Use of Electromyography in paediatric asthmatic patients

Master Thesis

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MST Medisch Spectrum Twente
een santeon ziekenhuis

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Abstract

Introduction Asthma is one of the most common chronic inflammatory diseases in children. One of the characteristics of asthma is that patients experience dyspnea due to bronchoconstriction, which can be objectified with spirometry. However, spirometry is not feasible in young, unsettled and/or sick children. Bronchoconstriction results in increased activity of the diaphragm to maintain airflow into the lungs. Electromyography(EMG) is used as an experimental tool to measure the activity of the diaphragm in paediatric patients with asthma, where an increase in diaphragm activity results in an increased amplitude. EMG could provide an easy, passive alternative for long-time continuous monitoring. Respiratory Inductance Plethysmography (RIP) is used to measure changes in thoracic dimensions and can be used as a reference method. In this study, activity of the diaphragm was measured continuously in spontaneous asthma attacks and during provoked asthma attacks as a tool to assess bronchoconstriction.

Methods Children with a known asthma diagnosis admitted to the paediatric out-patient clinic or ward of Medisch Spectrum Twente were measured continuously using a EMG and RIP bands integrated into a tight fitting shirt. Measurements are compared to spirometry measurements, where a drop of 13% or more in FEV_1 was classified as non-controlled asthma. Furthermore, EMG measurements taken during an Exercise Challenge Test (ECT) were analysed between controlled and non-controlled asthma groups. The same analysis was performed after creating 3 groups of equal size by the maximal FEV_1 decrease. EMG parameters were compared to spirometry parameters measured at the same timepoints.

Results Three children with a spontaneous asthma attack were included and measured continuously with the EMG amplifier. Two children also wore the shirt with integrated RIP bands. Respiratory rate calculated from EMG and RIP correlated, if both signals were artefact-free. Obtaining artefact-free signals proved to be difficult in awake children. EMG amplitude increased when lung function decreased.
EMG measurements from 70 asthmatic children after an exercise challenge showed an increase in EMG amplitude after exercise. This increase was significantly higher three minutes after exercise in children with non-controlled asthma (7.31 μV (4.09-11.52)) than in children with controlled asthma (2.70 μV (2.22-5.35)) ($P<0.001$). This amplitude remained higher after 200 μg of salbutamol was given for non-controlled asthma (4.23 μV (2.10-6.13)), while it returned to baseline values for controlled asthma (2.00 μV (1.39-3.02)) ($P=0.001$). Relative changes in EMG amplitude three minutes after exercise showed a moderate relation with the decrease in lung function (Pearson's R 0.639, $P<0.001$). EMG amplitude was still significantly increased 6 minutes after exercise, in subjects with a 24% decrease in FEV_1 after exercise (6.61 μV (4.09-10.60)), compared to subjects with a decrease between 6 and 24% (4.28 μV (2.26-5.61)) ($P=0.005$) and subjects with a 6% or less decrease (2.79 μV (1.92-4.00)) ($P<0.001$).

Discussion These results suggests that EMG can measure respiratory physiology. Continuous EMG measurements proved to be difficult in awake patients. Preferably, EMG could support clinical assessment in sleep.
Increasingly larger elevations in EMG amplitude were found in subjects with increasingly severe bronchoconstriction. Larger increases in EMG amplitude were found in non-controlled asthma, and this remained elevated after the use of bronchodilators, suggesting EMG is a more sensitive tool to measure respiratory function compared to spirometry.

Preface

Before you lies the thesis report to obtain my master's degree in Technical Medicine. I could say it took quite some time for me to finish my studies, but now the moment has finally arrived. In January 2022, I came to dr. Boony Thio and drs. Pascal Keijzer with the weird story of my years prior. I am very grateful to them for giving me the opportunity to prove myself. End March 2022, I started my internship, which marked the end of difficult times for me and the start of a new, exciting period.
From the start, I was eager to partake in the EMG research that is being conducted at the paediatric department. The technique and the opportunity to set up my very own research was the most interesting to me. The path forward was not always paved and straight, but managing the difficulties taught me a lot. It made me realise how I deal with them and how that could be improved.
Working with and for children has inspired me greatly throughout the internship and probably always will. The open-mindedness and honesty of younger children and the stubbornness of adolescents was great fun to negotiate with. During this internship, I felt part of the paediatric asthma care team. They provided me with lots of opportunities to develop myself clinically.

I want to thank my supervisors for their support. They helped me trough tough moments and taught me about paediatric asthma care, medical scientific research and myself.
Boony, thank you for your help and guidance throughout this graduation internship. You were always available for questions and you made me feel part of the team. Thanks to your expertise, I learned a lot about innovation inside a hospital and the walls you have to climb to reach whatever you're trying to achieve. I could come to you and discuss everything, my struggles, my achievements, and especially football.
Frans, thank you for being the critical eye during my graduation internship. You always knew how to inspire me. Due to your guidance and positive feedback, this thesis has gotten better over time. During the last months of my internship you made time in your busy schedule to discuss the contents of this thesis.
Pascal, thank you for you open-mindedness. You taught me all about diaphragmatic EMG. Your passion for the children and research is contagious. Thanks to you, I felt welcome immediately. Discussing crypto, football, anything, you kept it light. During tough times, you supported me and offered help. You still owe me a piece of your famous apple pie though, and I'm still waiting for an invitation to you house warming.
Ruby, thank you for you guidance in my path towards being professional. Your soft touch, sometimes mixed with some well-earned stern remarks, always hit me right where it should. You inspired me during this internship to make work of my professional development. You brought me back to earth when needed and taught me more about myself than I ever could imagine.
Professor Peter Veltink, thank you for taking over the role of chairman of my graduation committee on such a short notice.

My technical medicine colleagues, thanks to you it was a joy to make the journey to Enschede every day. Maybe there was a little too much chit-chat involved sometimes, but serious matters could always be discussed. Taking lunch in the sun, getting an ice cream from Van der Poel and the trip to Frank & Charlie for lunch are only a handful of the fond memories we made together.

Jamie, thank you for supporting me. I know these years have been tough on you and on the both of us. We encountered many obstacles, but we took them. And here we are, me finally graduating, married during this time, looking forwards to the future. Thank you for being there for me when I needed it.

Last but not least, mom, dad, how you put up with me through these years I don't know. You were always there for me. Thank you for being a listening eye and for the occasional well needed advice. This thesis marks the end of an era for us.

I hope you will enjoy reading this thesis.

Ruben Smink

List of abbreviations

ACh	Acetylcholine
AUC	Area Under the Curve
BHR	Bronchial Hyperresponsiveness
CO_2	Carbon dioxide
$D_{L_{O_2}}$	Diffusing capacity of oxygen
ECT	Exercise Challenge Test
EIB	Exercise Induced Bronchoconstriction
EMG	Electromyography
FEV_1	Forced Expiratory Volume in 1 second
FOT	Forced Oscillation Technique
ICU	Intensive Care Unit
IL	Interleukin
MEC-U	Medical research Ethics Committees United
NREM	Non Rapid Eye Movement
O_2	Oxygen
OCS	Oral Corticosteroids
P_A	Alveolar Pressure
$P_{A_{O_2}}$	Partial Pressure of Oxygen in the alveoli
$P_{C_{O_2}}$	Partial Pressure of Oxygen in the capillaries
P_B	Barometric Pressure
R_{AW}	Airway Resistance
REM	Rapid Eye Movement
RIP	Respiratory Inductance Plethysmography
ROC	Receiver Operating Characteristics
SABA	Short-Acting B2-Antagonist
SCN	Suprachiasmatic Nucleus
SWS	Slow Wave Sleep
TMSi	Twente Medical Systems international
$\dot{V}$	Flow
$\dot{V}_{O_2}$	Net diffusion of Oxygen

Contents

Chapter 1

Introduction

Asthma is one of the most common chronic inflammatory diseases in children.[1] Symptoms of asthma include shortness of breath, wheezing and coughing. It is characterised by bronchial hyperresponsiveness to various stimuli, resulting in diffuse airway obstruction. These stimuli include exercise, environmental stimuli such as pollen and external irritant factors such as cigarette smoke. The airway obstruction is reversible, either spontaneously or with adequate pharmacological treatment.[1, 2] One of the characteristics of asthma is that patients experience dyspnea during sleep, which can be burdensome on patients and their families.[3, 4]

1.1 Prevalence of asthma

In 2019, the total prevalence of asthma in the Netherlands was 586.000.[5] This number includes both children and adults. In children aged 1 till 14 in the Netherlands, prevalence of asthma ranges from 6 to 10 percent.[6, 7]

1.2 Control of asthma

The main goal of treatment of paediatric asthma is good control of symptoms and reduction of risks, according to current asthma guidelines.[2] When asthma is poorly controlled, children may experience more asthma symptoms, resulting in a decreased lung function and higher rates of asthma exacerbations. Exacerbations result in a lower quality of life.[8] In Dutch secondary care, approximately 47% of children have poorly controlled asthma.[9] Asthma symptom control can be achieved by the prescription of medication and a change in lifestyle to avoid triggers. Two types of medication can be administered, quick-relief medication and long-term asthma control (maintenance) medication. The maintenance medications should be taken regularly, to prevent the risk of an asthma attack. Furthermore, asthma control can be achieved by reducing the risk of exposure to external stimuli that can induce an asthma attack. Well-known risk reducing factors include minimizing exposure to cigarette smoke or dust mites.[9]

1.3 Asthma exacerbation

If asthma is not controlled effectively, exacerbations can occur. During an exacerbation, the bronchi produce larger amounts of mucus and the bronchial muscles will contract,

resulting in narrowing of the airway.[1] When an exacerbation is not treated adequately or directly, hospitalisation may be required. Exacerbations are often the first presentation of asthma.[2] Exacerbations are mostly treated by administration of quick-relief medications to make sure the airways are open and oxygen flow can be assured. When airflow is still limited after multiple administration of these medications, the use of prednisolone or dexamethasone can be considered. To prevent exacerbations in the future, the use of maintenance medication is highly recommended.[2, 10]

1.4 Assessment of asthma control

Controlled asthma can be distinguished from non-controlled asthma by the use of spirometry during an Exercise Challenge Test (ECT). A decrease of 13% or more in Forced Expiratory Volume in 1 second (FEV_1) after exercise is classified as non-controlled asthma.[2] Spirometry, however, requires participation of the patient and is difficult to achieve in younger children. Furthermore, during an exacerbation, spirometry cannot be measured reliably.[2, 11]

1.5 Asthma and sleep

There is a strong relation between asthma and sleep disorders and quality in children.[4, 12] These disorders are caused by symptoms of asthma occurring during the night. Exact causes are unknown, but it is discussed that bronchial hyperresponsiveness (BHR) and airway resistance increase overnight.[13] This does not have a significant effect in healthy individuals, but can cause symptoms in asthmatic patients. Moreover, children experience difficulty falling asleep and continuing to sleep.[2] An effect of the sleep disorders is that patients experience excessive daytime sleepiness due to sleep deprivation[12]. The combination of poorly-controlled asthma and sleep disorders could be a cause of the lower quality of life experienced by children with poorly controlled asthma.

1.6 Respiratory Inductance Plethysmography

Respiratory Inductance Plethysmography (RIP) is a technique used to measure ventilation using movements of the thoracic and abdominal wall. [14] By applying two elastic bands around the body at the height of the nipple line and umbilicus, expansion of these areas can be measured. [15] The elastic bands contain a sinusoidal wire coil, which is sensitive to longitudinal tension. The elastic bands are connected to a inductive transducer. The inductance of the coil changes when the length is altered, resulting in a frequency change. This change is used to monitor breathing patterns. [16] The use of RIP does not require cooperation of the patient.

1.7 Respiratory Electromyography

Electromyography (EMG) is a tool to measure the electrical activity of skeletal muscles. Muscles consist of multiple motor units, which can be activated and produce an electrical current. These currents themselves cannot be measured. However, the work required to propagate these currents over a distance, the electric potential, can be measured. Muscles

generate these potentials when they contract.[17]
By applying two electrodes bilaterally on the thorax at the height of the diaphragm, EMG can be used to examine breathing patterns in asthmatic children [18, 19]. A ground electrode is used to reference the bipolar electrodes. The EMG signals need to be processed before a respiratory waveform can be observed. Cardiac activity and motion artefacts need to be removed and the signal needs to be smoothed using moving average filtering.[20]
Exercise-induced bronchoconstriction can be identified by surface EMG measurements of the diaphragm.[21] Several parameters derived from EMG are strongly related to FEV_1.
One of the major downsides of using EMG is that it is highly sensitive to disturbances and artifacts. Slight movements of the subject and speech result in large potential differences. This will most likely be a major challenge when EMG is measured during the night, as children will not lay still during sleep.

1.8 Rationale

Spirometry can be challenging during an exacerbation and can not be used reliably in younger children. EMG could be a non-obtrusive, easy tool, that does not require participation of the patient. EMG is used as an experimental tool to measure the activity of the diaphragm in paediatric patients with asthma.[18, 19, 21–23] In current measurements protocols however, subjects need to be stationary, because slight movements could cause data that is cumbersome to process. Movements cause movement artifacts, which are challenging to process. However, during sleep children move far less compared to the wake state.
EMG during the night could give new insights in asthma control. Certain asthma symptoms could be detected and certain EMG parameters could predict changes in breathing patterns. EMG could be used as a simple tool to implement in home monitoring of asthma, especially if electrodes would be wireless.

The main research question is:
Can continuous electromyography of the diaphragm in asthmatic children be used to objectively monitor asthma control?

Secondary research questions are:

- Are continuous respiratory EMG measurements feasible in asthmatic children?
- Are parameters of EMG related to parameters derived from RIP?
- What differences are observed in EMG parameters before and after admittance of medication?

Additional goals

Furthermore, analysis of existing data from EMG measurements during exercise challenge testing is conducted.

The main question is:
Are parameters derived from diaphragm EMG related to changes in spirometry parameters?

Chapter 2

Background Information

2.1 Physiology of normal breathing

One of the crucial components necessary for survival is breathing, which is the primary mechanism that supplies the body with oxygen (O_2) and eliminates carbon dioxide (CO_2). The most important organs used in this mechanism are the lungs. In the smallest bifurcations of the lungs, the alveoli, gas exchange makes sure our body has oxygen available all the time.
Breathing can be substituted in two categories, ventilation and external respiration. Ventilation is transportation of air from outside the body into the lungs. External respiration is the mechanism of gas exchange that occurs in parts of the of the lungs, the alveoli. A schematic overview of the generations of the lungs is given in figure 2.1. [24]

2.1.1 Ventilation

Ventilation is the mechanism of transporting air from outside of the body into the lungs. Air is inhaled via the nose or mouth and flows to the trachea and mainstem bronchi, the first generation of the airways. The trachea is the first part of the so-called conductive airways. No gas exchange occurs in the conductive airways, their main purpose is to move air by convection to regions of the lungs where gas exchange occurs. All airways at which no alveoli are attached, and thus are not participating in gas exchange, are referred to as the anatomical dead space.[24]

Static properties of the lung

Lung volume is determined by the interaction between the lungs and the thoracic cavity. Naturally, the lungs have an inclination towards collapse, due to their elastic recoil. The thoracic wall, however, exerts the elastic recoil in the opposite direction. This result in a balance between the inward elastic recoil of the lungs and outward elastic recoil of the thoracic wall. The lungs and the thoracic wall are divided by the intrapleural space, between the visceral and parietal pleurae. The pressure in the intrapleural space, P_{IP}, is negative compared to barometric pressure. This results in the lungs not collapsing. A schematic overview of the thoracic cavity is given in figure 2.2.

The reason why air flows into the lungs is a negative pressure, compared to the barometric pressure, created in the lungs. This is done by the expansion of the thoracic cavity by muscles associated with breathing. By expanding this cavity, a more negative pressure is created, which allows air to flow into the lungs. Deflation of the thoracic cavity reverses

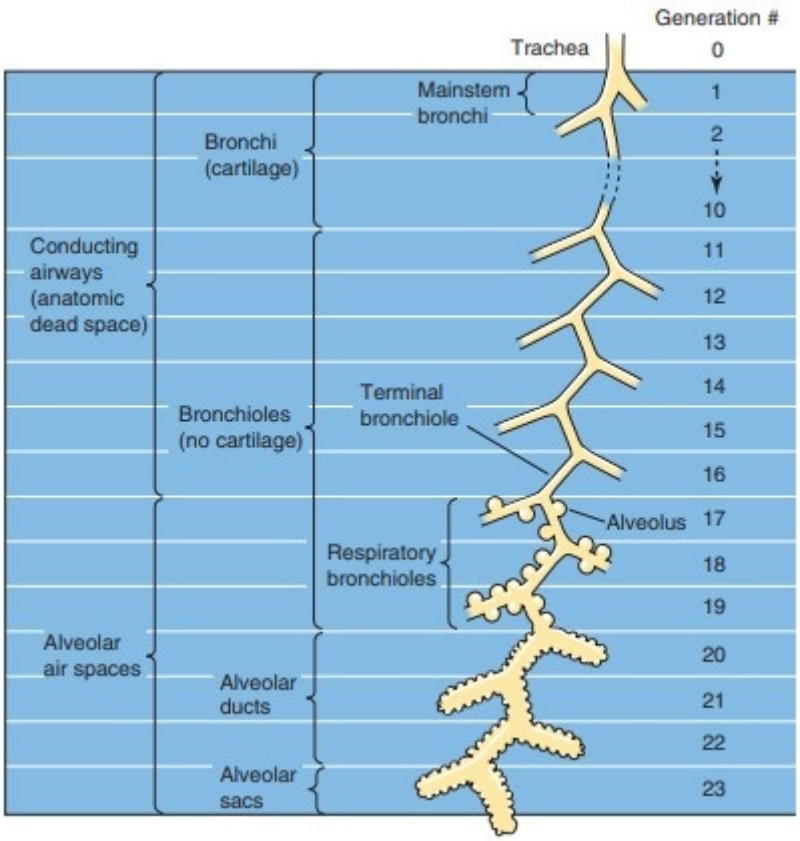

Figure 2.1: Generations of the airways. The airways are divided into conducting airways and alveolar air spaces. Alveoli are present from approximately the 17th generation, the respiratory bronchioles. Adapted from Medical Physiology [24].

this progress, the mechanism we call expiration. [24]
Muscles associated with breathing can be divided into the main respiratory muscles and accessory muscles. The main respiratory muscles are the diaphragm and intercostal muscles. Accessory muscles associated with breathing include the scalenes, the sternocleidomastoid muscles, neck and back muscles and the upper respiratory tract muscles. The main respiratory muscles are responsible for passive, non-forced inspiration. Accessory muscles are recruited during a forced inspiration.
Expiration is often passive. Relaxation of the diaphragm causes the pressure in the thoracic cavity to drop, causing the lungs to deflate, pushing the air out. There are no main respiratory muscles of expiration. Forced expiration can be obtained by contraction of intercostal, abdominal, neck and back muscles, the accessory muscles of expiration.[24] An overview of the muscles associated with breathing can be found in figure 2.3.

