GENERAL INTRODUCTION

The consumption of tobacco has reached the proportion of a global epidemic. Although, smoking tobacco is an unhealthy habit, the number of smokers still increases. The world health organization has predicted that by the year 2030 there will be around 2 billion smokers (http://www.who.int). Tobacco companies often use the argument that smoking benefits the economy. These arguments cannot outweigh the fact that tobacco is the second major cause of death and the fourth most common risk factor for disease worldwide. Smoking has been documented to be a risk factor of multiple cancers, particularly lung cancer and of chronic obstructive pulmonary disease (COPD). Smokers are also at far greater risk of organ damage and cardio-vascular diseases (1-5). Smoking is associated with a more rapid decline of renal function in patients with different renal diseases (6-8). Prospective studies have shown that smokers are more susceptible for developing diabetes (9).

In this thesis we will focus especially on the effects of smoking on mitochondrial function and oxidative stress in airway epithelial cells and discuss the potential of these phenomena in the pathogenesis of COPD.

Pathogenesis of COPD

Many epidemiological studies have shown that smoking is the most important risk factor for COPD (10). COPD is a chronic lung disease that includes two main illnesses: chronic obstructive bronchitis and emphysema. Chronic obstructive bronchitis involves abnormal inflammation, swelling, and excessive mucus production. Emphysema is a lung disease involving damage to the alveoli. Progressive destruction of alveoli and the surrounding tissue that supports the alveoli reduces the elasticity of the lung. In both diseases, there is chronic obstruction of the flow of air through the airways and out of the lungs, and the obstruction generally is permanent and progressive over time.

COPD is not only a disease of the lungs, but also extends to systemic (disease) manifestations. A chronic inflammatory response, with loss of skeletal muscle mass (muscle wasting) and loss of body weight have both been observed in these patients (11; 12). Metabolic data shows that muscles undergo a shift from oxidative to glycolytic metabolism. Under glycolytic action, less adenosine triphosphate (ATP) per mole of glucose is produced when compared to an oxidative metabolism. The functional consequences of these changes are reflected in significant changes in skeletal muscle energy metabolism and accelerated acidification (possible by a mitochondrial dysfunction) of patients with COPD (13). These phenomena are already clear when using an acute cigarette smoke (CS) exposure mouse model, where after smoking for 5 days a significant reduction in body weight occurs when compared to control mice (Fig. 1A, B).

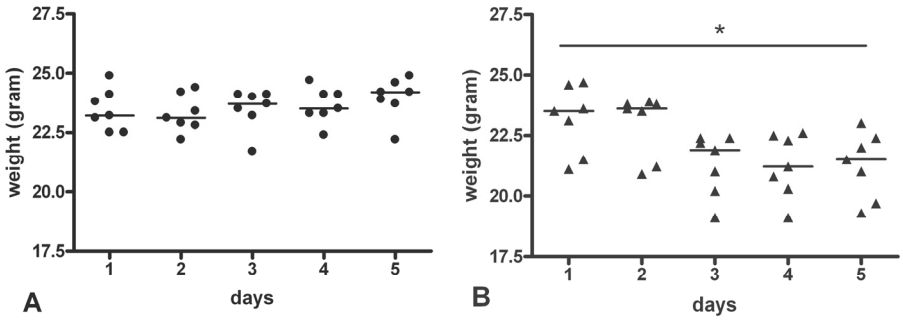

Figure 1. Effect of acute cigarette smoke on body weight in mice. BALB/C mice were exposed to air (A) or cigarette smoke (B) during 5 days. Results are expressed as mean±SEM of 7 mice in each group.
* $P < 0.05$ was considered significant.

Nowadays, it is recognized that COPD is multifactorial in origin, and that many (still unknown) factors play a role. Important factors in the pathogenesis of COPD are:

1) imbalance between reactive oxygen species (ROS) and antioxidants
2) chronic inflammation
3) imbalance between proteases and anti-proteases
4) increased tissue injury and cell death
5) new: disturbed mitochondrial function

CS-induced oxidative stress
Every day our airways are exposed to reactive environmental compounds, therefore the lung is always at risk of oxidative injury (13). To ensure an appropriate defense against this injury the lung is balanced with enzymatic and non-enzymatic antioxidant systems (Table 1).

Enzymatic antioxidant systems	***Non-enzymatic antioxidant systems***
family of superoxide dismutase	Glutathione
catalase	ascorbate (vitamin C)
glutathione peroxidase	α-tocopherol (vitamin E)
glutathione S-transferase	Bilirubin
thioredoxine	lipoic acid
	proteins that have oxidizable thiol groups
	transferring / ferritin
	urate

Table 1. Enzymatic and non-enzymatic antioxidants in normal subjects.

