD, new therapies to lower lipid levels are being developed, and include fibrates, thiazolidinediones, and although still in experimental studies also include liver X receptor agonists and ApoE-mimetic peptides. Furthermore, the effect of HDL-raising strategies, including apoAI-mimetic peptides, niacin and CETP inhibitors, on CVD is also under investigation. Whether COPD features are also
targeted by these compounds remains to be elucidated, however there are indications that they also lower inflammation. Niacin and fibrates for example, in addition to the advantageous effect on lipids, also lower inflammation (52, 53), and thus may lower COPD-related inflammation as well. Details on the mechanism and effect of these compounds on COPD outcome should therefore be studied in the future. As most of the lipid-lowering compounds are already used in the clinic for the treatment of CVD or diabetes, the effect on COPD features can be studies in for example clinical (observational) trials.

Although it is necessary to develop new therapeutic strategies that target both COPD and CVD, one of the first steps in inhibiting disease progression is an adjustment of lifestyle factors. More importantly, engaging a healthy lifestyle may at least reduce and even prevent COPD and CVD. Cigarette smoking is now considered as a negative habit, however was very popular some decades ago. Also, the percentage of smokers is higher amongst young adults and people with a low socioeconomic status. Unfortunately, due to the addictive nature, smoking cessation is difficult. As cigarette smoking is one of the most important risk factors for COPD and atherosclerosis, it should be further discouraged and preferably prohibited. Lately, smoking of e-cigarettes is very popular and is considered ‘safer’ than regular smoking and not until recently, regulation on the age and use of these e-cigarettes have been announced. Despite the fact that it was introduced as a method to quit smoking, it has rather replaced cigarette smoking and may even act as a stepping stone for starting smoking in adolescents. However, whether long-term e-cigarette smoking affects the lungs or the vascular system (but also other organs) is unknown and should be studied in the future.

Both smoking and a Western-type diet (a relatively high intake of carbohydrates and lipids) are associated with increased risk to develop COPD and CVD. Western-type diet intake is associated with an altered intestinal and pulmonary microbiome (54, 55). Chronic CS exposure in mice results in altered intestinal epithelial mucin expression, altered intestinal bacterial community and increased inflammatory gene expression profile (56). Epidemiological studies show that high dietary fibre intake improves lung function and prevents COPD development (57, 58). This effect is mediated through decreased oxidative responses combined with directing the intestinal microbiome towards production of advantageous metabolites. Furthermore, compared to healthy age-matched controls, COPD patients are less mobile, and therefore more inactive. Increasing activity and exercise in COPD patients may contribute to an increased well-being, but also lower circulating levels of pro-inflammatory cytokines and importantly, pro-atherogenic lipoproteins VLDL and (ox) LDL. Physical exercise in mice prior to CS exposure inhibited macrophage infiltration and oxidative damage to lipids and proteins in the lung (59). High lipid intake and oxidative stress induced by CS may also compromise immune cell function and lipid homeostasis in the lung. CS, for example, leads to foam cell formation in the lungs (60). Therefore, increasing exercise combined with a healthy diet may limit COPD and CVD severity and improve quality
of life. Altogether, one of the first steps in the treatment of COPD and CVD is improvement of lifestyle. This can be achieved through increased exercise, healthy diet and smoking cessation. Directly targeting the microbiome may also be regarded as a potential therapy for COPD with CVD in the future.

Finally, currently COPD and CVD are treated as separate modalities (and different specializations). However, COPD treatments may affect CVD and vice versa. Furthermore, optimal treatment of CVD in COPD patients may differ from that patients with a normal lung function. The heterogeneity of the patient population stresses the need for personalized medicine, which is increasingly recognized as being the cornerstone for optimizing treatment. Indeed, due to the complexity of both COPD and CVD, with different severities and comorbidities, in the future personalized medicine for COPD patients with CVD may be most effective.

### 7.3 BAT activation as therapeutic tool for COPD and CVD

Brown adipose tissue (BAT) is now increasingly recognized as an important organ in lipoprotein metabolism. BAT activation may be achieved by several mechanisms, including cold exposure and β3-adrenergic receptor agonism as described in Chapter 5 and 6.