Dynamic properties of the lung

When air is flowing into the lungs, a dynamic process, the flow of air from outside the body into the lower airways can be described using Ohm's law, adjusted to parameters specific to the lung:

$$\dot{V} = \frac{\Delta P}{R_{AW}} = \frac{P_A - P_B}{R_{AW}}, \tag{2.1}$$

where $\dot{V}$ equals the flow, ΔP equals the difference in pressure in the alveoli (P_A) and barometric pressure (P_B). R_{AW} is the airway resistance. During inspiration, the alveolar

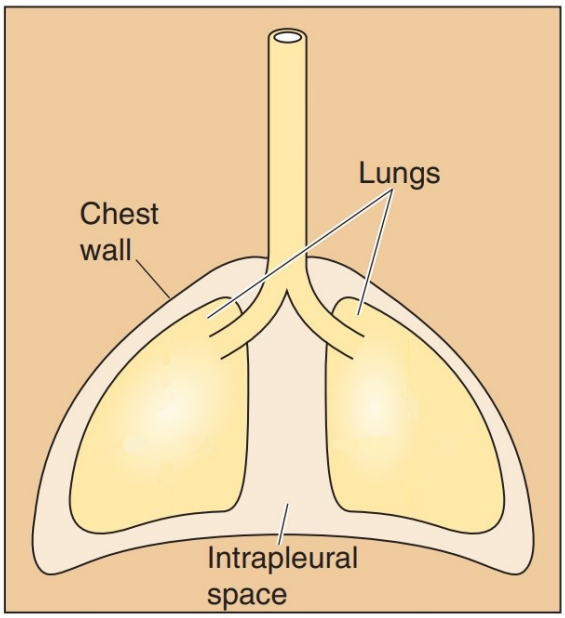

Figure 2.2: A schematic overview of the thoracic cavity. The lungs and thoracic wall are separated by the intrapleural space. Adapted from Medical Physiology [24].

pressure is negative compared to the barometric pressure, creating a negative flow, into the lungs. During expiration, the alveolar pressure is positive compared to the barometric pressure, resulting in a positive flow,
Total lung volume increases with inspiration, causing the pressure in the alveoli to drop below the atmospheric pressure. This increases the flow of air into the alveoli. An increase in the airway resistance will result in a higher ΔP needed to be able to maintain airflow.[24] When airflow is laminar, airway resistance of a singular circular tube can be described using Poiseuille's law:

$$R = \frac{8}{\pi} \cdot \frac{\eta l}{r^4}, \tag{2.2}$$

where R equals the resistance of a tube, η is the viscosity of the gas, l equals the length of the tube and r is the radius of the tube. This equation shows that a change in radius has a great impact on the airway resistance. A greater airway resistance results in a higher need of energy required to move air into the lungs, if the flow is to be maintained.
Airflow in the lungs is almost always laminar, but transitional airflow can occur during high flow in the first few generations of the tracheobronchial tree. Transitional airflow is a flow that contains characteristics of both laminar and turbulent flow. The turbulent flow in airways can mostly exists at bifurcations and in the trachea, which has a larger radius. Furthermore, an extremely high air velocity can occur in the trachea, for example during coughing or exercise. In turbulent flow, the ΔP in equation 2.1 is to be replaced, approximately, with $\sqrt{\Delta P}$, requiring a greater ΔP to maintain flow.[24] Therefore, in transitional flow, a higher ΔP is needed to produce the same airflow as in laminar flow. This means that a reduction in radius in airways requires even more energy to maintain an equal airflow.[24]

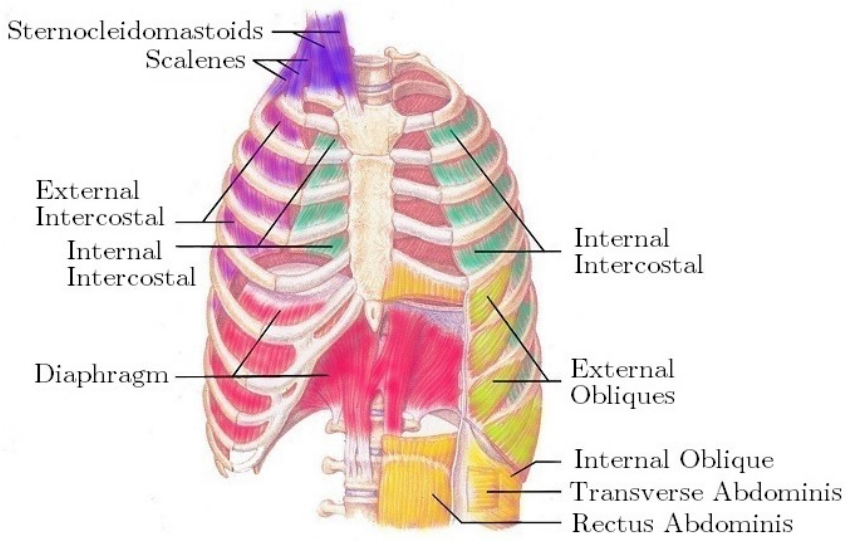

Figure 2.3: Muscles associated with breathing. The diaphragm and intercostal muscles are responsible for quiet inspiration. Accessory muscles are recruited when more force is needed. The muscles expand the thoracic cavity.

2.1.2 Respiration

Respiration is the term to describe gas movement across a membrane. External respiration is the mechanism involved in the gas exchange that occurs in the respiratory airways. This gas exchange occurs from approximately the 17th generation of the airways, the respiratory bronchioles. From this generation of the airways, alveoli are present in the airways, see figure 2.1. The lining of the alveoli is only one cell layer thick, which allows oxygen to diffuse out to the capillaries and carbon dioxide to diffuse into the alveolar spaces. The alveolar lining consists of two types of cells, the type I and type II alveolar pneumocytes, in equal number. However, type I cells are much thinner. Therefore, the surface of alveoli consists of type I cells for 90%. Type II cells are thicker and one of the main functions is to secrete pulmonary surfactant. Surfactant eases the expansion of the lungs by reducing surface tension and increasing compliance, the ability of the lungs to expand and stretch in response to changes in pressure. Therefore, less force is needed to keep the alveoli open.[24]
The flow of gas exchange over the alveolar wall, assuming the alveolar air, blood-gas barrier and pulmonary capillary blood is uniform in space and time, can be described by a simplified and adjusted version of Fick's law:

$$\dot{V}_{O_2} = \underbrace{\left[k\frac{A \cdot s}{a\sqrt{MW}}\right]}_{\mathrm{D}_{\mathrm{L}O_2}}(P_{A_{O_2}} - P_{C_{O_2}}), \tag{2.3}$$

where $\dot{V}_{O_2}$ equals the net diffusion of O_2 from the alveoli to the blood, $D_{L_{O_2}}$ equals the diffusing capacity of O_2, $P_{A_{O_2}}$ is the partial pressure of oxygen in the alveoli and $P_{C_{O_2}}$ the partial pressure of oxygen in the capillaries. The diffusing capacity is described using the first part of the equation, where k equals the proportionality constant describing the interaction of the gas with the barrier, A is the surface area available for diffusion, s is the solubility of the gas, a is the thickness of the barrier and MW is the molecular weight of the gas.

During inspiration, the surface area in the alveoli increases, causing the thickness of the barrier to decrease. As a result, the diffusing capacity increases till a maximum at the end of inspiration. When oxygen is diffused to the capillaries surrounding the alveoli, it binds to haemoglobin in erythrocytes to be transported to the rest of the body. [24]

2.2 Physiology of asthma

Asthma is one of the most common chronic inflammatory diseases in children. It is usually associated with chronic airway inflammation and airway hyperresponsiveness to certain stimuli. Symptoms include wheeze, shortness of breath and cough. Triggers to induce an airflow limitation can be exercise, allergen exposure or a respiratory viral infection. Symptoms may resolve spontaneously or in response to medication and can be absent for weeks or months. On the other hand, patients may experience periodic flare-ups (exacerbations) of asthma that can be life-threatening, if inadequately treated, and may be a significant burden to patients.[2]

2.2.1 Chronic inflammation

Asthma is characterised by chronic inflammation of the airways, due to an overreaction of the immune system to various allergens and/or pathogens. This overreaction stimulates the secretion of mucus, causing the airways to narrow. The reaction of the immune system is characterised by activation of T_H2 cells and inflammatory cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13. These cytokines amplify the inflammatory reaction by recruiting other immune cells such as eosinophils and mast cells. A schematic overview of the immune response can be found in figure 2.4. If the inflammation is not treated, damage to the airway epithelium can occur. This may result in airway remodeling and subsequently difficult-to-treat asthma.[2, 25–28]

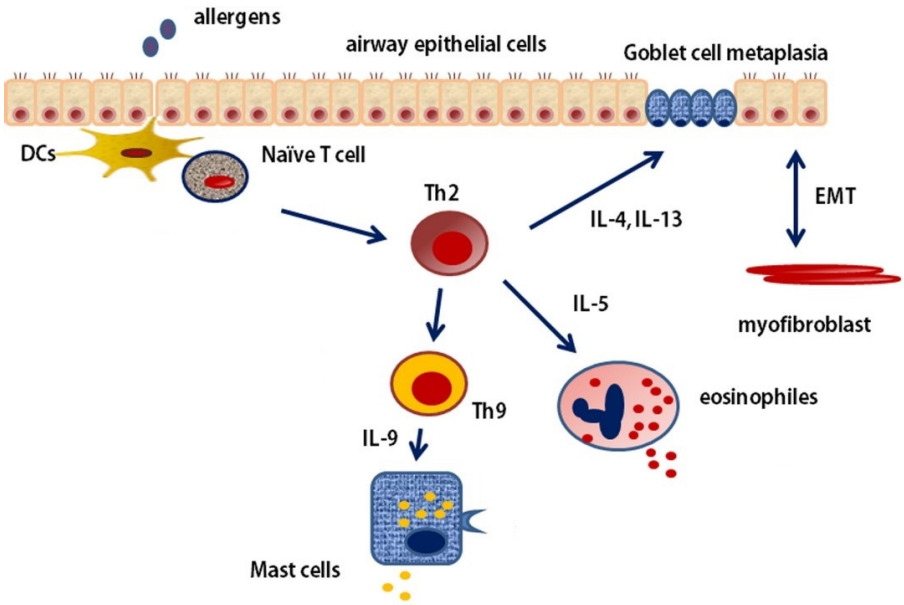

Figure 2.4: Eosinophilic immune response to pathogens involved in asthma. Adapted from [25].

2.2.2 Bronchoconstriction

Another characteristic of asthma is bronchoconstriction, which is also caused by the immune reaction to pathogens. Smooth muscles are found in the wall of the trachea and bronchioles. During exposure to a pathogen, the muscles may contract. An overview of this process is found in figure 2.5. If left untreated, inflammation can lead to hypertrophy and hyperplasia of smooth muscles. This, in combination with increased mucus secretion, results in a reduction of airway diameter. As can be derived from equation 2.2, a decrease in the radius of the airways causes the airway resistance to increase. Therefore, more energy is needed to be able to move air into and out of the lungs.[26]
Bronchoconstriction will result a limited airflow, especially during expiration. This ensues in air being trapped in the alveoli. Subsequently gas exchange will become more difficult, leading to a higher CO_2 and lower O_2 level in the blood (respiratory acidosis). The body responds by increasing breathing and heart rate. Furthermore, accessory muscles of breathing will be recruited. If bronchoconstriction persists, blood gas levels will deteriorate further and muscles associated with breathing will fatigue.[29]

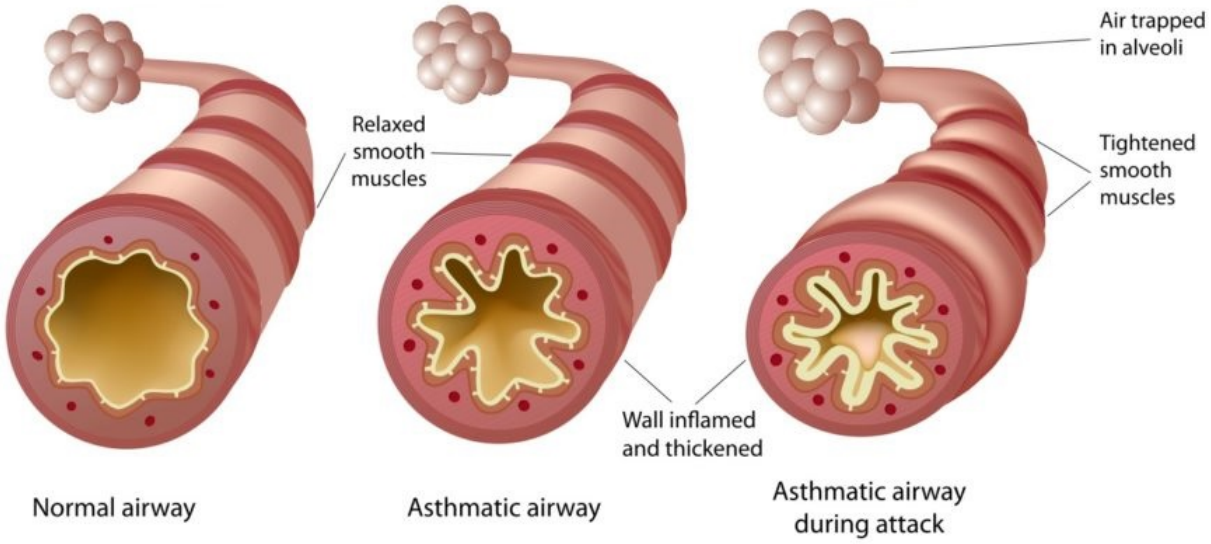

Figure 2.5: Cross sections of the airway. On the left, the normal aspect of the airway is displayed. In the middle, the asthmatic airway is displayed. A inflamed and thickened wall can be seen. On the right, the airway during an asthma attack is displayed. Along with the changes in the wall, the smooth muscles are tightened.

2.2.3 Treatment of asthma

The main long-term goal of treatment in asthma is control of symptoms and reduction of risks of an exacerbation. Control of symptoms in children is mostly achieved by the use of inhalation medication. The goal of this medication is to keep the airways open during exposure to stimuli. Reduction of risks is focused on trying to avoid certain stimuli and treatment of the airway inflammation.[2]

Asthma control medication

An important symptom of asthma is the shortness of breath experienced during bronchoconstriction. Therefore, it is important to relieve the bronchoconstriction and improve

airflow into the lungs. This can be achieved by using bronchodilator therapy, such as inhaled short-acting β2-antagonists (SABAs). One of the most used SABAs is salbutamol. SABA acts by binding to β-2 receptors in the smooth muscle of the airways. This will result in relaxing of the muscle, relieving part of the bronchoconstriction. This makes sure, airflow through the airways can be maintained, and gas exchange is not hindered. SABA act as symptom control medication, but do not act on the chronic inflammation that precedes smooth muscle contraction in asthma.[2, 30]
To be able to treat asthma, it is therefore essential to treat the chronic inflammation. This can be done by inhaling corticosteroids. Corticosteroids suppress T_H2-type cytokines, such as IL-5 and IL-13. Furthermore, the amount of inflammatory cells, such as eosinophils, lymphocytes or mast cells, will be reduced. Reducing the chronic inflammation results in a reduced reaction to pathogens and less bronchoconstriction due to a normal mucus secretion.[31]

Asthma exacerbation treatment

Exacerbations are longer periods of progressively increasing symptoms and decreasing lung function. They may occur with a pre-existing diagnosis, but are often the first presentation of asthma. Exacerbations may occur in response to exposure to external stimuli or due to poor medication adherence. Exacerbations can be severe and cause respiratory distress and, if not treated properly, asthma-related death.[2]
Exacerbations are usually treated self-management using an asthma action plan. This plan includes a regime with repeated administration of SABAs. If there is no improvement on the shortness of breath with the use of SABAs after repeated administration, a course of oral corticosteroids (OCS) can be described. This is usually prednisone, which is described for 3 to 5 days.[30]
If no improvement is seen on OCS and the action plan, or if the exacerbation is severe, resulting in a drop in blood oxygen levels, admission to a hospital might be necessary. If saturation levels are below 95% extra oxygen is administered via nasal cannulae or mask. Furthermore, nebulised SABAs can be administered, with nebulisation in a fixed time interval. In the case of life-threatening asthma, intubation and admission to the intensive care unit (ICU) might be necessary.[2]

2.3 Physiology of sleep

Sleep is a crucial mechanism of our body. The exact purpose of sleeping is unknown, but it is assumed that it is related to restoration in several biological processes. During sleep, hormones are released that help restore and repair damaged cells. Furthermore, sleep has an important function in certain brain functions. Memory consolidation and emotional regulation are examples of functions that are related to sleep and can be impaired if sleep is not sufficient.[32]
The sleep-wake cycle is regulated by the circadian rhythm. The circadian rhythm is the body's natural clock. It is influenced by external stimuli, such as temperature, exercise, and, most important, light. The circadian rhythm is regulated by the suprachiasmatic nucleus (SCN) in the brain, which regulates the release of hormones such as melatonin and cortisol. If light is detected, the release of these hormones is suppressed and the body stays awake. If signals of darkness are detected by the SCN, the hormones are released, which initiates sleepiness. The circadian rhythm has an influence on several physiological

processes, such as body temperature and hormonal secretory patterns.[32]
Sleep can be divided into two components, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep is characterised by reduced muscle activity and a decrease in cardiovascular and respiratory functions. This is mainly due to a change from neural to metabolic respiratory control.[33] NREM sleep is further divided into 3 stages, stage N1, stage N2 and stage N3. Stage N1 determines the onset of sleep and is characterised by slow eye movements and a slowing of the brain function. Usually, 5-10% of total sleep time is spent in this stage. Stage N1 is followed by stage N2. In this stage, brain function slows down even further, eye movements stop, the body temperature drops and heart rate and respiratory rate become regular. About 50% of total sleep time is spent in this stage. The last stage of NREM sleep is stage N3, which is also known as slow wave sleep (SWS). This is the deepest stage of NREM sleep. During this stage, the body repairs and generates tissues, builds bone and muscle and strengthens the immune system. Stage 3 sleep contributes for 15-20% to total sleep time.[32] REM sleep is characterised by rapid eye movements, muscle atonia and an increased brain function. During REM sleep the brain has an increased glucose consumption compared to wakefulness, while it is decreased in NREM sleep. Furthermore, blood pressure and heart rate become variable. During REM sleep, body temperature regulation is absent, due to the muscle atonia.[33]

2.3.1 Breathing during sleep

During NREM sleep there is an absence of important drives from the neuronal networks associated with breathing during wakefulness. This results in a decrease in respiratory rate, while tidal volume does not change.[32] Therefore, ventilation is reduced slightly in sleep, which increases arterial CO_2. Because neural regulation is reduced, metabolic regulation takes over. Drops in CO_2 can result in apneas during sleep. Furthermore, upper airway resistance increases during sleep. This is addressed to a reduced activity in the nervus hypoglossus, which innervates the genioglossus muscle. The reduced activity is primarily found in NREM sleep and activity may be absent in REM sleep. There is a difference in oral or nasal breathing. Oral breathing increases the upper airway resistance compared to nasal breathing. This difference occurs in sleep but is absent in wakefulness.[33, 34]
During sleep, general muscle activity is decreased significantly.[33, 35] Intercostal muscle activity, however, increases during NREM sleep, because of an increase in airway resistance. During REM sleep this activity is absent, parallel to general motor muscle function. Tonic muscle activity of the diaphragm is also reduced, but phasic activity is not inhibited in sleep. This is vital to be able to maintain respiratory function during sleep.[35]
The changes in breathing during NREM sleep result in a small reduction of $\dot{V}$ as described in equation 2.1 and $\dot{V}_{O_2}$ as described in equation 2.3. During stage N3 sleep, $\dot{V}$ decreases compared to stage N1 and stage N2. Changes of the airway flow and airway resistance during NREM sleep result in a lower partial pressure of O_2 in the alveoli and capillaries.[35]
In REM sleep, $\dot{V}$ and $\dot{V}_{O_2}$ are irregular due to the irregular nature of breathing in this stage.[35] There is more variation in RR and, therefore, ventilation in REM sleep, compared to NREM sleep.[32] Variations are substantional and may result in either tachypnea or apneas.[35]

2.4 Asthma and sleep

As described above, there are changes in breathing while sleeping, resulting in a changed respiratory physiology. Asthma, on the other hand, allows little change in respiration without implications. This results in a strong correlation between non-controlled asthma and sleep disorders and quality in children. [10,11] Nocturnal asthma symptoms include shortness of breath, coughing, wheezing and nighttime awakenings.[36]
Lung function has been known to fluctuate throughout the circadian cycle, with peak lung function at 16.00 hrs and minimal lung function at 4.00 hrs. These fluctuations are present in healthy individuals, but are more pronounced in patients with asthma.[37, 38] Furthermore, the airway is more hyperresponsive at this time and response to bronchodilator therapy is reduced.[38]
A possible trigger for bronchoconstriction in asthma during the night is body temperature. In asthma, cold air could be a trigger for bronchoconstriction. During the night, the body temperature drops, which also results in airway cooling, along with a cooler environment. This could play a significant role in triggering nocturnal asthma.[38, 39]
Furthermore, there is a variation in airway inflammation during the circadian cycle. It has been hypothesised that this is due to the variation in hormones during this cycle. However, the specific mechanism associated with these variations is unknown.[37, 38]

2.5 Principles of electromyography

Muscles contract following a small electrical current. These currents cannot be measured, but the potential difference between two points can be measured. Electromyography (EMG) is a technique to measure these electrophysiological potentials in muscles.

