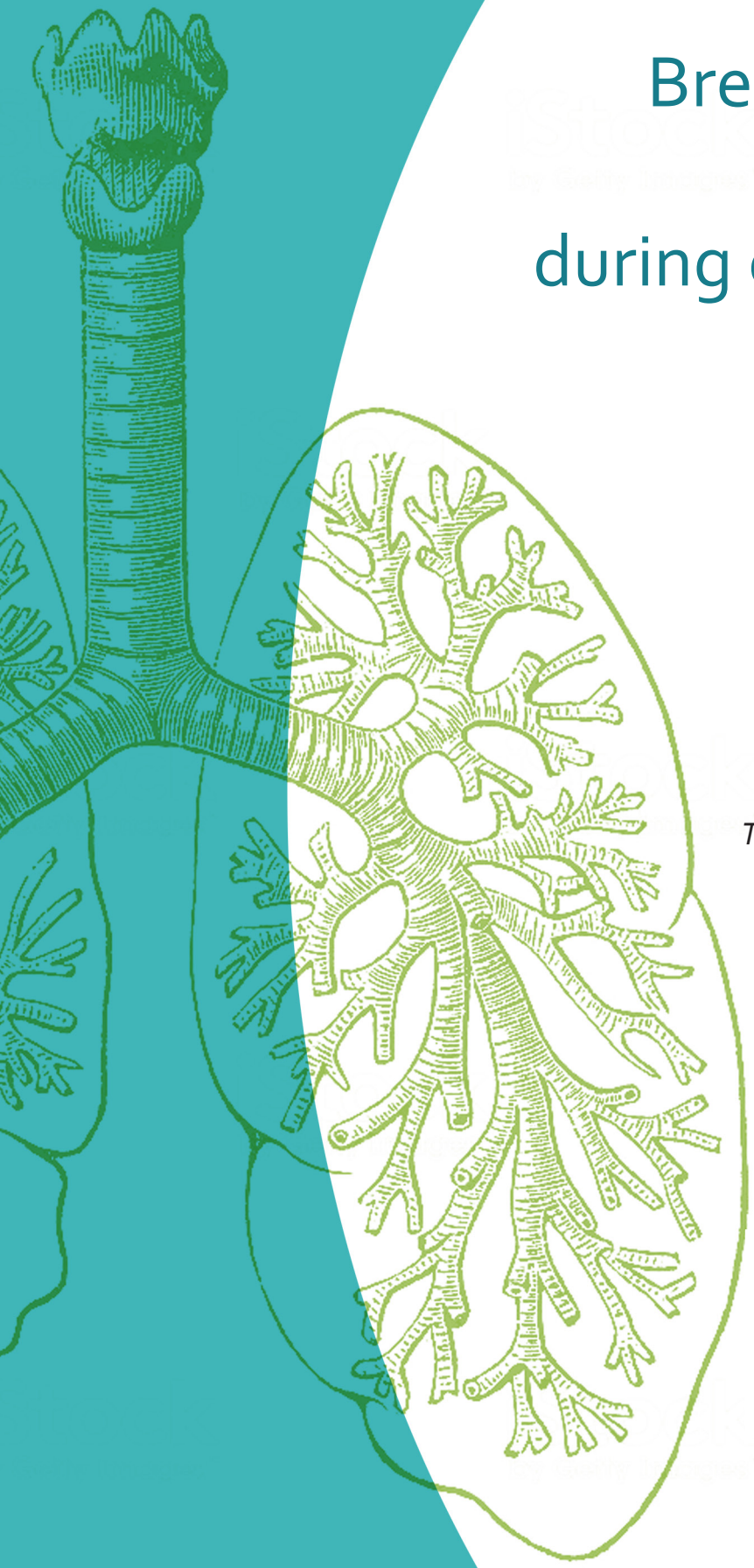

Breathing patterns of asthma patients during cycling ergometry

Arjen Pelgröm

Master thesis

27-10-2021

Examination Committee

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een santeon ziekenhuis

UNIVERSITY OF TWENTE.

You cannot teach a man anything; you can only help him discover it in himself

Galileo Galilei

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Bedankt allemaal!

Abstract

Introduction

In the asthma population, 29% of patients suffer from dysfunctional breathing (DB) often leading to exercise induced dyspnoea (EID). Most of the time, EID is attributed to exercise induced bronchoconstriction (EIB), but the possible effect of DB on EID in patients with asthma is often overlooked, potentially leading to suboptimal treatment. Therefore, this study aims to investigate whether asthma patients have different, and possibly dysfunctional, breathing patterns than healthy subjects. Additionally, it will be investigated whether breathing patterns can be quantified and related to certain forms of DB.

Methods

Breathing patterns of 15 healthy subjects and 12 asthma patients were analysed in rest and during maximal cycling ergometry using the Hexoskin sports vest with integrated respiratory inductance plethysmography (RIP) sensors. Subjects were classified in different groups based on the change of the new parameter BPratio (which is the tidal volume (Vt) divided by the breathing frequency (Bf) and the predicted vital capacity (VCpred)) and Ldis (new parameter to quantify the level of asynchrony of breathing) as a result of increasing exercise load.