#### 7.3.1. The effect of cigarette smoke on BAT

Smokers have a lower body weight and appetite compared to non-smokers, but a relatively unfavourable lipoprotein profile (e.g. high levels of (ox)LDL and VLDL). CS exposure lowers body weight and food intake, whilst increasing energy expenditure and expression of the classical marker of brown adipocytes uncoupling protein 1 (UCP1) (61-63). CS exposure lowered plasma triglycerides (TG) in wild-type mice, which was even more pronounced in hypertriglyceridemic human APOC1 transgenic mice. CS exposure increased the uptake of fatty acids (FA) from TG-rich lipoprotein-mimicking particles by BAT, suggesting that CS exposure increases BAT activity (Khedoe P., Boon M. et al., unpublished data). The precise mechanism of this increased energy expenditure upon CS exposure is not known precisely. One of the mediators may be nicotine, which regulates appetite and reduces food intake, through increasing levels of the anorexigenic adipokine leptin, whilst lowering levels of the orexigenic neuropeptide y (NPY) (61-63). Nicotine acts by activation of nicotinic receptors in the brain, leading to sympathetic activation, release of norepinephrine, which can bind to β-adrenergic receptors on BAT and induce BAT activation. Furthermore, nicotine can also directly activate nicotinic cholinergic receptors on adipocytes and thereby induce UCP1 expression (64-66). Although nicotine stimulation did not alter expression of genes related to thermogenesis or lipolysis in mouse T37i brown adipocytes, stimulation with CSE
General discussion and future perspectives

causd a dose-dependent decrease in lipoprotein lipase (LPL) expression (Khedoe P., Boon M. et al., unpublished data). *In vivo*, intranasal CSE instillation in *E3L* mice induced a dose-dependent decrease in LPL activity, which may be mediated via nicotine (67). Lower LPL activity may result in attenuated processing and subsequent uptake of TG-rich lipoproteins. Smoking may modify these lipoproteins (*e.g.* oxidation), leading to increased atherosclerosis development. To determine the effect of CS exposure on BAT function and lipoprotein metabolism, future (chronic) studies should be performed with main-stream CS exposure. Because of the difference in lipoprotein profile between humans and mice, it would be more relevant to study the effect of CS exposure on BAT activation and lipoprotein metabolism in both ‘healthy’ smokers and smokers with disease.

Several epidemiological studies show that smokers have a lower body weight compared to matched control non-smokers (67, 68). In general, smokers and COPD patients have a relatively unfavourable lipid profile, with high levels of (ox)LDL and VLDL, even though their body weight may be lower compared to age-matched controls. This may partly be explained by the low LPL activity, which inhibits fat storage in adipose tissue, whereas ROS may modify these lipoproteins, enhancing the development of atherosclerosis. Although in humans, BAT activation after CS has not been shown directly, a study in the Netherlands Epidemiology of Obesity (NEO) cohort showed that resting energy expenditure in smokers is higher compared to individuals who never smoked (69). Future studies on the relation between cigarette smoke, COPD and lung function, and body weight, lipoprotein profile and CVD can be studied well in for example the NEO study. This study is ideal to study the effect of various medications, obesity, inflammation and other parameters. As the subjects in this study are still being followed up, the relation of lipoprotein metabolism, smoking and COPD can also be investigated in humans in more detail. Furthermore, biomarkers of BAT activation, which may be correlated with a favourable lipoprotein profile, may also be taken into account in the NEO study. Exosomal microRNAs (miRNA), which are released from adipocytes, are changed after BAT activation. The exosomal miRNA-92a was found to be negatively correlated with BAT activation and therefore may serve as biomarker for BAT activation (70). Future research is needed to identify new biomarkers for BAT activation, which can then also be measured in clinical studies.

7.3.2. BAT activation as target for CVD

In this thesis we focussed on FA as fuel for BAT activation, where FA derived from TG-rich particles are stored in intracellular lipid droplets in brown adipocytes, and released again upon BAT activation. Uptake of FA from circulating TG-rich particles is thus an important step in replenishment of intracellular FA-stores. In Chapter 5, we showed that BAT takes up FA mostly after lipolysis, by using TG-rich lipoprotein mimicking particles. Although we did not use endogenous particles, the TG-rich lipoprotein mimicking particles closely
represent endogenous TG-rich particles (71). In fact, a recent study has demonstrated that radiolabeled endogenous VLDL is also selectively lipolyzed by LPL in BAT (Hoeke G. et al. unpublished). This process may also occur in humans, where TG-rich lipoproteins are recruited to BAT upon cold exposure enabling LPL-mediated FA release and thermogenesis. TG-rich lipoprotein remnants are probably subsequently cleared by the liver as shown in mice in Chapter 6 and (71). Hoeke G. & Nahon K. et al. (unpublished data) showed that short-term mild cooling of lean young adults leads to an increased concentration of large VLDL particles in serum, possibly through increased hepatic VLDL production, likely serving as substrate for BAT activation. As a result, subjects showed increased levels of small LDL which contained higher levels of cholesterol. Also, for the first time shown, short term mild cooling increased the particle concentration of small HDL, which was accompanied by higher ABCA1-dependent cholesterol efflux in vitro, indicating improved HDL function (Hoeke G. & Nahon K. et al., unpublished data). Bartelt A. et al. also showed that BAT activation through cold exposure and pharmacological stimulation in mice altered HDL composition, leading to increased cholesterol efflux (unpublished data). These data indicate that BAT activation also modulates lipoprotein metabolism in humans and, more importantly, can improve HDL function, which may be beneficial for treatment of CVD. It may be worthwhile in the future to examine whether in humans there is a difference in cold-induced BAT activation and effects on TG-rich lipoproteins and HDL composition and function between healthy subjects and patients with dyslipidemia and/or CVD.