While the adverse effects of CS are well established, there is still an incomplete understanding of the mechanisms by which smoking leads to the development of COPD. CS contains >4000 compounds (14; 15). The smoke can be separated into a gas and particulate phase, whereas many substances are partitioned between these two phases. Both phases contain high levels of reactive components and radicals [1]. In the gas phase high levels of ROS and reactive nitrogen species (RNS) are found. In the particulate phase, highly reactive components like polycyclic aromatic hydrocarbons, aldehydes, phenols, heavy metals and amines are present. These components are candidates that either induce endogenous reactions that produce high levels of ROS or react directly with the antioxidant defenses (16). It is well established that smoking cigarettes contributes to an imbalance between ROS and antioxidant defenses (17). If ROS increase or if antioxidants decrease, oxidative stress will arise.

COPD patients show evidence of increased oxidative stress suggesting that antioxidants may be insufficient to prevent oxidative damage from CS (18). ROS species such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot OH$) are associated with lung injury via several mechanisms as described in table 2. We believe that these species either directly inhaled via the gas phase of CS or endogenously produced in reaction to CS are a major cause of COPD.

Several mechanisms of ROS-mediated damage
damage to lipids, nucleic acids, proteins
depletion of antioxidants
initiation of redox-cycling reactions
enhancement of the respiratory burst in macrophages and neutrophils
inactivation of protease inhibitors
increased expression of inflammatory mediators

Table 2. Mechanisms by which ROS induce lung injury.

Inflammation and oxidative stress

Inflammation is a protective mechanism to remove the causative stimuli as well as initiate the healing process of the tissue. Prolonged inflammation, also called chronic inflammation, leads to a progressive shift in inflammatory cells to the tissue. Airway epithelial cells participate in this inflammatory response seen in COPD. When stimulated with pro-inflammatory mediators like ROS, redox-sensitive transcription factors like NFκB and AP-1 which lead to the expression of inflammatory genes are activated (19). The release of inflammatory mediators such as TNF-α, IL-8 and LTB4 by the activated epithelial cells stimulates the influx of inflammatory cells to the lung. Inflammatory cells release proteolytic enzymes like

[1] Free radicals are highly unstable species with unpaired electrons

neutrophil elastase (16) and also generate oxidants, which further enhance oxidative stress and tissue damage (Fig. 2).

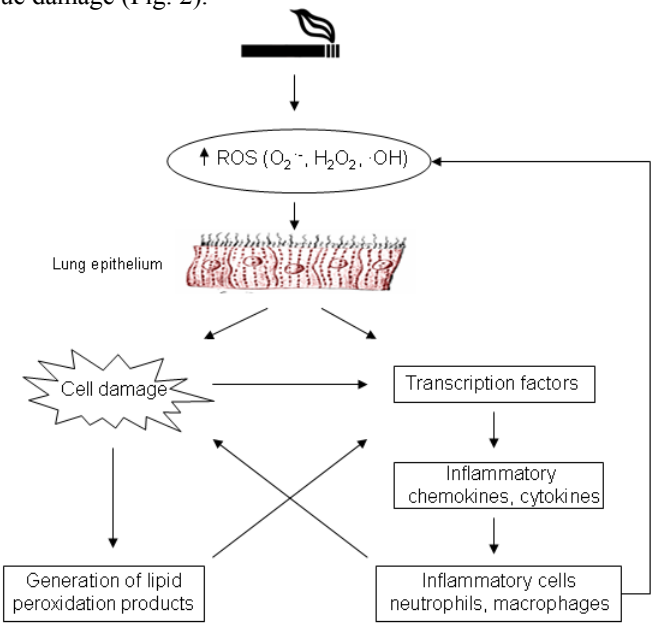

Figure 2. Mechanism of ROS mediated inflammation by cigarette smoke.

Many cells contribute to the inflammatory reaction in COPD and there seems to be a relationship between the severity of the disease and the content of inflammatory cells in the lung. For example the number of macrophages in the airways is increased in COPD. CS activates macrophages to produce and release TNF-α, IL-8, LTB4 and GROα, important chemoatractants for neutrophils. It is well established that in patients with COPD, recruitment of neutrophils and decline in lung function are related (20).

Cell death pathways in COPD

In the airways of smokers, accumulated phagocytic cells are seen at sites of inflammatory tissue injury. Apoptosis is considered to play an important role in effective repair of the injured airway, and in the resolution of inflammation. Apoptosis, or programmed cell death, is a highly regulated process that allows a cell to self-degrade in order to eliminate unwanted or dysfunctional cells. The apoptotic cell will be phagocytosed by macrophages before the cell's contents leak into the surrounding tissue (21). Apoptosis can be triggered in a cell through either the extrinsic pathway or the intrinsic pathway.

