# Redox regulation of metabolism in asthma

# Impact

## Background

Asthma is defined as a heterogeneous pulmonary disease, characterized by chronic inflammation, which affects more than 300 million people worldwide (1). The pathophysiology of asthma consists of structural changes in the airways that may induce symptoms including chest tightness, frequent coughs and wheezes as well as airway obstruction together with variable airflow limitation (2). These typical asthma symptoms vary over time and intensity and can affect daily life activities and reduce the quality of life in asthmatic patients. Asthma is generally not seen as a disease with high mortality, but according to the WHO, at least 350,000 deaths are attributed to asthma annually (3, 4). Current available therapeutics target symptoms but do not cure disease, and unfortunately, a significant patient population remains for whom these treatments are not effective. The fundamental causes of asthma are still not fully understood but are likely a combination of genetic profile and external factors. Moreover, it has to be taken into account that every individual is different in terms of their physiological and genetic profile. To better categorize asthmatic patients, several subtypes of asthma exist called endotypes and phenotypes, although it is hard to define patients into endotypes and phenotypes as overlap exists (5). Especially patients with severe asthma do not respond to current treatment, have uncontrolled disease and are hard to define into subgroups. To obtain better therapeutics, it is critical to understand the fundamental causes and the underlying (molecular) mechanisms of asthma pathophysiology. This way, asthmatics can be better classified in order to personalize treatment.

## Research and relevance

Abnormal cellular metabolism is implicated in the pathogenesis of several diseases, including diabetes, cancer as well as multiple chronic lung diseases (6). Metabolomic approaches on blood, and urine revealed that fatty acid and lipid metabolism were affected in asthmatics (7-9), but the metabolic alterations in lung tissues and specifically in epithelial cells, that drive the inflammatory response in asthmatics, remained largely unexplored. The first main objective of this thesis was to examine if alterations in cellular metabolism contribute to asthma pathology and secondly, whether metabolic changes are regulated by changes in the oxidative environment, notably by redox perturbations. The main results of

this thesis demonstrate that increases in the glycolysis pathway, which is a process that involves the breakdown of glucose to extract energy for cell metabolism, contribute to pathophysiological manifestations underlying asthma including increased lung inflammation and worsened lung function. Moreover, our results demonstrate that a pro-inflammatory mediator, Interleukin-1ß (IL-1β), is an important signal that induces these increases in glycolysis during asthma which corresponded with increased levels of lactate, which is an indicative of disturbed cell metabolism and worsened disease. Interestingly, our data displayed that IL-1β was associated with neutrophilic asthma (has been linked to more severe disease), rather than eosinophilic asthma, which is in line with published results showing that IL-1β is an important neutrophil activator (10). Additionally, IL-1β increases the expression of the epithelial cell-derived cytokine TSLP in epithelial cells, which is linked to steroid resistance and severe airway inflammation. This thesis moreover highlights the importance of the glycolysis pathway during asthma by showing that the key, rate-limiting, enzyme of the glycolysis pathway, called Pyruvate Kinase M2, contributes to asthma pathology. Our second objective was focused on Glutathione-S-transferase Pi (GSTP), a redox-based enzyme that is highly expressed in the lung and in epithelial cells, which also functions in cellular detoxification by neutralizing toxic and carcinogenic compounds in our body. GSTP catalyzes an oxidative process that changes the function of proteins, thereby affecting processes including metabolic pathways (11). Interestingly, our results displayed that GSTP worsens asthma by changing cellular metabolism, notably by affecting the glycolysis enzyme Pyruvate Kinase M2.