2.5.1 Bio-electricity

The source of the electrical current in muscles can be found along the cell membrane. A potential difference, the membrane potential, exists over the cell membrane, which is regulated by the concentration of specific ions in the intra- and extracellular matrix. The most important ions are sodium (Na^+), potassium (K^+) and chloride (Cl^-). The membrane potential can vary for specific cells in the human body. In rest, there is a difference of approximately 70-90 mV in skeletal muscles, where the intracellular matrix is negatively charged relative to the extracellular matrix. In this state, the cell is polarised. In rest, when no electrical current is present, the membrane potential can be described as follows:

$$V_m = \frac{RT}{F} ln \left(\frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o} \right), \tag{2.4}$$

where R is universal gas constant, T is the temperature in Kelvin, F is the Faraday constant, P_X equals the permeability coefficient of ion X and $[X]$ equals the concentration of ion X where subscript o denounces the concentration in the extracellular matrix and subscript i the concentration inside the cell.[24, 40]
Without a trigger, most cells will stay in this polarised state. The process of the cell reversing membrane potential from negative to positive is called depolarisation. The equilibrium is achieved again by repolarisation. In muscle cells, depolarisation occurs

when neighbouring cells depolarise. By depolarisation of neighbouring cells, the membrane potential decreases. When this reaches a certain threshold, the cell itself will also depolarise. Depolarisation is achieved by opening of voltage-gated Na^+ channels, causing an influx of sodium ions into the cell, resulting in a more positive intracellular charge. This leads to the opening of more ion channels, causing the rapid reversal of the membrane potential, resulting in an action potential.[17, 24, 40]

Bio-electricity in muscles

Skeletal muscles consist of elongated cells, called muscle fibers. These muscle fibers contain a parallel array of cylindrical elements called myofibrils, which is a chain of repeating units called sarcomeres, divided by Z disks. Sarcomeres consist of thin and thick filaments. The thin filaments, consisting primarily of actin molecules, originate from the Z-disk. Next to the actin, thin filaments consist of troponin and tropomyosin molecules. The thick filaments, consisting of multiple myosin molecules, lie between individual thin filaments. An overview of the different molecules in a myofibril can be found in figure 2.6. The head of these molecules bind along actin of the thin filaments. When these heads are activated, they slide along the length of the actin molecule and bind to another binding site, causing the sarcomeres to shorten.[24]

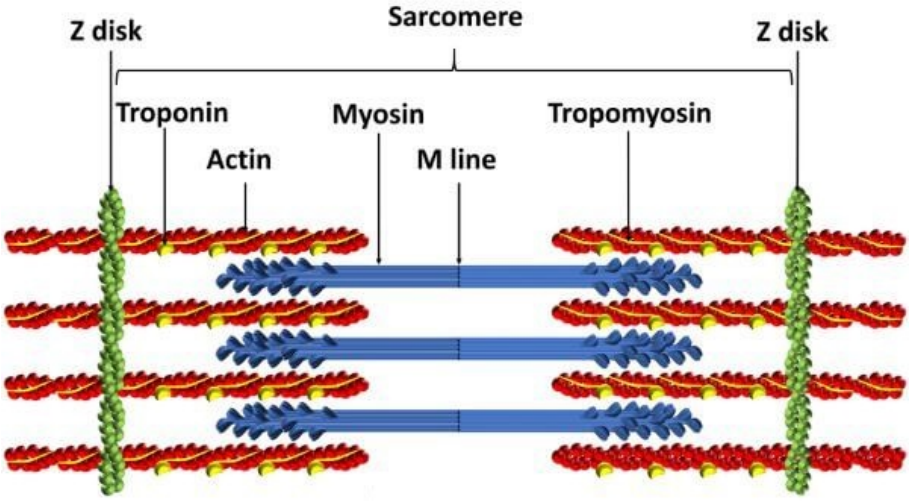

Figure 2.6: Schematic depiction of a sarcomere. The actin filaments are connected to the Z-disks. The myosin filaments can slide along the actin filaments to shorten the sarcomere, causing a muscle contraction.

In muscles calcium (Ca^{2+}) is an important ion, next to the ions described in the previous section. When a muscle cell depolarises due to its neighbor depolarising, ion channels are opened and Ca^{2+} enters the cell along the concentration gradient. Because of the rise of Ca^{2+} concentration in the cell, the calcium regulatory proteins, troponin and tropomyosin are activated. This makes sure myosin heads can interact with actin filaments, allowing them to slide towards the center of the sarcomere.[24]
Muscle fibers cannot generate their own action potentials, but can be activated by a motor nerve axon, which contacts the fiber to form a synapse called neuromuscular junction. In the neuromuscular junction, acetylcholine (ACh) is released from the motor nerve terminal. This ACh binds to nicotinic receptors on the muscle fiber, which depolarises the membrane at this site. When the threshold is met, an action potential is triggered.[24]

2.5.2 Potential differences

The small electrical current generated to contract muscles cannot be measured. However, the difference in charge between two points can be measured. This is called the potential difference, which is defined as the work required to move a unit of charge from one point in a space to another.[40]

2.5.3 Surface electromyography

Surface electromyography (sEMG) can be used to non-invasively measure potential differences in muscles. [17, 20] Electrodes are placed on skin above the muscle of interest, measuring underlying action potentials. These potentials are measured in reference to a reference electrode placed in an inert location on the body.[17, 40] When using sEMG, muscle activity is measured not directly at the source, but at a distance. This results in an electrical current measured at the electrode that consist of multiple potential differences from underlying muscle cells. In relation to the linearity of conductive tissue, this is called the superposition principle. This principle states that the electrical current measured at a distance is the sum of the potential gradients of individual sources:

$$F(a+b) = F(a) + F(b) \tag{2.5}$$

$$F(ab) = aF(b) \tag{2.6}$$

In practice, the measured signals from sEMG are the sum of all electrical activity in the underlying tissue.[40]

2.5.4 sEMG of respiratory muscles

Surface electromyography is used as an experimental tool to measure activity of respiratory muscles.[18, 19, 21–23] Muscles associated with breathing can be measured transcutaneously using adhesive electrodes. Muscles primarily measured are the diaphragm and intercostal muscles.[41] sEMG can be used to obtain respiratory waveforms in a clinical setting, and is used to examine breathing patterns in asthmatic children. [18, 19, 21]. One of the challenges of EMG in general and respiratory sEMG in particular is the sensitivity to disturbances. Due to the superposition principle described in the section above, other electrical signals are also recorded. An example is the signal from the heart, which has a great influence on the sEMG signal, which hinders interpretation of respiratory signals. [20]

2.6 Respiratory Inductance Plethysmography

Respiratory Inductance Plethysmography (RIP) is a non-invasive technique to measure respiratory function monitoring changes in thoracic and abdominal dimensions. RIP consists of two elastic bands, one around the thorax and the other around the abdomen, containing a sinusoidal wire coil with an alternating current. Changes of the thoracic or abdominal cross-section alters the inductance of these wire coils. This change of inductance is measured by RIP. [16]

Chapter 3

Methods

3.1 Continuous EMG recordings

A study was performed at the paediatrics ward of Medisch Spectrum Twente (MST) in Enschede. The proposal of this study was reviewed and approved by the Medical Research Ethics Committee United (MEC-U) and can be found in Appendix B.

3.1.1 Patient inclusion

The study was performed at MST in Enschede. Children aged 4 and above with paediatrician diagnosed asthma or BHR that are being admitted to the paediatrics ward or assessed at the outpatient paediactric clinic, were asked to participate. Inclusion and exclusion criteria are given in Table 3.1.

Table 3.1: Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Paediatrician diagnosed asthma or BHR	ICD or pacemaker
Ages 4 - 17	Admittance to ICU
	Parents and/or child cannot understand or speak Dutch
	Patient does not fit in one of the available Hexoskin shirts

Sample size calculation

Sample size calculation is based on results from Keijzer et al.[21]. Because data from this study has a non-normal distribution, non-parametric tests needed to be used to calculate a sample size.
Sample sizes are based on the difference in EMG peak height at lowest spirometry. Alpha was set to 0.05 and beta to 0.90. A higher statistical power was chosen because the study is performed on children, urging a higher certainty.
The mean peak EMG height difference for subjects with uncontrolled asthma at lowest spirometry was 4.85 μV, with an estimated SD of 4.95 μV. Calculation revealed that 15 subjects were required for significant statistical results.
A higher sample size of 20 was chosen because the measurements taken during this study would not guarantee a subject has an asthma attack during admission.

The sample size calculation has been discussed with and was conducted with the aid of one of the epidemiologists at MST.

3.1.2 Measurement devices

EMG measurements were performed using the Mobi-6 (Twente Medical Systems International B.V. (TMSi), Oldenzaal, The Netherlands), a portable amplifier. This amplifier was chosen because of its high input impedance. A ground lead and a bipolar lead were connected to the amplifier. The leads were attached to Ag/AgCl adhesive electrodes (3M, Saint Paul, Minnesota, USA). Data was transferred wirelessly to a computer and processed in Matlab (Version R2022a, The Mathworks Inc., Natick, Massachusetts, United States). RIP measurements were performed using a Hexoskin shirt (Carré Technologies Inc., Montreal, Canada). This is a stretchable shirt with built-in sensors that is commercially available. These sensors include two RIP belts, that are used to monitor respiration. These belts are positioned at the height of the thorax and abdomen. Data was anonymously synchronised to a server and was downloaded for further analysis. To be able to accommodate for different body sizes, 3 sizes Hexoskin shirts were used, Junior 2XS, Junior S and Junior L.

3.1.3 Measurement procedure

Children with paediatrician diagnosed asthma or BHR that were examined at the paediatrics ward or outpatient clinic of MST were eligible to participate in the study. The EMG amplifier was placed next to the patient. The ground electrode was positioned on the sternum or connected to a wristband. The bipolar leads were positioned bilaterally at the midclavicular line on the diaphragm. The Hexoskin shirt was also put on by the patient. Figure 3.1 gives a schematic overview of the electrode and belt positioning. The black dot depicts the ground electrode when placed on the sternum, the red dots depict the bilateral electrodes. The wristband is not depicted in this overview.
An overview of the steps in the measurement procedure are:

1. Patient was eligible for participation
2. Electrodes were positioned on the patient and leads were connected to the amplifier and Hexoskin shirt was put on
3. Measurement was started, no additional instructions were given
4. Measurement ended when the subject was discharged

3.1.4 Data analysis

Data extracted from the EMG amplifier was saved using a Matlab algorithm. Further data analysis was performed using Matlab. ECG artefacts were removed using a method based on an algorithm provided by TMSi. P- and T- waves were removed using high-pass filtering. QRS-complexes of the ECG were detected using a detection algorithm. The QRS-complexes were removed from the signal and replaced by a running average. The signal was smoothed using two moving average filters to obtain the breathing wave patterns.
Data extracted from the Hexoskin shirt was processed using Matlab. The respiratory

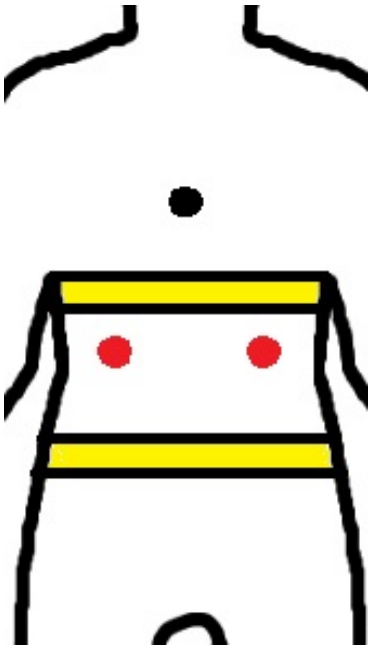

Figure 3.1: Schematic overview of electrode and belt positioning. The black dot depicts the ground electrode, the red dots depict the bilateral electrodes. The yellow bands depict the RIP belts, integrated in the Hexoskin shirt. The wristband is not depicted.

rate was calculated and compared to the respiratory rate calculated from the EMG data. When this respiratory rate correlates, it can be reasoned the EMG signals originate from the diaphragm.
Furthermore, epochs containing movement artefacts were removed.

3.1.5 Signal analysis

Parameters EMG amplitude, Area under the Curve (AUC) and respiratory rate were calculated.
The amplitude was calculated by detecting minima and maxima in the respiratory signal. The mean difference between a maximum and its preceding and following minima is the amplitude of one breath. A mean amplitude was calculated for every epoch. This results in the following calculation,

$$EMG_{Amp} = \frac{(EMG_{max} - EMG_{min1}) + (EMG_{max} - EMG_{min2})}{2}, \tag{3.1}$$

where EMG_{Amp} equals the EMG amplitude of one breath, EMG_{max} is the maximum, and EMG_{min1} and EMG_{min2} the minima preceding and following the maximum.
Area under the Curve was calculated by integration of the respiratory signal from one minimum to the following. The area under the line between the two minima was substrated from this integration. This results in the following equation:

$$AUC = \int_{min1}^{min2} f(t)dt - \int_{min1}^{min2} g(t)dt, \tag{3.2}$$

where $f(t)$ equals the respiratory signal and $g(t)$ equals the line between the two minima.
Respiratory rate was determined by the difference between the minima.

3.2 EMG measurements during elective ECT

3.2.1 Measurement procedure

EMG measurements were taken alongside a standard ECT. This ECT is an elective test to measure the effect of exercise on lung function. Bronchoconstriction measured during this test is classified as Exercise Induced Bronchoconstriction (EIB).
The ECT consists of spirometry and forced oscillation technique (FOT) measurements and a 6 minute exercise. The following steps were taken during this test:

- Spirometry and FOT measurement at baseline
- Exercise on a treadmill for 6 minutes in an air-conditioned room
- 1 minute after exercise: spirometry measurement (T1)
- 3 minutes after exercise: spirometry measurement (T3)
- 5 minutes after exercise: FOT measurement
- 6 minutes after exercise: spirometry measurement (T6)
- Admission of 200 μg of salbutamol
- 5 minutes after salbutamol: spirometry and FOT measurement

This standard protocol can be extended with spirometry measurements at 9, 12, 15 and 18 minutes after exercise. This is done when the FEV_1 at 6 minutes is decreasing compared to previous measurements. The protocol ends when the FEV_1 measured at a certain timepoint is improving over the previous timepoint.
The EMG measurements were taken before every spirometry step in the protocol. The test subject was asked to sit still for 45 seconds, with hands on their knees. No specific instruction was given on breathing.
Measurements were performed using the Mobi-6, as described above. The only difference with the procedure described above was that the ground electrode is always attached to a wristband instead of the adhesive electrode on the sternum.
Furthermore, patient characteristics age, sex, height and weight were collected.

3.2.2 Data analysis

Raw measurements were taken from the database and processed using Matlab as described above. EMG parameters were calculated at every timepoint: baseline, 1, 3 and 6 minutes after exercise and after salbutamol. This was done for every epoch by calculating the height, width and AUC per breath and taking the mean over all breaths.

3.2.3 Statistical analysis

Statistical analysis was performed using SPSS (Version 28, IBM Corp., Armonk, New York, United States). Patient characteristics and EMG parameters were tested for normality using Shapiro-Wilk test.
All parameters were divided into two groups, controlled and non-controlled asthma, as

classified by the outcome of the ECT. A FEV_1 decrease of 13% or more was classified as non-controlled asthma. Furthermore, parameters were divided into three groups equal in size, each characterised by a specific range of FEV_1 decrease. This is done to be able to distinguish by severity of the asthmatic reaction.
For all statistical tests, a level of significance of 0.05 was chosen.

Patient characteristics

Patient characteristics were statistically analysed to investigate whether the controlled and non-controlled asthma groups were equal. Normally distributed parameters were tested using an independent samples T-test. Not normally distributed parameters were tested using the Mann-Whitney U test. Furthermore, the patient characteristics of the 3 groups divided by FEV_1 decrease were tested using an ANOVA test if the parameters were normally distributed or a Kruskal-Wallis test if the parameters were not normally distributed. If the groups were found to be statistically significantly different, differences between the individual groups are tested using T-tests or ANOVA tests, for normally and not normally distributed data respectively.

Comparison between controlled and non-controlled asthma

EMG parameters were statistically analysed at every timepoint between controlled and uncontrolled asthma patients. Furthermore, every parameter was compared to its baseline. If the parameters were normally distributed, independent samples T-test would be applied. If parameters proved to be not normally distributed, Mann-Whitney U tests would be applied.

Comparison between 3 groups divided by lung function decrease

Statistically significant differences in parameters between the 3 groups were tested using an ANOVA test if parameters were normally distributed and a Kruskal-Wallis test if parameters were not normally distributed. If these differences were found to be statistically significant, differences between individual groups were analysed. For this analysis, a T-test was used if the data was normally distributed and a Mann-Whitney U test was used if the data was not normally distributed.

Comparison between spirometry and EMG parameters

Absolute and relative differences of EMG parameters were calculated for the timepoints of lowest FEV_1 from spirometry, maximal change in EMG parameters and at T3, 3 minutes after exercise. Linear regression models were computed, and coefficients of determination obtained for these differences. Furthermore, receiver operating characteristic (ROC) curves were computed with varying cut-off values of these parameters. These ROC-curves are computed with 13% and 30% decrease in FEV_1 as determination of good and bad outcomes.

Chapter 4

Results

4.1 Continuous EMG measurements

Three subjects were included in this study. Table 4.1 provides an overview of patient characteristics.

Table 4.1: Patient characteristics

Patient	Sex	Age (years)	Length (cm)	Weight (kg)
1	Male	5	110.0	20.8
2	Male	7	121.0	22.5
3	Male	13	154.3	60.0

4.1.1 Patient 1

A 5-year old boy presented himself at the outpatient clinic with progressive dyspnea and coughing for several days. Frequent use of salbutamol did not relieve symptoms.
EMG and RIP measurements were conducted for 40 minutes. During these measurements, spirometry was performed before and 5 minutes after administration of 200 µg salbutamol. Four artefact-free epochs were extracted, an example is given in Figure 4.1. Parameters from EMG, RIP and spirometry are given in Table 4.2.

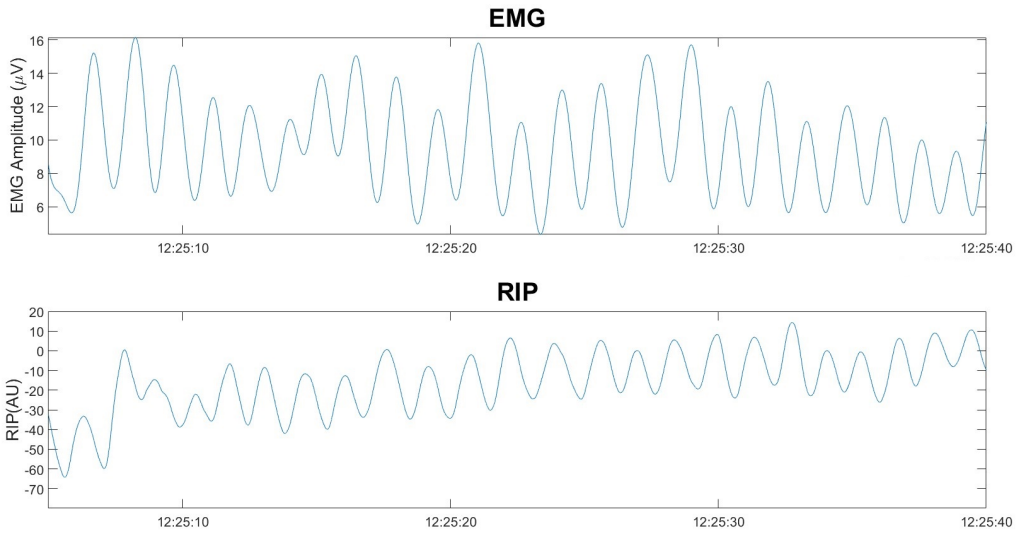

Figure 4.1: EMG and RIP of patient 1 for the same epoch

Table 4.2: Parameters derived from EMG and RIP, and percentage predicted FEV_1 for patient 1 at different timepoints.

