Chapter 1

General Introduction
Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a lung disease characterized by chronic airflow limitation which is generally progressive and associated with enhanced chronic inflammatory responses in the airways and lungs to noxious particles and gases (1). COPD is a leading cause of morbidity and the only chronic disease with ongoing increase in mortality. In 2002, COPD was the fifth leading cause of death, but its prevalence is increasing and it is expected that it will be the fourth leading cause of death in 2030 (2). The worldwide prevalence of COPD in adults aged 40 years and older is 9-10% (3).

The main symptoms of COPD are chronic and progressive dyspnea, cough and sputum production (4). Although COPD is a pulmonary disease, it is highly associated with systemic manifestations and comorbidities like diabetes, cardiovascular disease and skeletal muscle wasting (5,6). COPD is diagnosed by measuring of airflow limitation by spirometry, expressed as the Forced Expiratory Flow in one second (FEV₁) divided by the Forced Expiratory Flow (FVC) (also known as the Tiffeneau index). A postbronchodilator FEV₁/FVC < 0.70 confirms the existence of irreversible airflow limitation and strongly suggest a diagnosis of COPD (7). The disease severity of COPD is classified by the GOLD grading system based on FEV₁ % predicted: stage I (mild, FEV₁ ≥ 80% predicted); stage II (moderate, FEV₁ 50-80% predicted); stage III, (severe, FEV₁ 30-50% predicted); stage IV, (very severe, FEV₁ ≤ 30% predicted) (7).

Although the GOLD classification is generally maintained, it has been demonstrated that different kinds of phenotypes can be recognized. For this reason, assessment of disease severity was reconsidered and a new classification was composed in which besides airflow limitation, also patient’s symptom severity and risk of adverse future events (exacerbation risk) are taken into account (1). In this classification severity stages are summarized in groups A-D as follows: A – low risk (GOLD I-II, 0-1 exacerbations per year), less symptoms (mMRC grade 0-1 or CAT score <10); B – low risk, more symptoms (mMRC grade ≥2 or CAT score ≥10); C – high risk (GOLD III-IV, ≥2 exacerbations per year and/or ≥1 hospitalized exacerbation per year), less symptoms; D – high risk, more symptoms. It was considered that this classification might reflect the complexity of COPD better than solely the use of the classification of airflow limitation in stages I-IV. However, it turned out that the clinical presentation and course of COPD is too heterogeneous to be classified in a two dimensional scale. Furthermore, it was shown that mortality was even better predicted using the GOLD grading system rather than using the new ABCD groups (8).

Currently, COPD is thus still is a complex heterogeneous disease with various clinical expressions, which makes it therefore of importance to combine clinical, physiological, immunological and radiographic parameters for optimal characterization of COPD patients. This may already now, and even more so in the future, lead to better treatment targets to ameliorate disease severity and prevent further progression of the various types of COPD.

Risk factors

Cigarette smoking is the main cause of COPD. It has been shown in multiple studies that smokers show a higher prevalence of respiratory symptoms and abnormal lung function values, accompanied by a faster decline in lung function with higher age as compared to non-smokers.
(9). Of all COPD cases, it is estimated that 80% of COPD patients are smoking related. Although smoking is the most important cause of COPD development, other factors can increase the risk and the prevalence of COPD in non-smokers as well. For example, some genetic factors have been described to underlying COPD, from which polymorphisms in the alpha-1 antitrypsin gene is the first and most well-known factor causing alpha-1 deficiency and thereby increased tissue break down (10,11). Furthermore, bronchial hyperresponsiveness, childhood asthma, impaired lung growth (from gestation until adolescence), passive exposure to cigarette smoke, smoking during pregnancy, and occupational exposures like organic and inorganic dusts and chemical agents and fumes are associated with airflow obstruction and chronic respiratory symptoms (7,12,13).

**Susceptibility to COPD**

Not all of the individuals with the same smoking history will actually develop COPD, i.e. only about 15-20 % of all smokers. It still remains unclear for which reasons COPD is manifested only in this small proportion of smokers. Probably these so-called ‘susceptible’ smokers are more sensitive to the detrimental effects of cigarette smoking than ‘non-susceptible’ smokers. The exact mechanisms underlying this susceptibility are unknown. However, it is likely that genetic background is an important underlying factor. Several family studies have investigated the genetic predisposition in relation to smoking-related COPD. Silverman et al. showed that first-degree relatives of early-onset COPD probands had significantly lower FEV$_1$ and FEV$_1$/FVC values than control subjects, despite similar packyears of smoking (14). In a follow-up study they showed that these relatives also had increased bronchodilator responsiveness compared with controls (15). Another study also demonstrated significant familial risk of airflow obstruction in smoking siblings of patients with severe COPD (16). Interestingly, airway wall thickening and emphysema are independently aggregating within families of COPD patients (17). Furthermore, parental history of COPD is a strong risk factor for COPD, independently of the family history of smoking, personal lifetime smoking, and environmental smoke exposure during childhood (18). Taken together, the combination of smoking and familial COPD occurrence strongly associates with a higher risk to develop COPD. Although a familial risk for COPD may help identifying susceptible smokers for COPD it is not 100% predictive, hence a more discriminative biomarker is still needed.