As mentioned, asthmatics with severe disease often do not respond to current treatments. Severe asthma is associated with increased mortality and hospitalization, reduced quality of life and increased health care costs, and accounts for approximately 5-10% of all confirmed asthma cases in developed countries (12). As this thesis highlights the importance of cellular metabolism in asthma, monitoring changes in cellular metabolism in patients (with severe disease) in the clinic could be useful to gain better insights into the underlying mechanisms and may lead to new and better therapeutics. Results in this thesis showed that the pro-inflammatory mediator IL-1β drives increases in glycolysis in association with increased lactate levels in sputum of asthmatic patients. Therefore, lactate

as well as IL-1β levels in sputum as well as in blood could be non-invasive, quick and reliable biomarkers to identify patients with altered cell metabolism (glycolysis) and improve diagnostics and characterization of patients into endotypes and phenotypes.

Our results moreover highlight the importance of the key glycolysis enzyme Pyruvate Kinase M2 in asthma pathology, and showed that its function can be altered by the redox-based enzyme GSTP. Inhibition of GSTP moreover displayed reduced lactate levels and inflammation from epithelial cells. GSTP and PKM2 are also shown to be present in extracellular fluids including bronchoalveolar lavage and sputum. Therefore, Pyruvate Kinase M2 and/or GSTP could be potential targets for the development of anti-inflammatory therapies and inhibition of glycolysis for the treatment of asthmatics with high IL-1β levels and increased glycolysis.

**Target groups and activity**

Screening for alterations in cellular metabolism will not only be beneficial for asthmatics. It has for instance been shown that inhibition of glycolysis may have therapeutic benefit in lung fibrosis in animal models (13). Inhibition of glycolysis has also been tested in other metabolic disorders such as cancer and inflammatory diseases, and increased IL-1ß levels have been associated with more inflammation in patients with severe persistent allergic rhinitis as well as in COPD patients (14, 15). Moreover, it was found that patients with COVID-19 displayed higher IL-1ß levels in plasma than healthy subjects (16). Moreover, lung immune cells (that also play important roles in asthma) from COVID-19 patients displayed altered metabolism, increased levels of Pyruvate Kinase M2, as well as lactate levels. It is thought that these increases in cellular metabolism contribute to disease pathology, and severity of disease (e.g. more symptoms and hospitalization) and underline the importance of this research.

While there is debate whether asthma cases are increasing or not, it is worrisome that asthma is often underdiagnosed as well as undertreated. The development of asthma is complicated since it is often related to other factors including immunological factors, age, gender and obesity. Obesity-related asthma is linked to more severe disease and up to 30% of obese asthma patients do not respond to steroids (17). According to the CDC, the

prevalence of obesity is increasing and is currently approximately observed in 42,4% of the global population. Aging is another factor that is increasing, and asthma in elderly patients presents itself differently, with higher mortality rates. Therefore, deaths or incidents due to obesity or aging are not always directly linked to asthma. IL-1ß has already emerged as a biomarker in obesity (18), and it is suggested that IL-1ß participates in fundamental inflammatory processes that increase during the aging process (19).

It is important that target groups will be informed about research findings to incorporate potential novel and improved strategies in the clinic. Results from this thesis will be published in scientific journals which are available to a broad spectrum of people. However, patients without a background in science or healthcare rarely have access to or read these articles and they should be informed as well. The connection and communication between scientists and other healthcare professionals including medical doctors and nurses is essential. Moreover, patients including their families, friends and/or caretakers should be informed about novel research in an understandable way via healthcare professionals, health magazines or via (social) media sources. Important is that people with scientific backgrounds communicate the findings on these platforms, as data often is misunderstood or miscommunicated via the media. The Longdagen ('Days of the Lungs') is a conference held in the Netherlands where scientists, doctors, nurses and patients come together and discuss the latest findings regarding lung diseases. More gatherings like these should be organized and advertised as well as routine social meetings with patients, families and/or caretakers and pulmonary professionals including doctors and scientists would be essential to share most valuable information regarding better treatment strategies. Social media platforms such as twitter, linkedIN, and TED talks nowadays also share research outcomes, and improve the dissemination of research. Research funders can be of help to research organizations to develop knowledge uptake skills and promote research communication (20).

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