In the extrinsic pathway, signal molecules from the TNF family excreted by inflammatory cells, bind to transmembrane death receptors on epithelial and/or inflammatory cells and initiate a cascade of reactions leading to apoptosis. The

intrinsic pathway can be triggered by oxidative stress which results in mitochondrial dysfunction. This pathway is characterized by dissipation of the mitochondrial membrane potential ($\Delta\psi_m$) and transient opening of the mitochondrial permeability transition pore (MPTP) (22; 23). Transient opening of the MPTP causes swelling of mitochondria and release of apoptogenic factors like cytochrome c which initiate together with adenosine triphosphate (ATP) apoptosis. Caspases, belonging to the family of cysteine proteases, are of central importance in both pathways by activating DNases, inhibiting DNA repair enzymes and breaking down structural proteins in the nucleus (24). Finally the cell is fragmented into compact membrane-enclosed structures, called 'apoptotic bodies' which contain cytosol, the condensed chromatin, and organelles. The apoptotic bodies are phagocytosed by macrophages and removed from the tissue without causing an inflammatory response. Because of the tissue damage and airway inflammation present in COPD it can be hypothesized that in stead of the physiological apoptotic response to airway damage, cell death by necrosis occurs leading to secondary inflammation. This is in discrepancy with the severe inflammation seen in patients with COPD. Necrotic cell-death results in loss of membrane integrity, swelling and disrupture of cells. During necrosis, early $\Delta\psi_m$ depolarization, permanent opening of the MPTP and marked decline in ATP synthesis are seen (25). Consequential loss of membrane integrity and release of cellular content into the environment results in damage of surrounding cells and a strong inflammatory response (26). In vitro models of COPD have shown the induction of both apoptosis and necrosis in airway epithelial cells (27-29). Because mitochondria are involved in both cell death processes, it is to be assumed that an inefficient mitochondrial function plays a key role in the development of COPD.

Role of mitochondria in the pathogenesis of COPD

Mitochondria are the energy-producing organelles of our cells. Through oxidative phosphorylation, a series of four enzyme complexes (complex I-IV) transfer electrons and pump protons across the inner mitochondrial membrane. The proton motive force generated is used to drive the synthesis of chemical energy in the form of ATP. ATP is a multifunctional nucleotide that is used in energy-consuming processes like apoptosis. This molecule is the main source of energy in organisms and critical for life. It has been demonstrated that CS can disrupt $\Delta\psi_m$ in monocytes and macrophages in vitro and in vivo (30; 31).

Mitochondria consume approximately 85% of the oxygen during the production of ATP. During normal oxidative phosphorylation, ~4.0% of the oxygen is converted into ROS. Mitochondria are key players in the management of intracellular ROS-metabolism. This is best illustrated by the fact that all major antioxidants are functionally present within the mitochondria. Glutathione, catalase, thioredoxin, superoxide-dismutase and also, as described in chapter 3 heme oxygenase-1 (HO-1), are all linked to mitochondrial function. However when these enzymes cannot neutralize ROS or when exogenous and/or endogenous ROS production increase, oxidative damage occurs. Miro *et al.* showed that the function of complex IV of the electron transfer chain was inhibited in blood lymphocytes of smokers leading to increased production of endogenous ROS (32). Several *in vitro* studies demonstrated that treatment with specific blockers of the electron transfer

chain causes mitochondrial ROS production and alteration in mitochondrial morphology. These observations are similar to the increased levels of oxidative stress parameters and mitochondrial dysfunctions as documented in patients with COPD (12; 33; 34).

A third, very important function of mitochondria, is their crucial role in the regulation of apoptotic cell death through the release of apoptogenic factors (e.g. cytochrome-c, apoptosis inducing factor) in response to specific stimuli (35). In this thesis we show that CS is able to influence mitochondria and alter their role in the regulation of cell death.

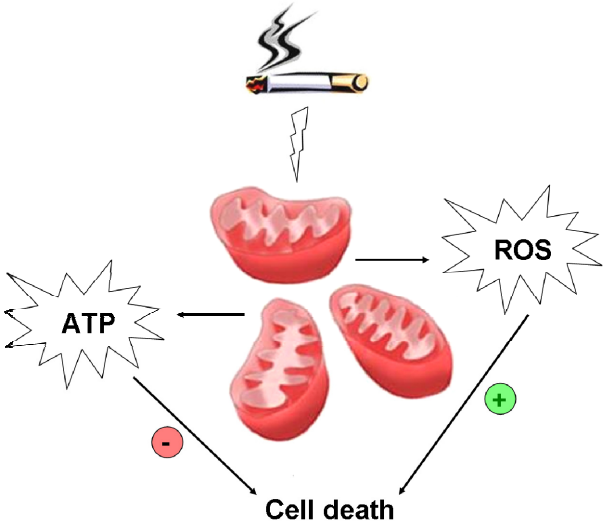

Figure 3. Influence of cigarette smoke on mitochondrial function.

These three -basic for life- features illustrated in figure 3: energy production, ROS management and regulation of cell death make the mitochondria potentially the "powerhouse" of disease (36-38).