	Before salbutamol	**After salbutamol**		
		5 min	**10 min**	**15 min**
EMG Height (μV)	9.99	8.73	7.51	3.82
EMG Width (s)	1.24	1.46	1.43	1.24
AUC ($\mu V \cdot s$)	4.23	5.07	3.90	1.87
RIP Width (s)	1.26	1.44	1.42	1.21
FEV_1 (%)	39	50	-	-

4.1.2 Patient 2

A 7-year old boy presented himself at the outpatient clinic with dyspnea and coughing, mainly during the night. He frequently uses salbutamol and recently had a treatment with antibiotics from the GP.
EMG and RIP measurements were conducted for 35 minutes. No specific instructions were given to the patient. Spirometry is performed before and 5 minutes after administration of 400 µg salbutamol. No completely artefact-free periods were found in the EMG data, because of constant movement of the patient. Furthermore, the patient did not like the fit of the Hexoskin shirt. Therefore, the shirt was touched and pulled on constantly. RIP showed 3 artifact-free periods, one is shown in Figure 4.2. No parameters of EMG and RIP could be calculated before administration of salbutamol. An overview of the calculated parameters at these epochs can be found in Table 4.3

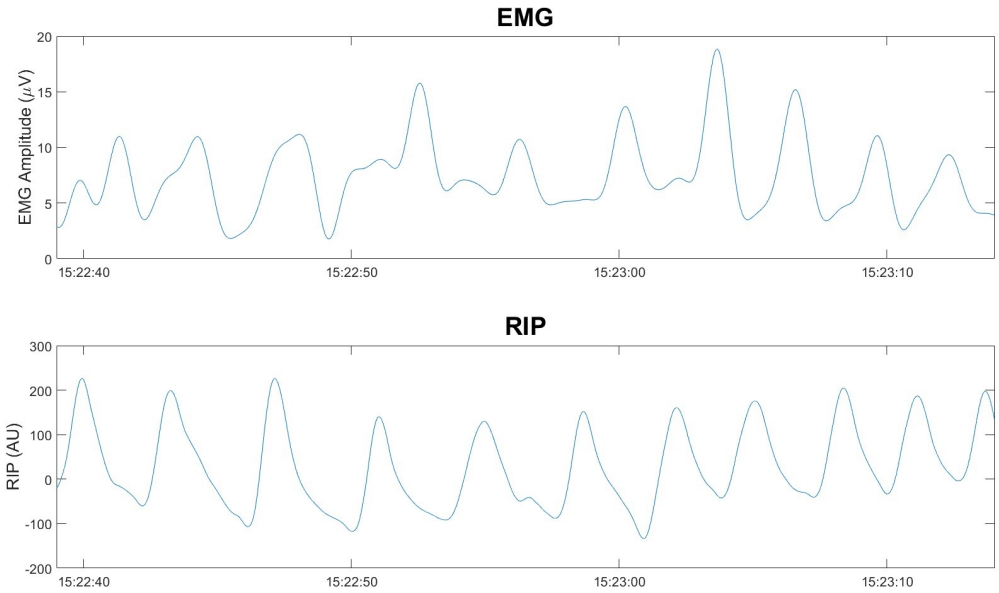

Figure 4.2: EMG and RIP of patient 2 for the same epoch

Table 4.3: Parameters derived from EMG and RIP, and percentage predicted FEV_1 for patient 2 at different timepoints.

	Before salbutamol	**After Salbutamol**		
		5 min	**7 min**	**10 min**
EMG Height (μV)	-	6.38	7.84	7.11
EMG Width (s)	-	1.99	1.75	2.30
AUC ($\mu V \cdot s$)	-	5.61	3.09	7.43
RIP Width (s)	-	3.69	3.19	3.04
FEV_1 (%)	99	107		

4.1.3 Patient 3

A 13-year old boy presented himself at the outpatient clinic with dyspnea. He had not taken the prescribed asthma control medication for several months. Since then he experienced progressive dyspnea and difficulty sleeping. Coughing and deep in- and expiration hurt at the sternum. After spirometry at the outpatient clinic, lung function was found to be low even after the administration of 400 μg salbutamol. He was administered to the ward for nebulisations with salbutamol.
At the ward, EMG measurements were started after the first nebulisation with 5mg of salbutamol. No fitting Hexoskin shirt was available. Therefore, no RIP measurements were taken. Artefact-free epochs were found before, during and after the second nebulisation and before and after the fourth nebulisation. After the nebulisations, spirometry measurements were taken. During a follow-up visit 7 days after the nebulisations, EMG and spirometry measurements are taken. Table 4.4 shows the calculated parameters of the artefact-free epochs. Figure 4.3 shows the EMG measurements before the second nebulisation, after the third nebulisation and at follow-up.

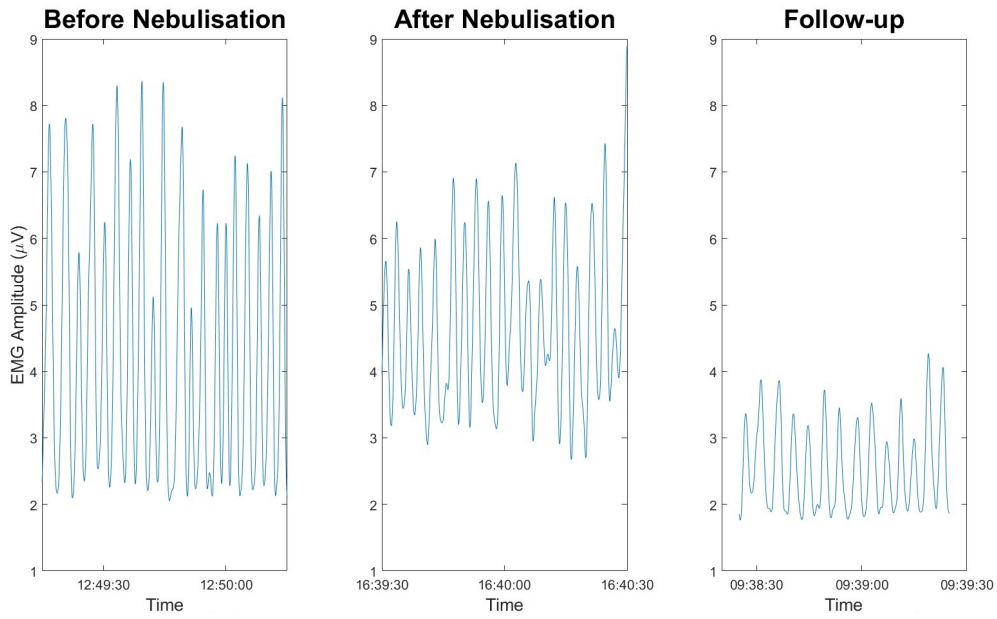

Figure 4.3: Three epochs of EMG, left figure is before the second nebulisation, middle figure after the third nebulisation and right figure during follow-up.

Table 4.4: Parameters derived from EMG and percentage predicted FEV_1 for patient 3 at different timepoints.

	Before salbutamol	**After salbutamol**	**Second nebulisation**			**Fourth nebulisation**		**Follow-up**
			Before	**During**	**After**	**Before**	**After**	
EMG Height (μV)	-	-	5.19	6.15	5.21	4.12	3.25	1.98
EMG Width (s)	-	-	2.42	3.02	2.68	2.37	2.78	3.29
AUC ($\mu V \cdot s$)	-	-	5.24	7.06	5.73	3.19	3.65	2.15
FEV_1 (%)	24	25	34	-	34	47	53	103

4.2 EMG measurements during ECT

From a period from March 2021 till May 2023, 70 test subjects with an asthma diagnosis were included in this analysis. Informed consent was obtained from all subjects. Patient characteristics are shown in Table 4.5. Normal distribution was found and a T-test was applied, where it is shown no statistical significant differences are found between the groups. For analysis of the 3 groups, Table 4.6 shows the patient characteristics of the test subjects between the 3 groups. An ANOVA-test showed no statistical significant differences between the groups.

Table 4.5: Patient characteristics from the included test subjects (n=70) divided in controlled (n=34) and non-controlled (n=36) asthma and results of the T-test.

	Controlled (n=34)		**Non-controlled (n=36)**		
	Mean	**SD**	**Mean**	**SD**	**P-value**
Age (years)	12.2	2.4	12.1	3.4	0.871
Height (cm)	153.0	15.2	157.4	15.3	0.238
Weight (kg)	45.0	13.9	48.9	16.3	0.297
Sex (% female)	29%		33%		

Table 4.6: Patient characteristics from the included test subjects divided in 3 groups. Group 1 are test subjects with a maximal FEV_1 decrease of 6%, Group 2 a FEV_1 decrease between 6 and 24% and Group 3 a FEV_1 decrease more than 24%.

	Group 1 (n=23)		**Group 2 (n=23)**		**Group 3 (n=24)**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P-value**
Age (years)	12.6	2.2	12.0	2.4	12.0	3.9	0.710
Height (cm)	156.8	13.2	151.6	16.6	157.5	15.8	0.356
Weight (kg)	46.9	13.0	45.4	18.1	48.7	14.3	0.754
Sex (% female)	32%		25%		38%		

4.2.1 Processed EMG

Processed EMG data at baseline (Base), 1 (T1), 3 (T3) and 6 (T6) minutes after exercise and after salbutamol (Salb) is shown in Figure 4.4 for a controlled asthma test subject. Figure 4.5 shows the the processed EMG at the same timepoints for a non-controlled asthma test subject.

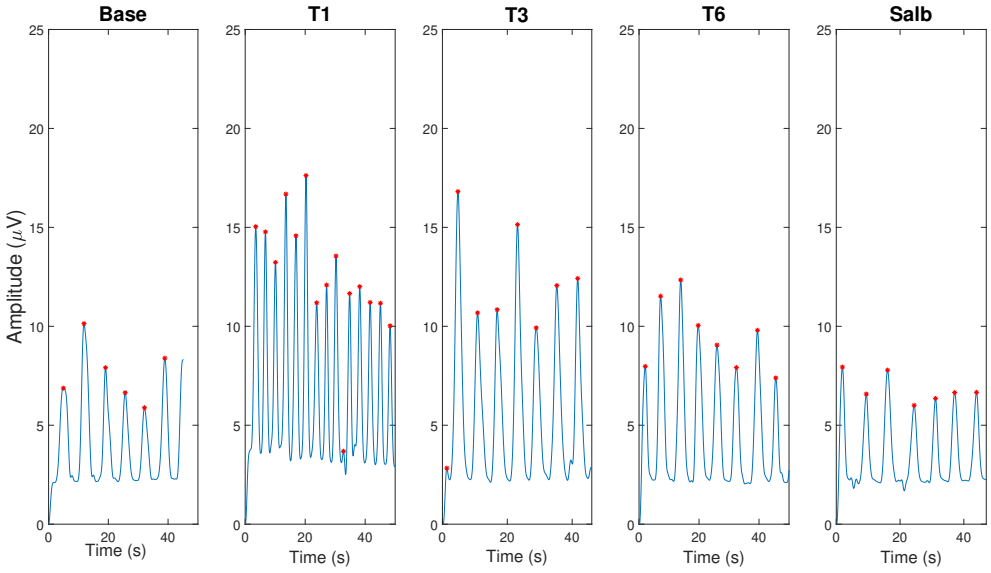

Figure 4.4: Processed EMG data at baseline, T1, T3, T6 and after salbutamol for a controlled asthma test subject.

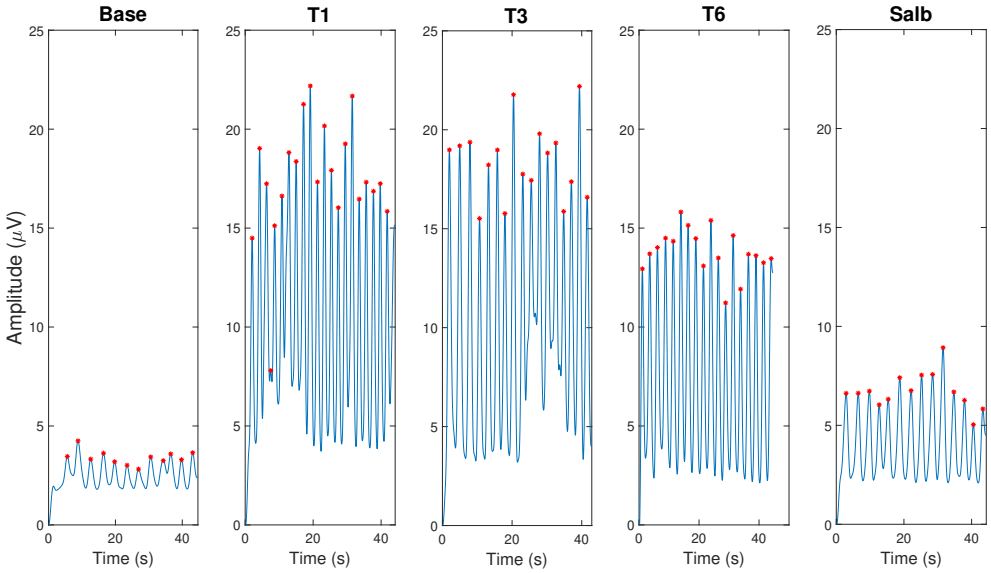

Figure 4.5: Processed EMG data at baseline, T1, T3, T6 and after salbutamol for a non-controlled asthma subject.

4.2.2 Analysis between controlled and non-controlled asthma

When the data was split in two groups, controlled and non-controlled asthma, it could be seen there are no significant differences in EMG Height at baseline measurements. Significant results were found at T1, T3 and T6 and after salbutamol. No statistically significant differences were found for EMG Width, in EMG AUC the same statistically significant differences were found. The results of the Mann-Whitney U tests for EMG Height used in this statistical analysis are shown in Table 4.7. Results for EMG Width and EMG AUC can be found in Appendix A.

Table 4.7: Median, IQR and significances of EMG Height compared between controlled and non-controlled asthma at Baseline, T1, T3, T6 and after salbutamol. Asterisks depict significant differences.

		Diagnosis	**Median**	**IQR**	**Significance**
Height (μV)	**Base**	Controlled	2.09	1.29-2.95	0.237
		Non-controlled	2.36	1.47-4.19	
	T1	Controlled	4.09	2.52-7.47	<0.001*
		Non-controlled	9.18	5.27-12.71	
	T3	Controlled	2.70	2.22-5.35	<0.001*
		Non-controlled	7.31	4.09-11.52	
	T6	Controlled	2.98	1.77-4.43	<0.001*
		Non-controlled	5.60	3.86-8.95	
	Salb	Controlled	2.00	1.39-3.02	0.001*
		Non-controlled	4.23	2.10-6.13	

These results are visually represented in Figure 4.6. Figures for EMG Width and EMG AUC can be found in Appendix A.

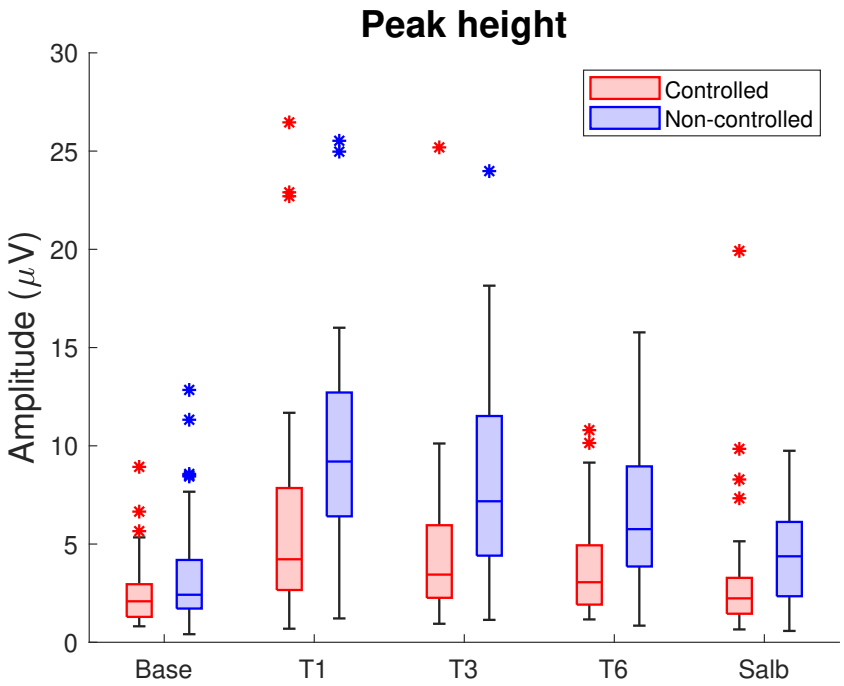

Figure 4.6: Boxplots for EMG Height at Baseline, T1, T3, T6 and After salbutamol split in a controlled asthma (n=34) and non-controlled asthma (n=36) group. Asterisks depict outliers.

Parameters were statistically compared to the baseline measurements with Mann Whitney U tests in Table 4.8 for EMG Height. Statistically significant differences were found at T1, T3, and T6 for controlled asthma test subjects and at T1, T3, T6 and after salbutamol in non-controlled asthma subjects. Tables for EMG Width and AUC can be found in Appendix A. For EMG Width, statistically significant differences are found only at T1 for both groups, for EMG AUC statistically significant differences are found at T1, T3 and T6 for both groups.

Table 4.8: Median, IQR and significance compared to baseline for EMG Height (μV) in controlled and non-controlled asthma.

		Median	IQR	Significance
Controlled	**Base**	2.09	1.29-2.95	-
	T1	4.09	2.52-7.47	<0.001*
	T3	2.70	2.22-5.35	<0.001*
	T6	2.98	1.77-4.43	0.002*
	Salb	2.00	1.39-3.02	0.959
Non-controlled	**Base**	2.36	1.47-4.19	-
	T1	9.18	5.27-12.71	<0.001*
	T3	7.31	4.09-11.52	<0.001*
	T6	5.60	3.86-8.95	<0.001*
	Salb	4.23	2.10-6.13	0.009*

4.2.3 Analysis between three ranges of decrease in lung function

The data was split in 3 groups equal in size, characterised by a specific range of decrease in FEV_1 after exercise. Group 1 includes test subjects with a less than 6% decrease in FEV_1. Group 2 includes test subjects with a decrease between 6 and 24% in FEV_1 after exercise. Group 3 includes test subjects with a decrease greater than 24% in FEV_1. Boxplots by group depicting the results for EMG Height are shown in Figure 4.7. Figures for EMG Width and EMG AUC can be found in Appendix A.

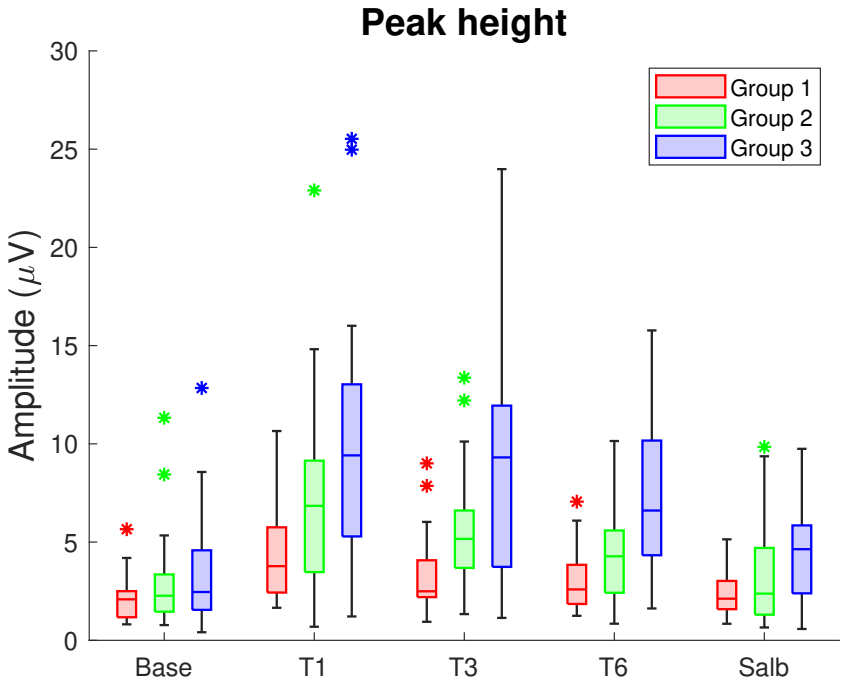

Figure 4.7: Boxplots for EMG Height at Baseline, T1, T3, T6 and After salbutamol split in group 1 (FEV_1 decrease $\leq$ 6%) (n=23), group 2 (FEV_1 decrease between 6% and 24%) (n=23) and group 3 (FEV_1 decrease $\geq$ 24%) (n=24). Asterisks depict outliers.

Statistically significant differences between group were tested using a Kruskal-Wallis test. Significant differences were found at T1, T3, T6 and after salbutamol. The results of the statistical analysis for EMG Height is found in Table 4.9. The results for EMG Width and AUC can be found in Appendix A. No statistically significant differences are found for EMG Width. For EMG AUC, statistically significant differences are found at T1, T3 and T6.

Table 4.9: Median, IQR and significances of EMG Height, Width and AUC compared between the 3 groups at Baseline, T1, T3, T6 and after salbutamol. Asterisks depict significant differences.