**Acute effects of cigarette smoking**

To understand the underlying mechanisms of smoking-induced COPD it is valuable to investigate the very first airway responses to cigarette smoke. Several studies have investigated the effects of acute smoking on inflammation and oxidative stress in humans, animals and in vitro models. A few years ago, van der Vaart et al. extensively reviewed the acute effects (<24 h) of cigarette smoking (19). In animal models, alveolar macrophages and neutrophils were increased in lung tissue, whereas mast cells were increased in the airways within 6 hours after acute smoking. In contrast, eosinophils were decreased 6 to 24 hours after smoking. Alveolar macrophages and neutrophils were increased in BALF varying from 1 to 24 hours after acute smoking. Unfortunately, only a few studies are available investigating the
effects of acute smoking in humans. In summary, these studies showed that neutrophils are increased or unchanged in bronchoalveolar lavage fluid (BALF), that there are no effects on the number of monocytes or leucocytes in BALF, and that the local concentration of radio-labelled neutrophils in the lung increases after smoking. Furthermore, there are indications that the epithelial barrier is impaired and fibroblast function decreased. With regard to the systemic compartment, peripheral blood neutrophils were increased, whereas eosinophils decreased.

**Acute smoking in susceptible individuals**

To understand the mechanisms underlying the development of smoking-related COPD it might be attractive to perform an acute smoke model in young individuals who are susceptible to COPD and compare the effects with those in non-susceptible individuals. As mentioned before, young susceptible individuals can be identified by a high familial risk for COPD. Probably, these young susceptible individuals already have abnormal responses to cigarette smoke compared to non-susceptible individuals. Available acute smoking studies have generally investigated old smokers or COPD patients (age >40 years) with a high smoking history. However, in these groups repetitive acute effects of cigarette smoke have already accumulated and thus contributed to structural changes and irreversible lung damage. Therefore, it seems more attractive to investigate the acute effects of cigarette smoking in 'naive' individuals in whom the lungs and the systemic inflammatory component are not yet affected by chronic smoke exposure.

**Inflammation and COPD**

The progressive airflow limitation that occurs in many patients with COPD is accompanied by an increased inflammatory response of the lungs to noxious agents. This airway inflammation persists with aging in COPD. Inflammation is treated with corticosteroids that suppress virtually every step of the inflammatory pathway. However, in contrast with asthma, the majority of COPD patients are less responsive to corticosteroid treatment, even at high doses of inhaled or oral steroids. Inhaled corticosteroids (ICS) have no effects on disease progression or mortality in COPD, but reductions in symptoms and exacerbations were observed in subgroups of patients and health status has been shown to improve after ICS treatment as well (20-22). Besides these clinical beneficial effects, some studies have demonstrated anti-inflammatory effects in some COPD groups as well (23-26). The GLUCOLD study investigated mild-to-moderate COPD patients and found besides a reduction in FEV₁ decline, an improvement in airway hyperresponsiveness, positive effects on health status, and a reduction in inflammatory cells in the airways (23). Bronchial T-lymphocyte and mast cell numbers were reduced after treatment as well as sputum cell counts. Another study showed a reduction in the absolute numbers of biopsy leukocytes (CD45⁺), CD8⁺ cells, and CD4⁺ cells accompanied by decreases in cells expressing genes for the proinflammatory mediators IFN-γ and TNF-α, and a reduced number of neutrophils and eosinophils in sputum (24). Also other studies have confirmed anti-inflammatory effects of ICS treatment assessed in bronchial biopsies of COPD patients, including reductions in CD8⁺ lymphocytes, mast cells and macrophages (25,26). It is not fully understood why there is such a diverse responsiveness to corticosteroid treatment between
different groups of patients and why, despite the depression of inflammation that appears to occur, there is not a clear effect on FEV1 decline and mortality. Several molecular mechanisms contributing to corticosteroid resistance have been described in the literature, including genetic susceptibility, defective GR binding and nuclear translation, transcription factor activation, or abnormal histone acetylation (27). Also cigarette smoking has found to be associated with reduced corticosteroid responsiveness in asthma (28-30), and in COPD (31). Because of the high variety of phenotypes within COPD it is hard to call corticosteroid unresponsiveness as a general characteristic of COPD, especially given the sometimes reported long-term positive responses in a subset of patients, or the beneficial effects in most patients when treating exacerbations. Furthermore, we can speculate on whether corticosteroid unresponsiveness is a characteristic of COPD by being a result of the disease, or that it earlier in life also contributes to the development of COPD.