In Chapter 6, we showed that pharmacological BAT activation, by means of a β3-adrenergic receptor agonist in E3L.CETP mice, increased energy expenditure and decreased plasma TG and cholesterol levels. Furthermore, BAT in E3L.CETP mice selectively takes up FA from TG-rich lipoproteins, which facilitates hepatic clearance of the cholesterol-enriched remnants. Most importantly, this all resulted in reduced hyperlipidemia and atherosclerosis. However, the β3-adrenergic receptor agonist did not reduce atherosclerosis and hypercholesterolemia in Apoe−/− and Ldlr−/− mice, suggesting that the hepatic apoE-LDLR clearance pathway should be functional for these effects. In humans, the hepatic apoE-LDLR clearance pathway is functional, opposite to Apoe−/− and Ldlr−/− mice, and therefore, BAT activation may prove to lower atherogenic lipoproteins and atherosclerosis also in humans. Recently, a human randomized controlled trial started in the LUMC to study the effect of the β3-adrenergic receptor agonist mirabegron (72), on BAT activity in South Asians, which have a higher risk to develop diabetes and CVD compared to white Caucasians. If BAT activation in this study leads to favourable lipoprotein profiles and lipids, subsequent studies in patients with CVD can then address the effect of mirabegron on atherosclerosis. Furthermore, in future studies BAT activating agents can be combined with other lipid-lowering strategies to maximize the beneficial effects on dyslipidemia and atherosclerosis.
Besides activation of classical BAT, another way to induce increased energy expenditure may be browning of white adipose tissue (WAT), which occurs e.g. upon β3-adrenergic receptor agonism as shown in mice in Chapter 6. Especially since humans (in developed countries mostly) have high levels of WAT relative to BAT, induction of browning in WAT is an attractive target to lower the incidence and severity of CVD. Finally, whereas BAT visualization generally occurs through invasive and radioactive $^{18}$F-FDG PET-CT scans, it has recently become possible to visualize BAT by MRI (73). Studies are ongoing adapting this technique to measure BAT activity. Such a non-invasive technique can be used to measure BAT activity several times compared to $^{18}$F-FDG PET-CT scans, which can only be done twice per year. Furthermore, MRI scanning is more convenient and safe for the subject or patient as well as the researcher. Furthermore, as described in section 7.3.1, identification of new biomarkers for BAT activation may lead to more convenient ways to measure BAT activity in patients or subjects.

7.4 Concluding Remarks

In this thesis we showed that mouse models are useful platforms to study the mechanisms that contribute to the increased risk of COPD patients to develop CVD. More importantly, these mouse models can be used to develop new combined treatments which target COPD and CVD, and take into account the possibility that optimal treatment of CVD in presence of COPD may differ from CVD without COPD. Although the first step to treatment of COPD and CVD is an overall improved lifestyle, an additional treatment with MSC may lower inflammation, emphysema and atherosclerosis. As COPD patients with CVD have heterogeneous symptoms, it is also important in the future to focus more on personalized medicine to maximize treatment effects. Furthermore, it was recently shown that BAT activation, either through cold exposure or by pharmacological agents, improves the lipoprotein profile in mice and humans, and lowers atherosclerosis in mice. BAT activation, therefore, may in the future be targeted as a therapeutic tool in treatment of CVD. These developments indicate that new and better treatments strategies for patients with COPD and CVD are on the horizon.
Chapter 7

References


9. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease. 2010;Chapter 3: Chemistry and Toxicology of Cigarette Smoke and Biomarkers of Exposure and Harm.


General discussion and future perspectives