		Group	Median	IQR	Significance
Height (μV)	**Base**	Group 1	2.13	1.19-2.64	0.397
		Group 2	2.27	1.42-3.38	
		Group 3	2.46	1.47-4.71	
	T1	Group 1	3.93	2.61-6.35	0.003*
		Group 2	6.84	3.22-9.25	
		Group 3	9.41	4.65-13.14	
	T3	Group 1	2.56	2.23-4.25	<0.001*
		Group 2	5.16	3.45-6.81	
		Group 3	9.31	3.62-12.14	
	T6	Group 1	2.79	1.92-4.00	<0.001*
		Group 2	4.28	2.26-5.61	
		Group 3	6.61	4.09-10.60	
	Salb	Group 1	2.18	1.66-3.20	0.044*
		Group 2	2.38	1.30-5.18	
		Group 3	4.64	2.37-5.90	

Statistically significant different parameters from Table 4.9 were further analysed between individual groups using Mann-Whitney U tests. The results of this analysis can be found in Table 4.10 for EMG Height parameters. Statistical significant differences were found between groups 1 and 2 for T3. Between groups 1 and 3, significant differences were found for T1, T3, T6 and after salbutamol. Between groups 2 and 3, significant differences were found for T3 and T6. Tables for EMG AUC can be found in Appendix A, the same groups were statistically significantly different.

Table 4.10: Results of Mann-Whitney U tests between groups for statistically signficant different timepoints in EMG Height. Table a. shows the results for T1, Table b. shows the results for T3, Table c. shows the results for T6 and Table d. shows the results for After salbutamol. Asterisks depict significant differences.

a. T1

	Group 2	Group 3
Group 1	0.056	0.001*
Group 2	X	0.067

b. T3

	Group 2	Group 3
Group 1	0.017*	<0.001*
Group 2	X	0.023*

c. T6

	Group 2	Group 3
Group 1	0.124	<0.001*
Group 2	X	0.005*

d. Salb

	Group 2	Group 3
Group 1	0.774	0.008*
Group 2	X	0.127

EMG Height was statistically compared to the baseline measurements in Table 4.11. Statistically significant differences are found at T1, T3 and T6 for Groups 1 and 2. For Group 3, statistical significant differences are found at T1, T3, T6 and after salbutamol. Tables for EMG Width and AUC can be found in Appendix A. For EMG Width, statistically significant differences are found at T1 for all 3 groups. For EMG AUC, statistically significant differences are found at T1 for Group 1, and at T1, T3 and T6 for Groups 2 and 3.

Table 4.11: Median, IQR and significance compared to baseline for EMG Height (μ V) for the 3 groups. Asterisks depict significant differences.

		Median	**IQR**	**Significance**
Group 1	**Base**	2.13	1.19-2.64	-
	T1	3.93	2.61-6.35	<0.001*
	T3	2.56	2.23-4.25	0.011*
	T6	2.79	1.92-4.00	0.019*
	Salb	2.18	1.66-3.20	0.232
Group 2	**Base**	2.27	1.42-3.38	-
	T1	6.84	3.22-9.25	<0.001*
	T3	5.16	3.45-6.81	<0.001*
	T6	4.28	2.26-5.61	0.019*
	Salb	2.38	1.30-5.18	0.848
Group 3	**Base**	2.46	1.47-4.71	-
	T1	9.41	4.65-13.14	<0.001*
	T3	9.31	3.62-12.14	<0.001*
	T6	6.61	4.09-10.60	<0.001*
	Salb	4.64	2.37-5.90	0.019*

4.2.4 Comparison to spirometry

A comparison between the maximal change in FEV_1 after exercise and relative change of EMG Height at 3 minutes after exercise is shown in Figure 4.8. Linear regression analysis yielded a coefficient of determination of 0.410.

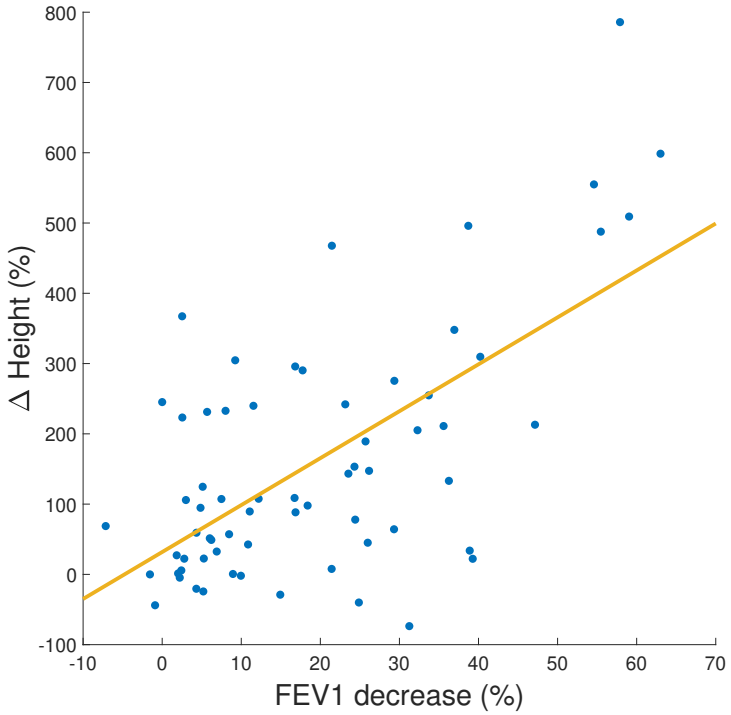

Figure 4.8: Comparison of change in FEV_1 at maximal decrease after exercise and relative change in EMG Height at T3, along with the linear regression trend line.

ROC-curves are constructed for different thresholds of FEV_1 decrease after exercise, which are shown in Figure 4.9 EMG Height changes at 3 minutes after exercise were used in the construction of these ROC-curves. Optimal sensitivity and specificity and AUC for these curves can be found in Table 4.12.

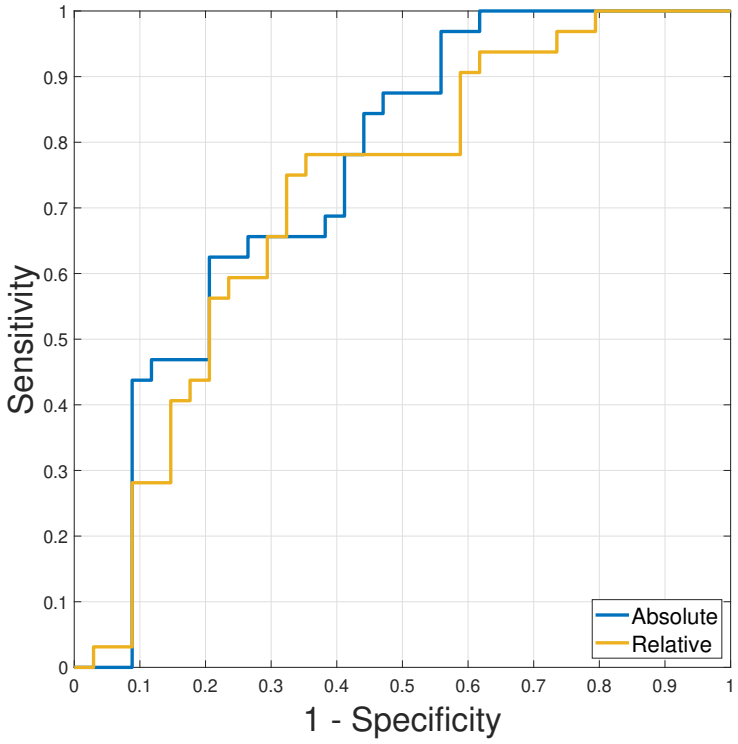

Figure 4.9: ROC-curves comparing relative and absolute ΔHeight to decrease in FEV_1 at T3.

Table 4.12: Sensitivity and specificity at the optimal operating points and AUC for the ROC-curves of the changes in EMG Height at 3 minutes after exercise.

	Sensitivity/ specificity	**AUC**
Absolute	62.5%/79.4%	0.753
Relative	75.0%/67.7%	0.720

Comparison to lowest spirometry

A comparison between the maximal change in FEV_1 after exercise and the absolute change of EMG Height at the same timepoint is shown in Figure 4.10. Linear regression analysis yielded a coefficient of determination of 0.280.

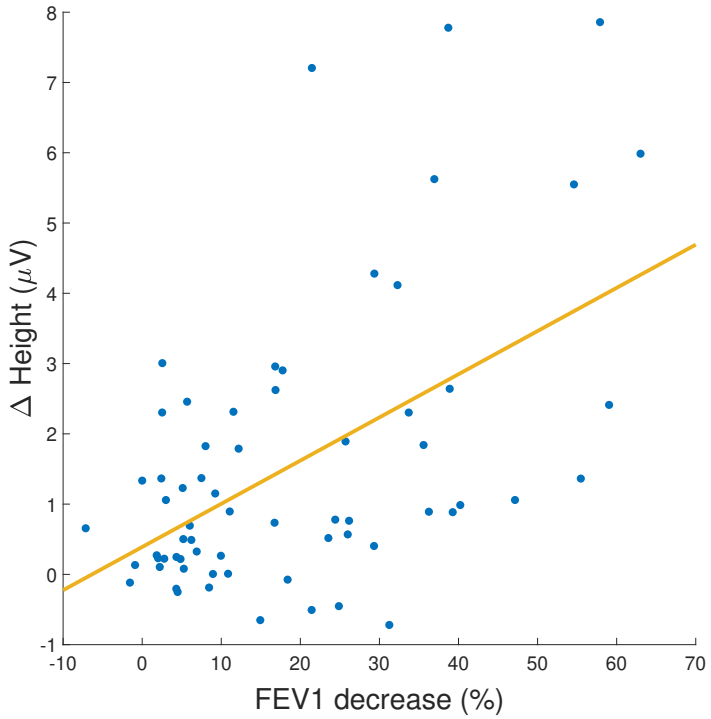

Figure 4.10: Comparison of change in FEV_1 at maximal decrease after exercise and absolute change in EMG Height at the same timepoint, along with the linear regression trend line.

ROC-curves of EMG Height changes at the timepoints of lowest spirometry after exercise can be found in Figure 4.11. Optimal sensitivity and specificity and AUC for these curves can be found in Table 4.13.

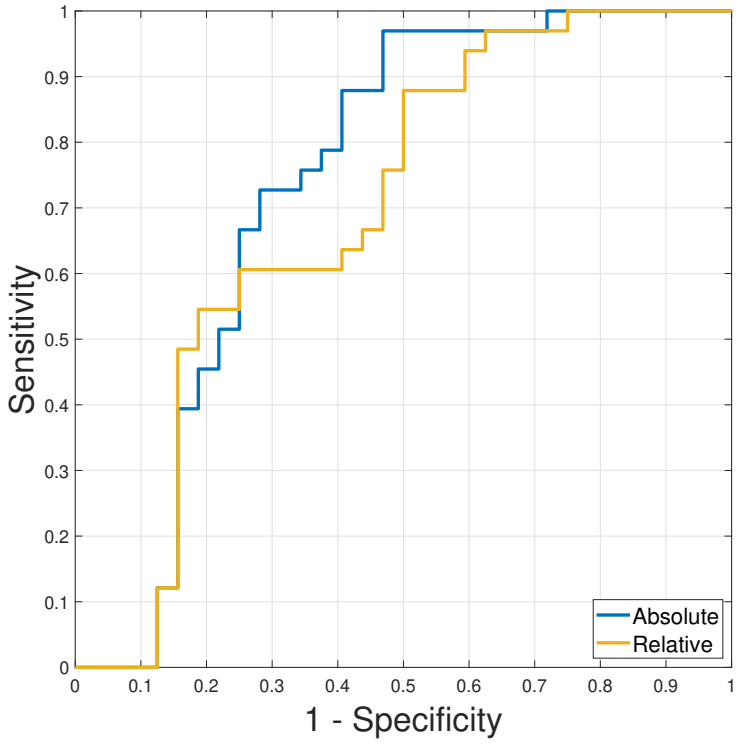

Figure 4.11: ROC-curves comparing absolute and relative ΔHeight to decrease in FEV_1 at lowest spirometry

Table 4.13: Sensitivity and specificity at the optimal operating points and AUC for the ROC-curves of the changes in EMG Height at lowest spirometry after exercise.

	Sensitivity/ specificity	**AUC**
Absolute	97.0%/53.1%	0.740
Relative	87.9%/50.0%	0.695

Comparison to maximal change in EMG parameters

A comparison between the maximal absolute change in EMG Height after exercise and the change in FEV_1 at the same timepoint is shown in Figure 4.12. Linear regression analysis yielded a coefficient of determination of 0.2073.

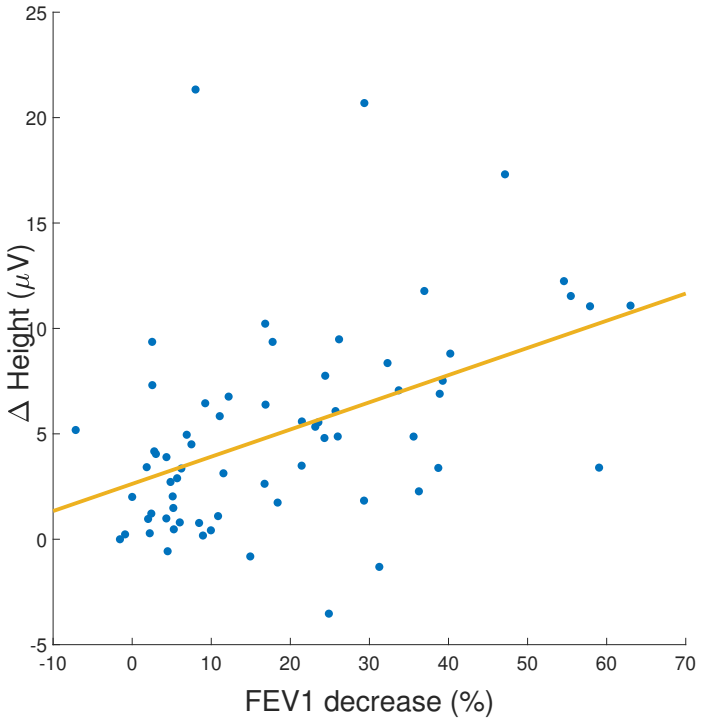

Figure 4.12: Comparison of maximal absolute change in EMG Height and change in FEV_1 at the same timepoint, along with the linear regression trend line.

ROC-curves of maximal EMG Height changes after exercise can be found in Figure 4.13. Optimal sensitivity and specificity and AUC for these curves can be found in Table 4.14.

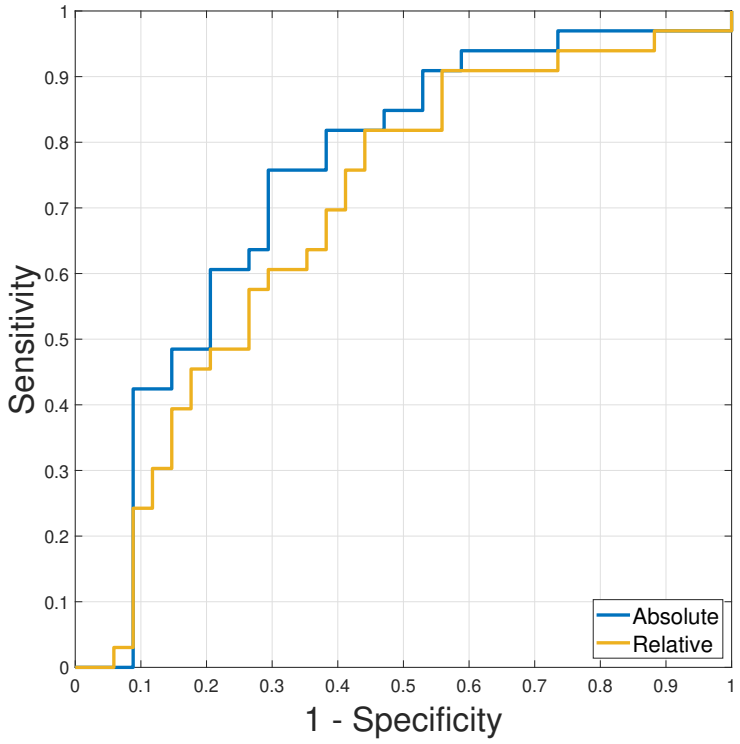

Figure 4.13: ROC-curves comparing absolute and relative ΔHeight at maximal EMG Height change to decrease in FEV_1 at the same timepoint.

Table 4.14: Sensitivity and specificity at the optimal operating points and AUC for the ROC-curves of the maximal changes in EMG Height.

	Sensitivity/ specificity	**AUC**
Absolute	75.8%/70.6%	0.745
Relative	81.8%/55.9%	0.693

Table 4.15 shows the Pearson correlation coefficients and their significance for the relative and absolute changes in EMG Height at T3, at lowest spirometry after exercise and at maximal change in EMG Height after exercise, compared to the decrease in FEV_1 after exercise. Scatterplots for the absolute change at T3, relative change at lowest spirometry and relative change at maximal EMG Height change can be found in Appendix A.

Table 4.15: Pearson coefficients and significance for absolute and relative changes in EMG Heigth at T3, lowest spirometry and maximal EMG Height change.

Parameter		**Pearson's R**	**Significance**
T3	Absolute	0.552	<0.001*
	Relative	0.639	<0.001*
Low spirometry	Absolute	0.460	<0.001*
	Relative	0.527	<0.001*
Max EMG Height	Absolute	0.455	<0.001*
	Relative	0.448	<0.001*

Chapter 5

Discussion

In this thesis, the use of EMG to assess paediatric asthma was investigated. Continuous measurements were conducted during out- and inpatient clinic visits. Furthermore, EMG measurements during an elective ECT, where asthmatic reactions are provoked, were analysed.

5.1 Clinical patients

Patient 1

From the 40 minutes of EMG data of this patient, only 4 epochs of considerable length were found to be free of artefacts. The patient was not given specific instructions to sit still. This resulted in constant moving of the patient, which is reflected in the number of artefacts in the data. This is a known short-coming of respiratory EMG [20].
This patient showed an increase of 30% in FEV_1 after 200 µg salbutamol. This increase in lung function coincided with a decrease in EMG Height of 1 µV at 5 minutes after bronchodilator therapy till 6 µV at 15 minutes after. No significant changes were found in EMG Width, which was low throughout the measurements. This suggests a high respiratory rate, which was also observed during the visit. EMG Width and RIP Width are comparable in all analysed epochs, suggesting EMG measures respiratory function.
Visually analysing both RIP and EMG from the same time frame, shows a slight difference in peak locations. This may suggest a discrepancy in time vectors in data analysis, but could also suggest that activity of the diaphragm does not directly relate to changes in thoracic cross-sectional area, which is conceivable from a mechanical point of view.

Patient 2

From the 35 minutes of EMG data, no artefact-free epochs of considerable length were found. This was due to the constant moving of the patient during the measurements. Furthermore, the Hexoskin shirt was found to be bothersome by the patient. Therefore, the patient constantly plucked the shirt, which resulted in artefacts in the RIP data. However, RIP showed fewer artefacts than EMG, resulting in 3 epochs of considerable length to be analysed. Respiratory rate, calculated from RIP Width, is found to be constant during these epochs.
The patient presented himself with dyspnea, but lung function measured by spirometry was found to be within normal limits with a FEV_1 99% of the predicted value, while earlier spirometry measurements in this patient showed baseline values of 106% of predicted

FEV_1. After the use of 400 µg salbutamol, FEV_1 increased with 8% till 107% of predicted. The normal baseline lung function and the lack of response to salbutamol indicated the symptoms were not related to bronchial obstruction.

Patient 3

Patient 3 was the only subject that was admitted to the ward, as the lung function of this patient was not responding to 400 µg of salbutamol given by a spacer at the outpatient clinic. Therefore, more intensive treatment and monitoring was necessary by the use of nebulisations. EMG measurements were started only after the first nebulisation. From the improvement in spirometry parameters, it can be seen the nebulisations were effective and were therefore continued. The lung function of this patient kept improving, and after a fourth nebulisation, the lung function was deemed good enough for discharge. Overall, there was an increase of 121% of the lung function from the moment the patient presented himself till discharge. The patient was seen 7 days after discharge and the FEV_1 had increased to 103% of predicted, in accordance with earlier measurements of the patient. EMG parameters showed a decline in EMG Height and AUC, when the lung function improved. During the nebulisation the patient had a forced in- and expiration, which resulted in a higher EMG Height, Width and AUC. This suggests the patient was breathing in deeper and had a lower respiratory rate during the nebulisations, compared to non-forced breathing between the nebulisations.