**Advanced Glycation End products and their receptor**

Advanced glycation end products (AGEs) are a heterogeneous group of compounds that are formed from nonenzymatic glycation and oxidations of proteins and lipids which is irreversible (32). Besides these endogenous pathways, tobacco smoking is an important exogenous source of AGEs formation (33,34). Under normal circumstances, AGEs are slowly formed and they accumulate in the body during aging, however, this process is accelerated in inflammatory conditions and oxidative stress. The best known AGEs are Nε-(carboxymethyl)lysine (CML), Nε-(carboxyethyl)lysine (CEL) and pentosidine. AGEs have damaging effects on tissues in which they accumulate, by altering protein function, cross-linking proteins, and by binding the receptor for AGEs (RAGE) (35,36).

RAGE is a multi-ligand receptor of the immunoglobulin super family and ligand binding activates inflammatory and tissue remodeling processes. RAGE is implicated in the pathogenesis of several chronic diseases including cardiovascular diseases and metabolic and neurodegenerative disorders. Interestingly, RAGE is highly expressed in the lung compared to other organ tissues (37). RAGE exists in two isoforms; membrane-bound (mRAGE) or in a soluble form without the transmembrane domain (sRAGE). sRAGE can be generated through alternative splicing or by cleavage of the cell-bound receptor at the cell surface (38,39). The ligand binding domain between the different isoforms is similar. Therefore it is thought that sRAGE acts as a decoy receptor, preventing the interaction of ligands with mRAGE.

The presence of chronic inflammation and oxidative stress in COPD, local as well as systemic, may lead to increased formation and accumulation of AGEs in COPD. Unfortunately, until now, AGEs are rarely studied in COPD. Only a few studies have shown that accumulation is elevated in lung tissue and plasma of COPD patients (40,41). Importantly, sRAGE levels are decreased in COPD patients (41-45) and there are indications that RAGE expression is elevated in human lung tissue (40,46). These data may indicate an important role for the AGE-RAGE interaction in the pathogenesis of COPD. As AGEs formation is highly associated with smoking, we hypothesize that the AGE-RAGE interaction is increased in ‘susceptible smokers’ thereby contributing to COPD development. Therefore, further research is needed.
Aims of this thesis
In summary, it is of importance to gain more insight in the underlying mechanisms driving the development of COPD in ‘susceptible’ individuals. We already know that familial COPD is an important predictor of COPD development, however, a more discriminative biomarker to establish susceptibility to COPD would be welcome in the field of preventive medicine. The first aim of this thesis is to investigate if there exist differential local and systemic inflammatory responses between young individuals being susceptible or non-susceptible to develop COPD after exposure to a disease-specific challenge; smoking three cigarettes in one hour. Additionally, we examined AGEs, RAGE and their interaction, and corticosteroid (in) sensitivity in smoking and never-smoking young and older healthy controls and COPD patients, providing more insight in the origins and pathology of COPD.

Outline of the thesis
Chapter 2 presents the research protocol of the clinical study investigating acute and chronic effects of cigarette smoking in young and old individuals who are susceptible or non-susceptible to develop COPD. In Chapter 3 results of the acute smoking study are presented in which the local and systemic inflammatory responses to cigarette smoking were investigated in young and old individuals who are susceptible and non-susceptible to the development of COPD. The results of a study on accumulation of Advanced Glycation End-products (AGEs) in the skin of young and old healthy smokers and never-smokers, and COPD patients are presented in Chapter 4. Chapter 5 presents data on AGEs and RAGE in plasma, sputum, bronchial biopsies and the skin of young and old healthy smokers and never-smokers, and COPD patients. In Chapter 6 data of the skin blanching test are presented, investigating corticosteroid sensitivity in the skin of healthy controls and COPD patients. Chapter 7 presents a study on the differential effects of inhaled corticosteroids in smoking and ex-smoking COPD patients. Chapter 8 contains the summary and a general discussion with future perspectives of research.
REFERENCES