Overall results

Feasibility of diaphragm EMG during wakefullness is low, because of constant moving of the test subjects. This resulted in movement artefacts. Furthermore, respiratory rate measured with RIP and EMG are comparable, indicating both measure respiratory function. These results have been shown in adults in previous research to classify apneas during sleep.[42] EMG Height decreases with improving lung function. This is in accordance with earlier research. [19, 21] An increase in EMG amplitude is a result of more motor units of the diaphragm firing at the same moment. This means, the diaphragm exerts more force to pull air into the lungs. In asthma patients, higher EMG amplitude is an effect of the bronchoconstriction that occurs.

5.2 EMG during elective ECT

EMG data of 70 patients with a known asthma diagnosis, obtained during a standard ECT at the outpatient clinic of MST were analysed. Analysis was done by dividing the patients in two and three groups. The two groups were controlled and non-controlled asthma, defined by a cut-off of 13% decrease in FEV_1 after exercise. The three groups were defined by equal group size, resulting in a group with a 6% or less decrease in FEV_1, a group with a decrease between 6 and 24% and a group with a decrease in FEV_1 greater than 24%.
Statistical comparison on the patient characteristics was performed to determine if groups are equal. For the two groups, no statistical significant differences were found. The same result was found when comparing the three groups.

5.2.1 Controlled and uncontrolled asthma

Two groups were defined as explained above. The boxplots demonstrate there was an increase in EMG Height at 1 minute after exercise that was more pronounced for the non-controlled asthma group. The controlled asthma group returned to baseline more quickly at 3 minutes and 6 minutes after exercise. Furthermore, a return to baseline was seen for the controlled asthma group after salbutamol, while the non-controlled asthma group still had an increased EMG Height compared to baseline. The boxplots for EMG Width showed a decrease at 1 minute after exercise for both groups, with a return to baseline at the other timepoints.
Statistical analysis between the two groups at the various timepoints showed a statistical difference in EMG Height and AUC at all timepoints, except baseline. No statistical differences were found in EMG Width at any timepoint for both groups. These results suggest more diaphragm activity is needed for non-controlled asthma patients after exercise and after salbutamol compared to controlled asthma patients. No differences in respiratory rates between the two groups were found.

Comparison to baseline EMG

When comparing the EMG parameters at different timepoints to their baseline, EMG Height changed significantly at 1, 3 and 6 minutes after exercise for both groups. However, after salbutamol, patients with controlled asthma returned to baseline, while patients with non-controlled asthma still had a higher amplitude. This suggests, a higher diaphragm activity was still present after salbutamol, while lung function had returned to baseline. In EMG AUC, statistical significant changes were found at 1, 3 and 6 minutes after exercise for both groups. However, both groups showed no statistical significant difference after salbutamol, in contrast with EMG Height. Furthermore, statistical significant changes were found in EMG Width at 1 minute after exercise. This can be explained by a higher respiratory rate after exercise for both groups, that has returned to baseline two minutes later.

5.2.2 Division into three ranges of decrease in lung function

Three groups were defined as described above. A visual inspection of the boxplots for EMG Height showed an increase at 1 minute after exercise for all groups, which was increasingly pronounced for the groups. At 3 minutes after exercise, group 1 had almost returned to baseline, while the EMG Heights of group 2 and 3 remained elevated. At 6 minutes after exercise, group 1 and 2 were returning to baseline, while EMG Height in group 3 still remained elevated. After salbutamol, group 1 had returned to baseline, while group 2 and 3 still remained elevated.
When this was statistically analysed, it could be seen no statistically significant differences were found at baseline between the groups. Significant differences were found at 1, 3 and 6 minutes after exercise and after salbutamol. While analysing individual groups within significant different parameters, statistical significant differences at 1 minute after exercise were found for groups 1 and 3 only. At 3 minutes after exercise, there were statistical significant differences between all individual groups. At 6 minutes after exercise, the differences were significant between groups 1 and 3 and between groups 2 and 3. After salbutamol, statistical significant differences were found between group 1 and 3.
These results suggest diaphragm activity is increased significantly more for patients with

more severe EIB. Furthermore, this increased activity was continued throughout the measurements, resulting in significant differences at all timepoints. After salbutamol, the activity was still increased compared to the group with the least severe asthma. For group 2, the increase in activity after exercise was less pronounced, and only statistically significant compared to group 1 at 3 minutes after exercise. Furthermore, the return to baseline was quicker, resulting in significant differences with group 3 at 3 and 6 minutes after exercise. EMG Width showed a significant decrease in all groups at 1 minute after exercise, that returned to baseline at 3 minutes after exercise. Results for EMG AUC are comparable to the results for EMG Height, as these are complimentary parameters.
These results suggest more diaphragm activity is needed to pull air into the lungs when patients have more severe asthma. This activity is also increased if asthma is less severe, but is decreasing faster after exercise and returns to baseline after salbutamol. Patients with severe asthma still had a higher diaphragm activity after the use of salbutamol.

Comparison to baseline EMG

When compared to baseline, EMG Height was increased in all 3 groups after exercise. This increase remained at all timepoints after exercise. There were no significant differences compared to baseline after the use of salbutamol in group 1 and group 2, while group 3 still had an increased EMG Height. This suggests more activity of the diaphragm is present after exercise for all asthmatic patients. However, after the use of salbutamol, the diaphragm activity was still significantly higher for group 3, while FEV_1 had returned to baseline This suggests severely asthmatic patients still have a higher diaphragmatic activity even if there lung function has returned to baseline.
In EMG Width, statistical significant differences were found compared to baseline at 1 minute after exercise for every group. No other statistical differences were found. This suggests there was a higher respiratory rate after exercise which returned to baseline after three minutes. Furthermore, this shows that severity of asthma does not result in a difference in respiratory rate when an asthmatic reaction is provoked.
For the parameter EMG AUC, statistical significant increases compared to baseline were found at 1 minute after exercise for all three groups. For groups 2 and 3, these significant differences were also found for 3 and 6 minutes after exercise, while no statistical significant differences were found after salbutamol. These results suggest a higher activity is present after exercise for all asthmatic patients, while this activity remains increased in severe and less severe EIB.

5.2.3 Comparison to spirometry

Changes in EMG Height from baseline were compared to changes in FEV_1 from spirometry. When visually inspecting the scatter plot it can be seen that a increase in ΔFEV_1 results in a higher ΔHeight at T3. The ROC-curves showed no great differences were found between absolute and relative changes in EMG Height and between a cut-off of -13% and -30% decrease in FEV_1 for ΔHeight at T3 to classify controlled and non-controlled asthma. Linear regression showed a moderate degree of correlation for both absolute and relative changes in EMG Height at T3 compared to the maximal decrease in FEV_1 after exercise.

Comparison at point of lowest spirometry

Comparisons were made at timepoints where the lowest spirometry is measured. An increase in ΔFEV_1 resulted in a higher ΔHeight. ROC-curves yielded an AUC between 0.695 for a relative change in EMG Height at a cut-off of -13% in FEV_1 and 0.781 for an absolute change in EMG Height at a cut-off of -30% to classify controlled and non-controlled asthma. Linear regression showed a low correlation for an absolute change and a moderate correlation for a relative change in EMG Height.

Comparison to maximal change in EMG parameters

Furthermore, this analysis was performed at the timepoints where there is a maximal change in the EMG parameters. ROC-curves yielded an AUC between 0.693 for a relative change in EMG Height at a cut-off of -13% in FEV_1 and 0.756 for an absolute change in EMG Height at a cut-off of -30% to classify controlled and non-controleld asthma. Linear regression showed a low correlation for both absolute and relative changes in EMG Height.

5.2.4 Comparison to earlier results

An earlier study with a similar study design shows similar changes in EMG parameters. [21] In this study a greater difference is found between baseline and after exercise for parameters Height and AUC in non-controlled asthma patients compared to controlled asthma patients. The same results are found in this thesis. However, when comparing changes in EMG parameters to changes in FEV_1, AUC for ROC-curves with a value of 0.95 were found. In this study, the same parameter yielded an AUC of 0.70. This result could be explained by the use of a different measurement device and the use of different method to filter ECG from the EMG between the studies. ECG-removal is based on the same algorithm by O'Brien [43], but could be applied differently in both studies.
Increase in diaphragm activity after exercise is measured in all groups analysed. In healthy adults, this increased diaphragm activity is also seen.[44, 45]

5.3 Strengths and limitations

5.3.1 Patient inclusion

Patient inclusion and measurement is done by the same researcher. Therefore, there was a uniform execution of the measurement protocol.
During the inclusion of the research studying continuous measurements, no potential test subjects were admitted to the paediatric ward and were spending the night. At first, the goal of this research was to study the usability of long-time EMG measurements during sleep, because there is a strong relation between asthma and sleep disorders and quality in children.[4, 12] The reason for the lack of admissions to the paediatric ward during the inclusion peridd, was a well-controlled population of asthma patients under treatment at the MST. This was not anticipated at the start of the research.
Furthermore, none of the measured asthmatic patients were asleep during the measurements. Therefore, the use of long-time continuous measurements during sleep could not be studied. The reason for this was the duration of admittance for the single included test subject that was admitted to the ward. Due to the lack of inclusions and no nightly measurements, no statistical analysis could be performed on this data in this thesis.

However, measurements taken during the ECT are conducted by only two researchers who are skilled in the measurement protocol. This reduces the amount of possible errors in data acquirement.

5.3.2 Measurement device

The measurement device, the Mobi-6 amplifier, was chosen because of its high input impedance and compact design. A limitation for continuous measurements of this device is the use of leads from the electrodes to the amplifier. This limits a test subject in its movements and could cause loss of data due to the detachment of a lead from the electrode. For measurements during the ECT, this is no issue, because the test subject can be instructed to sit still for the duration of the measurement. For continuous measurements, however, the patient will move around occasionally, resulting in potential entanglement of the leads.
Another limitation of this amplifier is the need to be in close proximity of the research computer collecting the data, because no data can be stored on the device. This limits the locations at which measurements can be taken, because of privacy and security reasons.
Furthermore, the portable amplifier has a limited battery life. During long-time continuous measurements, a long battery life is preferable. This ensures no potential clinically interesting data is lost. If measurements during the night would be taken, a minimum of 10 hours of battery life would be recommended. Some younger children will sleep for at least that amount of time.

5.3.3 Analysis algorithm

The analysis algorithm used in these measurements is constructed by the research team. Therefore, all steps in artefact detection and removal, construction of the respiratory signal and calculation of the EMG parameters are known and could be adjusted when necessary.
There are some limitations on the analysis algorithm used in these measurements. One of the limitations is the lack of online processing and plotting of the data. This could give insight in whether a test subject is connected correctly to the measurement device and would give a physician the possibility to analyse the data visually in real-time. Deterioration of lung function could be detected bedside, which could prove to be useful during an asthma exacerbation.

5.4 Future perspectives

5.4.1 Continuous measurements

This thesis describes a method for long-time continuous measurements. However, this method has not been tested in asthmatic patients. Results of test measurements give rise to the idea that long-time continuous EMG measurements during sleep might be feasible. It would be interesting to be able to develop a method to non-invasively measure work of breathing during sleep. This could give insight in asthma control in paediatric patients. Because of the lack of admissions to the ward in the paediatric asthma population of the MST, measurements at home could provide a solution. Limitations of this are the privacy and security concerns described above. A possible solution for this could be a device that

stores the data on an internal storage, without compromising portability or size of the device.

5.4.2 Data analysis

Real-time analysis of EMG measurements could give a physician insight in the current respiratory condition of the patient. Actions on lung function deterioration could be made while they occur, resulting in quick relieve of symptoms for patients and greater insight in the effect of medication. Furthermore, signal quality can be analysed if real-time analysis is performed. If the signal is found to be of insufficient quality, actions could be taken to improve the quality. The downside of real-time analysis is the complexity to implement this. Numerous data analysing steps are taken before the respiratory waveform is constructed. The construction of a real-time analysis algorithm will demand the help of skilled software engineers.
Furthermore, use of machine learning methods could provide additional information to the physician. If deterioration of lung function could be predicted before it occurs, sufficient steps could be taken to prevent this. To be able to use these methods, it is necessary to obtain data of continuous EMG measurements of sufficient quality from patients with a loss in lung function during those measurements.

5.4.3 Home monitoring

A goal for the far future could be the implementation of continuous EMG measurements in home-monitoring. Especially during the night, no objective, non-invasive technique is yet available to monitor lung function. The use of EMG could provide solutions. To be able to use EMG in home monitoring, a measurement device needs to be used that guarantees sufficient data quality. Furthermore, it needs to be portable, preferably worn entirely on the body, without the use of long leads that are suspect for noisier signals.

Chapter 6

Conclusion

In this thesis the use of EMG of the diaphragm as a measurement device in paediatric asthma care has been investigated. The use of EMG measurements in children with a spontaneous asthma attack (n=3) admitted to the outpatient clinic or the ward for acute treatment of asthma symptoms has been studied by continuously measuring EMG and RIP. The feasibility of EMG as a continuous measurement tool to objectify asthma during spontaneous attacks showed to be difficult at least in wake patients without the proper instructions. However, when sitting perfectly still, which is hard in sick young children, EMG provided continuous signals during these attacks. The respiratory rate obtained from RIP and EMG are comparable, as long as signal quality of both measurements was sufficient. This indicates EMG of the diaphragm measures respiratory physiology. Artefacts in one or both of the signals will cause problems in the calculation of respiratory rate. Use of bronchodilator medication caused the EMG amplitude to drop. This corresponds to the theory that bronchoconstriction causes increased activity of the diaphragm.
Measurements during a provoked asthma attack after an exercise challenge test (n=70) showed an increased EMG amplitude after exercise. This increase was significantly greater in children with a 13% or more decrease in lung function (non-controlled asthma) and increased with severity of asthma. Respiratory rates increased for all asthmatic patients after exercise, but did not distinguish between controlled or non-controlled asthma. At three minutes after exercise, the respiratory rate changed to baseline values for all test subjects. The relation between diaphragm EMG parameters and changes in spirometry parameters were found to be moderate, with a relative change of EMG amplitude at 3 minutes after exercise yielding the most desirable results. After the use of bronchodilators, diaphragm activity returned to baseline for controlled asthma patients, but remained elevated in non-controlled asthma patients. This shows a still sustained increase in muscle activity, despite a return to baseline spirometry values, suggesting EMG is a more sensitive measurement tool for strain on the respiratory physiology than spirometry.

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Appendix A

Additional analyses

A.1 EMG Width

A.1.1 Analysis between controlled and non-controlled asthma

Table A.1 shows the result of the Mann-Whitney U test comparing the controlled and non-controlled asthma group. No statistical significant differences are found at all timepoints.

Table A.1: Median, IQR and significances of EMG Width compared between controlled and non-controlled asthma at Baseline, T1, T3, T6 and after salbutamol.

		Diagnosis	**Median**	**IQR**	**Significance**
Width (s)	**Base**	Controlled	2.84	2.36-3.09	0.624
		Non-controlled	2.96	2.39-3.23	
	T1	Controlled	2.36	1.77-2.57	0.221
		Non-controlled	2.40	1.94-2.78	
	T3	Controlled	2.70	2.31-3.23	0.690
		Non-controlled	2.75	2.20-3.25	
	T6	Controlled	2.70	2.38-3.47	0.792
		Non-controlled	2.84	2.26-3.61	
	Salb	Controlled	2.69	2.43-3.33	0.782
		Non-controlled	2.71	2.33-3.82	

The boxplots presented in Figure A.1 show a decrease in EMG Width 1 minute after exercise, that returned to baseline at 3 minutes after exercise. As shown in Table A.2, there is a statistically significant decrease in EMG Width 1 minute after exercise for both groups. No other significant differences are present. This suggests both controlled and non-controlled asthma patients have an increase in respiratory rate shortly after exercise, which returns to baseline within 3 minutes after exercise.

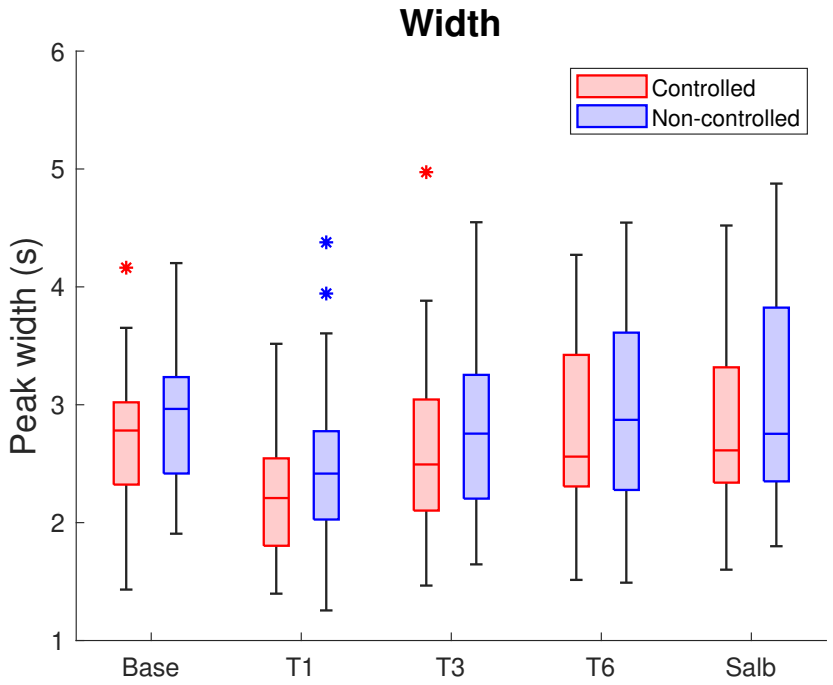

Figure A.1: Boxplots for EMG Width at Baseline, T1, T3, T6 and After salbutamol split in a controlled asthma (n=34) and non-controlled asthma (n=36) group. Asterisks depict outliers.

Table A.2: Median, IQR and significance compared to baseline for EMG Width (s) in controlled and non-controlled asthma

		Median	IQR	Significance
Controlled	**Base**	2.84	2.36-3.09	-
	T1	2.36	1.77-2.57	<0.001*
	T3	2.70	2.31-3.23	0.453
	T6	2.70	2.38-3.47	0.784
	Salb	2.69	2.43-3.33	0.478
Non-controlled	**Base**	2.96	2.39-3.23	-
	T1	2.40	1.94-2.78	0.004*
	T3	2.75	2.20-3.25	0.540
	T6	2.84	2.26-3.61	0.741
	Salb	2.71	2.33-3.82	0.612

A.1.2 Analysis between three ranges of decrease in lung function

When the data was split into the three groups of equal size, no significant differences were found between the groups for EMG Width, as can be seen in Table A.3.

Table A.3: Median, IQR and significances of EMG Width compared between the 3 groups at Baseline, T1, T3, T6 and after salbutamol.

		Group	Median	IQR	Significance
Width (s)	**Base**	Group 1	2.83	2.37-3.02	0.852
		Group 2	2.99	2.35-3.19	
		Group 3	2.96	2.42-3.26	
	T1	Group 1	2.20	1.78-2.55	0.323
		Group 2	2.39	2.05-2.75	
		Group 3	2.42	1.94-2.78	
	T3	Group 1	2.49	2.10-2.80	0.276
		Group 2	2.96	2.35-3.29	
		Group 3	2.66	2.18-3.30	
	T6	Group 1	2.52	2.24-3.27	0.488
		Group 2	2.98	2.30-3.59	
		Group 3	2.81	2.34-3.64	
	Salb	Group 1	2.71	2.52-3.30	0.963
		Group 2	2.71	2.33-3.37	
		Group 3	2.59	2.33-3.71	

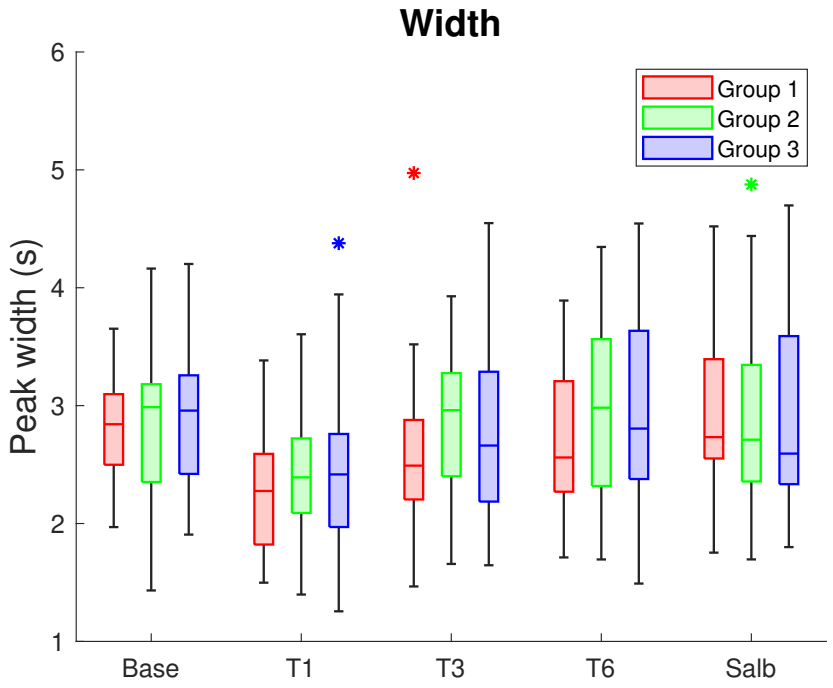

Figure A.2: Boxplots for EMG Width at Baseline, T1, T3, T6 and After salbutamol split in group 1 (FEV_1 decrease $\leq 6\%$) (n=23), group 2 (FEV_1 decrease between 6% and 24%) (n=23) and group 3 (FEV_1 decrease $\geq$ 24%) (n=24). Asterisks depict outliers.

The boxplots in Figure A.2 show a decrease in EMG Width at 1 minute after exercise for all three groups, with a return to baseline at 3 minutes after exercise. The statistical analysis presented in Table A.4, shows a statistically significant difference at 1 minute after exercise for all three groups. No other significant differences were found. This suggests children with more severe asthma do not have sustained higher respiratory rate after exercise.

Table A.4: Median, IQR and significance compared to baseline for EMG Width (s) for the 3 groups. Asterisks depict significant differences.

		Median	**IQR**	**Significance**
Group 1	**Base**	2.83	2.37-3.02	-
	T1	2.20	1.78-2.55	<0.001*
	T3	2.49	2.05-2.75	0.212
	T6	2.52	2.24-3.27	0.575
	Salb	2.71	2.52-3.30	0.681
Group 2	**Base**	2.99	2.35-3.19	-
	T1	2.39	2.05-2.75	0.024*
	T3	2.96	2.35-3.29	0.951
	T6	2.98	2.30-3.59	0.260
	Salb	2.71	2.33-3.37	0.715
Group 3	**Base**	2.96	2.42-3.26	-
	T1	2.42	1.94-2.78	0.022*
	T3	2.66	2.18-3.30	0.648
	T6	2.81	2.34-3.64	0.841
	Salb	2.59	2.33-3.71	0.841

A.2 EMG AUC

A.2.1 Analysis between controlled and non-controlled asthma

Table A.5 shows the result of the Mann-Whitney U test comparing the controlled and non-controlled asthma group. Statistically significant differences are found at T1, T3, T6 and after salbutamol, in accordance with the results for EMG Height.

Table A.5: Median, IQR and significances of EMG AUC compared between controlled and non-controlled asthma at Baseline, T1, T3, T6 and after salbutamol. Asterisks depict significant differences.

		Diagnosis	**Median**	**IQR**	**Significance**
AUC ($\mu V \cdot s$)	**Base**	Controlled	2.24	1.50-3.56	0.178
		Non-controlled	2.91	1.50-5.66	
	T1	Controlled	3.78	1.97-7.13	<0.001*
		Non-controlled	9.41	4.67-13.08	
	T3	Controlled	3.17	2.22-5.96	<0.001*
		Non-controlled	7.90	4.45-13.88	
	T6	Controlled	2.99	1.66-6.44	<0.001*
		Non-controlled	7.02	3.93-11.75	
	Salb	Controlled	2.44	1.31-3.37	0.004*
		Non-controlled	4.46	1.87-9.28	

The boxplots presented in Figure A.3 show an increase in EMG AUC 1 minute after exercise for both groups, that is more pronounced in the non-controlled asthma group. Furthermore, the AUC remains elevated for the non-controlled asthma group, while the controlled asthma group showed a trend towards baseline.

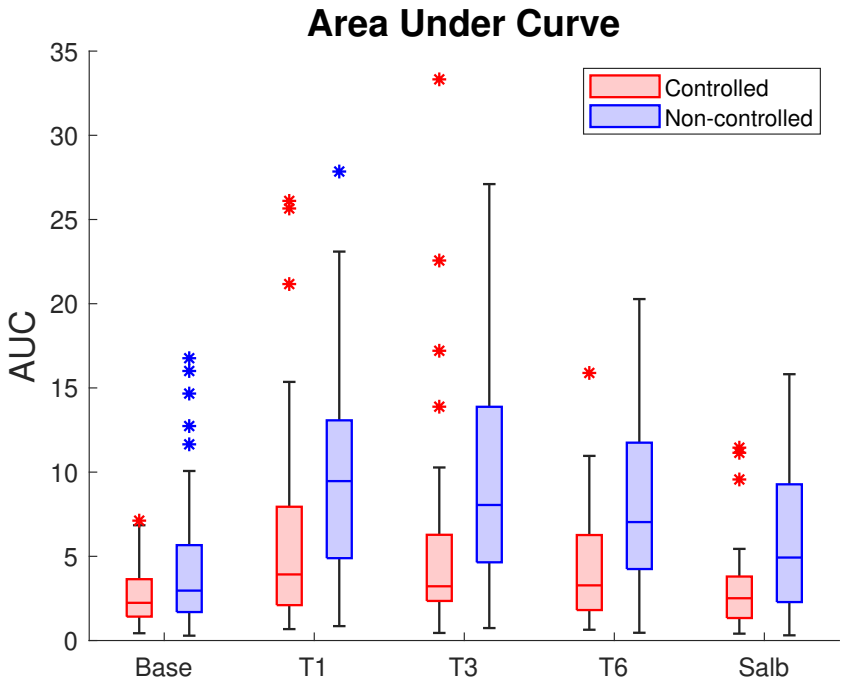

Figure A.3: Boxplots for EMG Area Under the Curve at Baseline, T1, T3, T6 and After salbutamol split in a controlled asthma (n=34) and non-controlled asthma (n=36) group. Asterisks depict outliers.

Comparing both groups to their baseline, as shown in Table A.6, statistically significant differences were found at T1, T3 and T6 for both controlled and non-controlled asthma. These results were similar to EMG Height, with the exception of a non-significant difference after salbutamol in the non-controlled asthma group. This can be explained by the lack of changes in the EMG Width, as the respiratory rate influences the EMG AUC, while it does not have an influence on EMG Height.

Table A.6: Median, IQR and significance compared to baseline for EMG AUC ($\mu V \cdot s$) in controlled and non-controlled asthma

		Median	IQR	Significance
Controlled	**Base**	2.24	1.50-3.56	-
	T1	3.78	1.97-7.13	<0.001*
	T3	3.17	2.22-5.96	<0.001*
	T6	2.99	1.66-6.44	0.009*
	Salb	2.44	1.31-3.37	0.465
Non-controlled	**Base**	2.92	1.50-5.66	-
	T1	9.41	4.67-13.08	<0.001*
	T3	7.90	4.45-13.88	<0.001*
	T6	7.02	3.93-11.75	<0.001*
	Salb	4.46	1.86-9.28	0.105

A.2.2 Analysis between three ranges of decrease in lung function

When the data was split into the three groups of equal size, statistically significant differences are found between groups at T1, T3 and T6, as shown in Table A.7. The boxplots in Figure A.4 show an increase in AUC after exercise, that was increasingly pronounced when the asthmatic reaction was more severe. This trend is seen troughout all measurements after exercise. Furthermore, an increase in AUC is still present after salbutamol for a more severe asthmatic reaction.

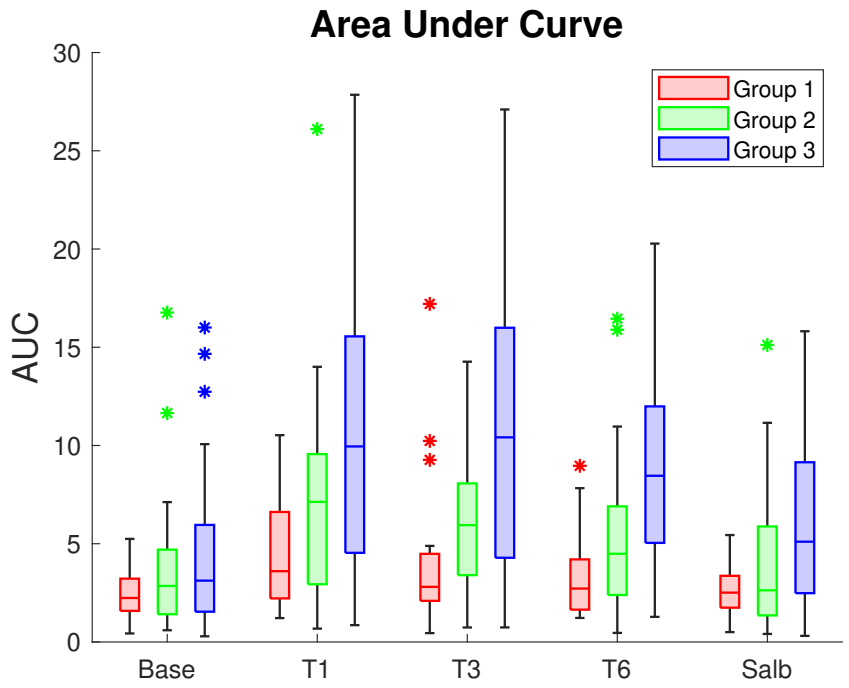

Figure A.4: Boxplots for EMG AUC at Baseline, T1, T3, T6 and After salbutamol split in group 1 (FEV_1 decrease $\leq$ 6%) (n=23), group 2 (FEV_1 decrease between 6% and 24%) (n=23) and group 3 (FEV_1 decrease $\geq$ 24%) (n=24). Asterisks depict outliers.

Table A.7: Median, IQR and significances of EMG AUC compared between the 3 groups at Baseline, T1, T3, T6 and after salbutamol. Asterisks depict significant differences.

		Group	Median	IQR	Significance
AUC ($\mu V \cdot s$)	**Base**	Group 1	2.39	1.55-3.22	0.492
		Group 2	2.62	1.35-4.39	
		Group 3	3.12	1.50-5.98	
	T1	Group 1	3.63	1.99-6.75	0.006*
		Group 2	7.13	2.85-9.63	
		Group 3	9.95	4.42-15.81	
	T3	Group 1	2.89	1.84-4.52	0.001*
		Group 2	5.95	3.22-8.08	
		Group 3	10.41	4.23-16.02	
	T6	Group 1	2.86	1.70-4.32	<0.001*
		Group 2	4.49	2.25-7.03	
		Group 3	8.46	5.02-12.05	
	Salb	Group 1	2.55	1.50-3.50	0.067
		Group 2	2.63	1.34-6.36	
		Group 3	5.10	2.38-9.21	

The statistically significant results between groups, as shown in Table A.7, are tested between groups using Mann-Whitney U tests. The results can be seen in Table A.8. Statistical significant differences between groups 1 and 2 were found for T3. Between groups 1 and 3, significant differences were found for T1, T3 and T6. Between groups 2 and 3, significant differences were found for T3 and T6. These results are in accordance with the results of EMG Height. They suggest a higher diaphragmatic activity after exercise in more severe asthmatic patients. Furthermore, the return to baseline is quicker if asthma is less severe.

Table A.8: Results of Mann-Whitney U tests between groups for statistically signficant different timepoints in EMG AUC. Table a. shows the results for T1, Table b. shows the results for T3 and Table c. shows the results for T6. Asterisks depict significant differences.

a. T1

	Group 2	**Group 3**
Group 1	0.093	0.003*
Group 2	X	0.067

b. T3

	Group 2	**Group 3**
Group 1	0.018*	0.002*
Group 2	X	0.032*

c. T6

	Group 2	**Group 3**
Group 1	0.197	<0.001*
Group 2	X	0.007*

When comparing the measurements to baseline within the same group, statistically significant differences are found at T1 for all groups and T3 and T6 for Group 2 and Group 3. This further amplifies the hypothesis a higher diaphragmatic activity is sustainably present in patients with more severe asthma, while patients with controlled asthma return to a normal diaphragmatic activity in 3 minutes after exercise.

Table A.9: Median, IQR and significance compared to baseline for EMG AUC ($\mu V \cdot s$) for the 3 groups. Asterisks depict significant differences.

		Median	IQR	Significance
Group 1	**Base**	2.39	1.55-3.22	-
	T1	3.63	1.99-6.75	0.003*
	T3	2.89	1.84-4.52	0.147
	T6	2.86	1.70-4.32	0.179
	Salb	2.55	1.50-3.50	0.970
Group 2	**Base**	2.62	1.35-4.39	-
	T1	7.13	2.85-9.63	0.002*
	T3	5.95	3.22-8.08	0.001*
	T6	4.49	2.25-7.03	0.021*
	Salb	2.63	1.34-6.36	0.986
Group 3	**Base**	3.12	1.50-5.98	-
	T1	9.95	4.42-15.81	<0.001*
	T3	10.41	4.23-16.02	<0.001*
	T6	8.46	5.02-12.05	<0.001*
	Salb	5.10	2.38-9.21	0.209

A.3 Comparison to spirometry

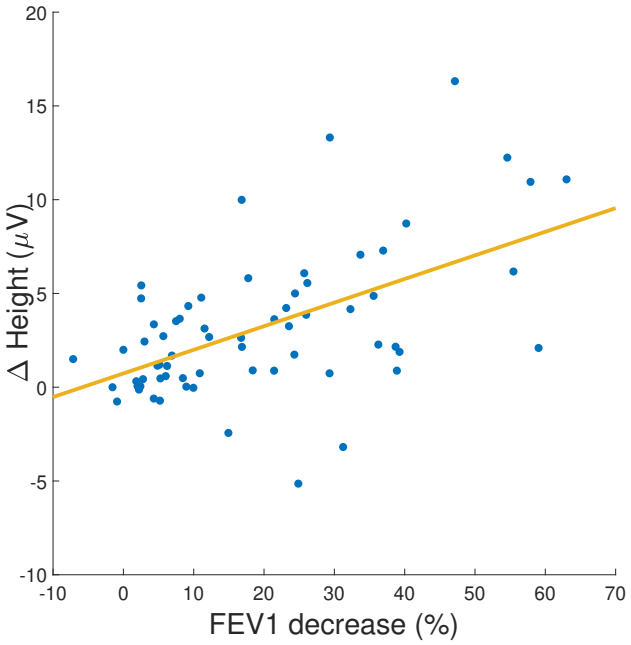

Figure A.5: Comparison of change in FEV_1 at maximal decrease after exercise and absolute change in EMG Height at T3, along with the linear regression trend line, yielding a coefficient of determination of 0.305.

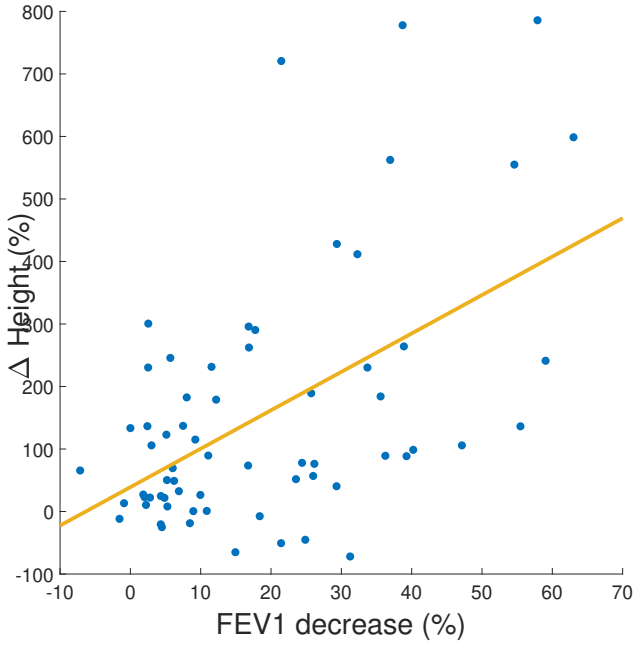

Figure A.6: Comparison of change in FEV_1 at maximal decrease after exercise and relative change in EMG Height at the same timepoint, along with the linear regression trend line, yielding a coefficient of determination of 0.281.

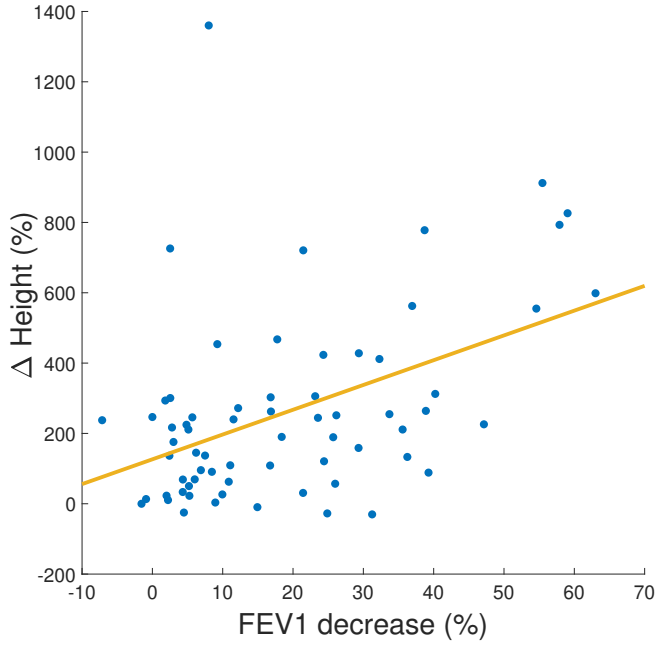

Figure A.7: Comparison of maximal relative change in EMG Height and change in FEV_1 at the same timepoint, along with the linear regression trend line, yielding a coefficient of determination of 0.202

Appendix B

Research protocol

Continuous EMG measurements in children with asthma during sleep

(April 2023)

- May 2015: adaptation section 11.5: text in accordance to old and new Measure regarding Compulsory Insurance for Clinical Research in Humans
- Sept 2015: adaptation section 9.1, 9.2 and 12.5: text in accordance to WMO amendment on reporting SAE and temporary halt (section 10 of WMO)
- Oct 2015: adaptation section 4.4 – comment [CCMO15], 8.2 and 10.1 with respect to methodology/statistics
- Sept 2018: adaptation section 12.1 and comment [CCMO46] due to applicability GDPR as of May, 2018

PROTOCOL TITLE 'Continuous EMG measurements in children with asthma during sleep'

Protocol ID	**EMGSleep**
Short title	**EMG in sleep**
EudraCT number	***Not applicable***
Version	**7**
Date	**21-04-2023**

Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	***Pascal Keijzer*** **P.Keijzer@mst.nl** ***053 – 487 2310***
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Sponsor (in Dutch: verrichter/opdrachtgever)	***Medisch Spectrum Twente*** ***Koningsplein 1*** ***7512KZ Enschede***
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PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department: **dr. L.G.M van Rooij** **Paediatrician**		
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AUC	**Area Under the Curve**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EMG	**Electromyography**
EudraCT	**European drug regulatory affairs Clinical Trials**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
ICD	**Implantable Cardioverter-Defibrillator**
ICU	**Intensive Care Unit**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
MST	**Medisch Spectrum Twente**
PIF	**Patient Information Form**
RIP	**Respiratory Inductance Plethysmography**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
SVM	**Support Vector Machine**
TMSi	**Twente Medical Solutions international**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Asthma is one of the most common chronic inflammatory diseases in children. One of the characteristics of asthma is that patients experience dyspnea during sleep, if the asthma symptoms are poorly controlled. The combination of poorly-controlled asthma and sleep disorders could be a cause of the lower quality of life experienced by children with poorly controlled asthma. Electromyography (EMG) is used as an experimental tool to measure the activity of the diaphragm in paediatric patients with asthma. EMG could provide easy, passive method for monitoring asthma during sleep.

Objective: To investigate whether electromyography of the diaphragm during sleep in asthmatic children can be used to objectively monitor asthma control.

Study design: The study will have a cross-sectional design.

Study population: Subjects aged 2 to 18 with paediatrician diagnosed asthma or bronchial hyperresponsiveness that are admitted to the paediatric ward of Medisch Spectrum Twente

Main study parameters/endpoints: The main study parameter will be the amplitude of the EMG signal. This corresponds to the maximal amount of action potentials in a single inhalation. This parameter will be compared before and after administration of medication.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The study provides no risks to subjects. The measurements will be done alongside regular care according to the current guidelines. There is no additional burden involved in this study.

1. INTRODUCTION AND RATIONALE

Asthma is one of the most common chronic inflammatory diseases in children.[1] Symptoms of asthma are shortness of breath, wheezing and coughing. It is characterised by bronchial hyperresponsiveness to various stimuli, resulting in diffuse airway obstruction. These stimuli include exercise, environmental pollutants such as pollen and external irritant factors such as cigarette smoke. The airway obstruction is reversible, either spontaneously or with adequate pharmacological treatment.[1,2] One of the characteristics of asthma is that patients experience dyspnea during sleep.[3]

Prevalence of asthma
In 2019, the total prevalence of asthma in the Netherlands was 586.000.[4] This number includes both children and adults. In children aged 1 till 14 in the Netherlands, prevalence of asthma ranges from 6 to 10 percent.[5,6]

Control of asthma
The main goal of treatment of paediatric asthma is good control of symptoms and reduction of risks, according to current asthma guidelines.[2] When asthma is poorly controlled, children may experience more asthma symptoms, resulting in a decreased lung function and higher rates of asthma exacerbations. This may cause a lower quality of life.[7] In Dutch secondary care, approximately 47% of children have poorly controlled asthma.[8] Asthma symptoms control can be achieved by the prescription of medication and a change in lifestyle to avoid triggers. Two types of medication can be administered, quick-relief medication and long-term asthma control (maintenance) medication. The maintenance medications should be taken regularly, to prevent the risk of an asthma attack. Furthermore, asthma control can be achieved by reducing the risk of exposure to external stimuli that can induce an asthma attack. The most well-known risk reducing factor is minimizing exposure to cigarette smoke.[8]

Asthma exacerbation
If asthma is not controlled effectively, exacerbations can occur. During an exacerbation, the bronchi produce larger amounts of mucus and the bronchial muscles will contract, resulting in narrowing of the airway.[1] When an exacerbation is not treated adequately or directly, hospitalisation may be required. Exacerbations are often the first presentation of asthma.[2] Exacerbations are mostly treated by administration of quick-relief medications to make sure the airways are open and oxygen flow can be assured. When the airflow is still limited after multiple administration of these medications, the use of prednisolone or dexamethasone can be considered. To prevent exacerbations in the future, the use of maintenance medication is highly recommended.[2,9]

Asthma and sleep
There is a strong relation between asthma and sleep disorders and quality in children.[10,11] These disorders are caused by symptoms of asthma occurring during the night. Exact causes are unknown, but it is discussed that bronchial hyperresponsiveness and airway resistance increase overnight.[12] This does not have a significant effect in healthy individuals, but can cause symptoms in asthmatic patients. Moreover, children experience difficulty falling asleep and continuing to sleep.[2] An effect of the sleep disorders is that patients experience excessive daytime sleepiness due to sleep deprivation[10]. The combination of poorly-controlled asthma and sleep disorders could be a cause of the lower quality of life experienced by children with poorly controlled asthma.

Respiratory Inductance Plethysmography
Respiratory Inductance Plethysmography (RIP) is a technique used to measure ventilation using movements of the thoracic and abdominal wall. [13] By applying two elastic bands

around the body at the height of the nipple line and umbilicus, expansion of these areas can be measured. [14] The elastic bands contain a sinusoidal wire coil, which is sensitive to longitudinal tension. The elastic bands are connected to a inductive transducer. The inductance of the coil changes when the length is altered, resulting in a frequency change. This change is used to monitor breathing patterns. [15] The use of RIP does not require cooperation of the patient.

Respiratory Electromyography
Electromyography (EMG) is a tool to measure the electrical activity of skeletal muscles. Muscles consist of multiple motor units, which can be activated and produce an electrical current. These currents themselves cannot be measured. However, the difference of these currents between two points, the electric potential, can be measured. Muscles generate these potentials when they contract.[16]
By applying two electrodes bilaterally on the thorax at the height of the diaphragm, EMG can be used to examine breathing patterns in asthmatic children [17,18]. A ground electrode is used to reference the bipolar electrodes. The EMG signals need to be processed before a respiratory waveform can be observed. Cardiac activity and motion artefacts need to be removed and the signal needs to be smoothed using moving average filtering.[19]
Exercise-induced bronchoconstriction can be identified by surface EMG measurements of the diaphragm.[20] Several parameters derived from EMG are strongly related to FEV1.
One of the major downsides of using EMG is that it is highly sensitive to disturbances and artifacts. Slight movements of the subject and speech result in large potential differences. This will most likely be a major challenge when EMG is measured during the night, as children will not lay still during sleep.

Rationale
Electromyography is used as an experimental tool to measure the activity of the diaphragm in paediatric patients with asthma.[17,18,20–22] In current measurements protocols however, subjects need to be stationary, because slight movements could cause unreadable measurements. During sleep, children might move frequently, causing movement artifacts. Furthermore, different positions during sleep cause different potentials in the EMG. EMG during the night could give new insights in asthma control. Certain asthma symptoms could be detected and certain EMG parameters could predict changes in breathing patterns. EMG could be used as a simple tool to implement in home monitoring of asthma, especially if electrodes would be wireless.

2. OBJECTIVES

Primary Objective: To investigate whether electromyography of the diaphragm during sleep in asthmatic children can be used to objectively monitor asthma control.

Secondary Objective(s):

1. To determine whether EMG measurements are feasible in paediatric patients during the night
2. To investigate if parameters of EMG are related to parameters of RIP
3. To determine differences in EMG parameters before and after admittance of medication
4. To investigate whether clinical worsening of symptoms of asthma during the nights can be predicted using EMG parameters

Feasibility of EMG measurements will be determined by analysing the EMG data. EMG measurements are highly sensitive and movement artifacts could make a measurement unreadable. We aim to investigate whether EMG measurements of the diaphragm in a long period of time during the night are suitable for analysis.

Children with proper asthma control will not experience problems during sleep. However, when asthma is poorly controlled, sleep can be disturbed. We aim to investigate whether respiratory signs such as coughing and dyspnea can be detected using EMG. EMG data will be compared with RIP. This technique will be used to determine if signals measured with EMG can be compared to the respiratory rate measured with RIP.

Administration of quick-relief medication is one of the strategies used in asthma care in patients admitted to the paediatric ward. A patient often experiences more difficulty breathing before the admittance of the medication compared to after the admittance. We aim to investigate if changes can be found in EMG parameters before and after admittance of these quick-relief medications. These EMG parameters will be related to the clinical asthma score.

Prediction of clinical worsening of symptoms of astham during a hospital admittance could result in a more adequate treatment during the stay. Using EMG, we aim to be able to predict this worsening minutes before it actually occurs.

3. STUDY DESIGN

The study will have a cross-sectional design. Subjects admitted to the paediatric ward of Medisch Spectrum Twente will be included. Subjects will be equipped with a portable EMG amplifier and RIP shirt before sleep. This study will be performed alongside normal care.

We aim to start inclusion in October 2022. Measurement devices and software are available.

4. STUDY POPULATION

4.1 Population (base)

The research population consists of children with paediatrician diagnosed asthma or bronchial hyperresponsiveness. When they are admitted to the paediatric ward of Medisch Spectrum Twente, patients are asked to be included. Minimal age is set at 2 years old, provided that they can fit in an available Hexoskin shirt. Both male and female patients are included.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Paediatrician diagnosed asthma or bronchial hyperresponsiveness
- Aged 2 till 18
- Admitted to the paediatric ward of Medisch Spectrum Twente

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Admittance to the ICU
- Patient has ICD or pacemaker
- Parents and/or patient cannot understand or speak Dutch
- Does not fit in one of the available Hexoskin shirts

4.4 Sample size calculation

For this study a sample size of 20 is chosen.

The required sample size is calculated on data from a study performed by Keijzer et al [29]. Sample sizes are based on the difference in EMG peak height at lowest spirometry. Alpha was set to 0.05 and beta to 0.90. A higher statistical power was chosen because the study is performed on children, urging a higher certainty.

The mean peak EMG height difference for subjects with uncontrolled asthma at lowest spirometry was 4.85 µV, with an estimated SD of 4.95 µV. Calculation revealed that 15 subjects were required for significant statistical results.
A higher sample size is chosen because the measurements taken during this study will not guarantee a subject has an asthma attack during sleep.

The sample size calculation has been discussed with and was conducted with the aid of one of the epidemiologists at Medisch Spectrum Twente.

5. TREATMENT OF SUBJECTS

This section does not apply to the proposed study, as it is not an intervention study

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

During this study, we will use the Mobi-6 amplifier (Twente Medical Systems international). This is an amplifier that can be used for the acquisition of (electro-) physiological signals. Electric signals can be measured via bipolar inputs on the device via electrode leads connected to disposable electrodes. Data is transferred via a Bluetooth connection to the computer for viewing or further analysis.

Respiratory Inductance Plethysmography (RIP) will be measured with the Hexoskin shirt (Carré Technologies Inc, Montreal, Canada). This is a stretchable shirt with built-in sensors that is commercially available. These sensors include two RIP belts, which can be used to monitor respiration. These belts are positioned at the height of the thorax and abdomen. Data is anonymously synchronised to a server and can be downloaded for further analysis.

7.2 Summary of findings from non-clinical studies

Not relevant for the Mobi-6 amplifier. The intended use of this device is to perform physiological measurements in a clinical setting.

The Hexoskin has been validated in a laboratory setting.[30] It is found that respiratory rate can be suitably measured using a Hexoskin shirt.

7.3 Summary of findings from clinical studies

The TMSi Mobi has been used in a clinical study in the proposed setting at MST approved by the METC Twente (nr. K19-29) and is listed in the Dutch trial register (Trial NL7850). The TMSi Mobi is currently used in a clinical study at MST approved by the MEC-U and is listed in the Dutch trial register (Trial NL9364).
Two other devices have been used to measure respiratory EMG in the proposed setting, the Porti device (Twente Medical Solutions international)[18,24-27] and Dipha (Demcon Macawi Respiratory Systems) [28,29].

In the past years, the Hexoskin shirt is tested in numerous studies. The studies vary in subject characteristics (cardiac, cognitive state, respiratory, sleep and stress). In most of the respiratory studies, the Hexoskin shirt is used as the 'gold' standard and the parameters are assumed to be stable and reliable [31–34].

7.4 Summary of known and potential risks and benefits

No risks are to be expected. The Mobi device collects data passively and sends it to a computer using Bluetooth.

No potential risks of wearing the Hexoskin shirt are known and expected. There are no potential risks mentioned or found in studies involving the Hexoskin shirt.

Expected benefits are for future settings. No interventions will be done based on the findings during or after measurements.

7.5 Description and justification of route of administration and dosage

Not applicable

7.6 Dosages, dosage modifications and method of administration

Not applicable

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable

7.8 Drug accountability

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The main study parameter will be the amplitude of the EMG signal. This corresponds to the maximal amount of action potentials in a single inhalation.

8.1.2 Secondary study parameters/endpoints (if applicable)

- The area under the curve (AUC) of the EMG signal. This is related to the total electrical activity measured. This is a secondary parameter to the amplitude.
- The peak height of the EMG signal. A sudden change in the height of the EMG signal can be related to coughing. This is a secondary parameter to the amplitude.
- The respiratory rate, which can be calculated from the EMG signal and RIP. This can give insight in when a subject experiences shortness of breath, a symptom of an asthma attack. Furthermore, respiratory rate will be used to correlate signals measured with EMG and RIP.
- Comparison of the EMG parameters before and after administration of medication.

8.1.3 Other study parameters (if applicable)

Not applicable

8.2 Randomisation, blinding and treatment allocation

All subjects are submitted to the ward and will undergo treatment following the current guidelines. Randomisation, blinding and treatment allocation are not applicable.

8.3 Study procedures

Children with paediatrician diagnosed asthma or bronchial hyperresponsiveness that spend the night at the paediatrics ward of MST will be asked to participate in the study. When informed consent is obtained, the patient will be included. The ground electrode will be positioned on the sternum using stick-on electrodes. The bipolar leads will be positioned bilaterally on the diaphragm using the same kind of electrodes. Leads will be attached to the EMG amplifier.
A fitting Hexoskin shirt will be chosen and worn by the subject. The EMG amplifier will be worn by the patient in the pouch provided by TMSi.
Measurements will be started before the patient is going to sleep and end the next morning, when the patient awakes. When the patient is admitted to the ward while sleeping, the measurement will be started when the patient arrives at the ward. Sleepless periods during the night will be part of the measurements. The measurement ends the morning after the measurement is started, when the patient is discharged or transferred or if the batteries of the amplifier run out of power.

The subject will undergo care following the current guidelines during the measurements. During the measurements, the clinical asthma score will be obtained. When medication is administered, this will be recorded. The most likely form of medication is the admittance of 400 ug salbutamol, or nebulisations of 2.5-5 mg of salbutamol.

EMG parameters will be obtained from epochs containing no artefacts for the duration of 1 minute, within 10 minutes before and after admittance of the medication. An artefact-free epoch as close to the admittance as possible will be used.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

Withdrawal of individual subjects is not expected, because the measurements will be taken alongside standard care. If a subject withdraws from the study, they can be replaced. Due to the broad inclusion criteria, it is expected that it does not impact study outcomes.

8.6 Follow-up of subjects withdrawn from treatment

Not applicable

8.7 Premature termination of the study

Premature termination of this study is not expected due to the non-invasive technique used. The technique does not impose risks to the subject.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to [the investigational product / trial procedure/ the experimental intervention]. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Due to the risk-free nature of this study, no DSMB will be installed.

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

All EMG parameters will be obtained from epochs containing no artefacts for the duration of 1 minute, within 10 minutes before and after admittance of the medication. An artefact-free epoch as close to the admittance as possible will be used.

EMG peak amplitude will be compared pre and post administration of medication using Paired T-tests or Wilcoxon Signed-rank tests, determined by the distribution of the variables. Normal distribution of all parameters will be determined using Shapiro-Wilk tests. Non-normal distributed variables will be analysed using Wilcoxon Signed-rank tests and normal distributed variables will be analysed using Paired T-tests. The EMG peak amplitude will be related to the clinical asthma score. Only the data from the first administration of medication will be used per patient. The clinical asthma score will be determined shortly before and 10 minutes after the administration of medication. It will be analysed if there is a relationship between the score and the EMG parameter before administration of medication using linear regression. A similar analysis will be done after the administration of medication.

10.2 Secondary study parameter(s)

Respiratory rate from EMG and RIP will be analysed at every minute during the measurements. Pearson's Correlation Coefficient will be calculated within subjects to analyse if respiratory rate measured with the two techniques is correlated.
EMG AUC and peak height will be analysed pre and post administration using Paired T-tests or Wilcoxon Signed-rank tests, determined by the distribution of the variables.

A machine learning algorithm will be created to be able to investigate whether changes in the EMG parameters in the time window from one hour before administration of medication till 10 minutes before, can predict the asthma score calculated before administration of medication. To be able to predict good outcomes, the asthma scores will be presumed good at from 70 minutes after administration of the medication, provided the child is sleeping comfortably and respiratory rate is in the normal range for the age group. Normal ranges are less than 30 min^{-1} for children aged 4 and 5, less than 26 min^{-1} for children between 6 and 12 years old and less than 23 min^{-1} for children aged 13 and above. EMG parameters will be obtained from 1 hour till 10 minutes before this time period. For the prediction analysis, a Support Vector Machine (SVM) will be constructed, with the clinical asthma score as the outcome. The parameters used as input are the parameters derived from the EMG signal.

10.3 Other study parameters

Not applicable

10.4 Interim analysis (if applicable)

One of the goals of this study is to investigate whether the use of EMG in a measurement during the night is feasible. Feasibility in this study is determined by analysing the data to investigate if artefacts distort the signal to such a degree that it is deemed unreadable. Respiratory rate will be analysed to investigate this feasibility. The correlation between the respiratory rates measured from EMG and RIP needs to be high. The correltation is deemed high if the correlation coefficient is higher than 0.75. A signal is declared unreadable if from 30% or more of the signal no respiratory rate can be calculated and if this respiratory rate is not correlated to the respiratory rate obtained by the RIP measurements. Conclusively, if 70% of the data within the patients is valid, the data within this patient is deemed feasible. All first 5 included subjects need to have valid data (70% or more) for the study to be deemed feasible. If feasibility is found to be insufficient after the first five subjects, the study will be terminated.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (7th revision ,2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

Subjects will be recruited from the paediatrics ward of Medisch Spectrum Twente in Enschede. Subjects between the age of 2 and 18 with paediatrician diagnosed asthma are eligible for participation. Potential subjects will be asked to participate in the study after admittance to the ward. PIFs and informed consent forms will be handed over to parents and child after arrival at the ward and information about the measurements will be given by the researcher. Measurements start when the subject goes to sleep. Time of consideration is between this point and the handing out of the forms.
Before the measurements start, the subject and parents will be asked whether they understood the information and if they have any questions. If both parents and subject agree to participate in the study, informed consent forms will be signed. Both parents and subject are allowed to ask for discontinuation of the measurements during the night. The measurements will then be terminated.
The following day, after the measurements are completed, a second informed consent is asked. If informed consent is not obtained, the subject will be excluded. A form to revoke informed consent will be handed to the subject and/or parents to be able to revoke the informed consent.

11.3 Objection by minors or incapacitated subjects (if applicable)

For this study, children between the ages of 4 and 18 will be included. Therefore, the code conduct for research with minors is applied. The study will be executed in accordance to this code of conduct.

Children will be informed about the goals and progress of this study. This is done by providing a PIF specified for children combined with images. Children under the age of 13 will be provided with an additional simplified PIF.

Both the subject and their parents are allowed to withdraw from the study during the measurements. This is also stated in the PIF and informed consent forms. Children under the age of 13 that have not signed an informed consent are still allowed to withdraw.

11.4 Benefits and risks assessment, group relatedness

The study provides no risks to subjects. The measurements will be done alongside regular care according to the current guidelines. There is no additional burden involved in this study. Children may be allergic to the stick-on electrodes, resulting in a rash.

The benefits of this study involve an easy, passive method for monitoring asthma during sleep. The measurement can be applied to obtain objective information about lung function, without the need of waking a child to perform spirometry. EMG might be applicable in early detection of asthma attacks and could be used as a home monitoring tool in asthma control.

There are no benefits at this time for participation in this study.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

11.6 Incentives (if applicable)

Participants will not be rewarded for participation.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Handling of personal data will comply with the European General Data Protection Regulation and Dutch Act on Implementation of General Protection Regulation (AVG, UAVG). Subjects will be entered into the database in chronological order. This data will be stored without patient's initials, patient number and date of birth. All patient data will be stored for 5 years in accordance to European and Dutch legislation. The investigators will have access to the source data. The key of the coding will also be safeguarded by the head investigator separate from the study data in the personal digital folder of the lead investigator at Medisch Spectrum Twente.

EMG data is saved on a password protected laptop used only for research purposes. Measurements are conducted using the same laptop, no data transfer is required. Hexoskin data is synchronised with an external server and is downloaded onto the same password protected laptop.
The investigators will have access to the data in order to perform analyses.

12.2 Monitoring and Quality Assurance

This is an investigator-initiated study and therefore study monitoring is under responsibility of the investigational site.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

We intend to publish the results in a thesis written by the coordinating investigator to obtain a Master's degree in Technical Medicine at the University of Twente. Furthermore, we intend to publish the results in a medical or scientific journal. On the website of the CCMO (www.ccmo.nl) the CCMO statement on publication policy can be found. This statement contains the basic principles of the CCMO's position of the disclosure/ publication of research results obtained from studies involving human subjects.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action
Not applicable

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism
Not applicable

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?
Not applicable

d. Selectivity of the mechanism to target tissue in animals and/or human beings
Not applicable

e. Analysis of potential effect
Not applicable

f. Pharmacokinetic considerations
Not applicable

g. Study population
Children with paediatrician diagnosed asthma or bronchial hyperresponsiveness

h. Interaction with other products
Not applicable

i. Predictability of effect
Not applicable

j. Can effects be managed?
Not applicable

13.2 Synthesis

We anticipate no additional risks for subjects in this study. During a stay in the paediatric ward, a paediatrician will always be available and registered nurses are present.

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