Results

Based on the BPratio subjects could be distributed in three groups according to the change in contribution of Vt and Bf. Asthma patients with worse asthma control than others (ACQ of 1.6 vs 0.14 ($p=0.137$), VAS for dyspnoea over past 7 days 16.5mm vs 2.0mm ($p=0.041$), and mini-AQLQ scores of 4.65 vs 6.67 ($p=0.111$)) tend to increase their Bf earlier than those with better asthma control. Based on the level of asynchronous breathing, quantified with Ldis, subjects with highly increasing Ldis during physical exercise showed a lowered performance level, while subjects with an almost stable Ldis showed elevated levels of dyspnoea at the end of exercise (VAS 91.5mm vs 52.9mm, $p=0.058$). These phenomena were more common in patients with asthma than in healthy subjects (33% vs 7%).

Discussion

It is hypothesized that subjects with worse asthma control developed a deviant natural breathing pattern (earlier increase of Bf) as a response to elevated stress perception. The differences in Ldis among subjects showed that both lowered and increased levels of asynchrony might be a sign of DB.

Conclusion

Based on Ldis it appears that asthma patients more often show deviant breathing patterns. To quantify the breathing patterns, this study introduces two new parameters (BPratio and Ldis). Subjects with deviant breathing patterns based on these parameters showed indications of dysfunctional breathing. This gives promising possibilities to analyse breathing patterns for the diagnosis of DB in asthma patients using these parameters, which could result in more optimal treatment of asthma patients suffering from EID.

List of abbreviations

ACQ	Asthma control questionnaire
ANOVA	Analysis of variance
AU	Arbitrary units
Bf	Breathing frequency
BMI	Body mass index
BPgroup	Group op subjects based on BPratio
BPratio	Breathing pattern ratio
CPET	Cardiopulmonary exercise test
DB	Dysfunctional Breathing
EIB	Exercise induced bronchoconstriction
EID	Exercise induced dyspnoea
FEV1	Forced expiratory volume in the first second
FVC	Forced vital capacity
HR	Heart rate
HRR	Heart rate reserve
Ldis	Lissajous distribution
LISgroup	Group of subjects based on Ldis
Mini-AQLQ	Mini-asthma quality of life questionnaire
QDC	Qualitative diagnostic calibration
RIP	Respiratory inductance plethysmography
RER	Respiratory exchange rate
VAS	Visual analogue scale
VCpred	Predicted vital capacity
V'CO2	Carbon dioxide production per minute
V'E	Expiratory minute volume
V'O2	Oxygen consumption per minute
Vt	Tidal volume

Contents

Chapter 1: Introduction

In 2017 roughly 273 million people worldwide suffered from asthma[1]. Asthma is defined by the Global Initiative for Asthma as follows: "*Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation."*[2] Symptoms are triggered and worsened by bronchial hyperresponsiveness caused by for example allergens, viral infections or non-allergic factors like medication, cold air or exercise. General treatment of asthma consists of maintenance medication with inhaled corticosteroids together with β2-agonists and lifestyle changes. Lifestyle changes are for example quitting smoking and avoidance of the earlier mentioned triggers.

Conversely, recent studies show that one trigger, physical exercise, should not be avoided, but must be encouraged[3-5]. Physical exercise in patients with asthma often triggers exercise induced dyspnoea (EID). As a result of this, patients with asthma may abstain from physical exercise leading to a worse physical condition, which consecutively leads to a greater manifestation of asthma symptoms[3–6]. As a result, the patients experience more dyspnoea during physical exercise and thus perform even less physical exercise, bringing them into a vicious cycle[5].

A common cause of EID is exercise induced bronchoconstriction (EIB). The prevalence of EIB in patients with asthma is estimated at 90%, therefore, EIB is one of the main contributors to EID in patients with asthma[7].

Another significant contributor to EID, but sometimes overlooked, is the breathing pattern[8]–[10]. An atypical breathing pattern that may lead to medical complaints is called dysfunctional breathing (DB). Notable is that the prevalence of DB is much higher in asthma patients than in the general population (29% vs 8%)[11]. Taking into consideration that DB is a common contributor to EID this strongly suggests that DB contributes to EID in patients with asthma.

The breathing pattern describes the strategy of a subject to increase their expiratory minute volume (V'E). In this study this is defined as the relative contribution over time of breathing frequency (Bf), tidal volume (Vt), abdominal breathing, and thoracic breathing to the V'E. In this way, the breathing pattern of patients with asthma during exercise has never been properly investigated. A small number of studies has been performed, but none of these studies describe the whole breathing pattern including the relative contribution of abdominal and thoracal breathing. For example, Azab et al. describes the Bf and Vt and spirometry parameters like forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) during exercise[12]. However, this is not compared to healthy subjects and no distinction was made between abdominal and thoracic breathing and the presence of DB was not investigated[12]. Nield et al. does compare the Bf and Vt of patients with obstructive lung disease to patients with restrictive lung disease and healthy subjects[13]. They also investigated several parameters at different percentages of maximal exercise, which is very useful to investigate whether Vt or Bf increases first. However, they do not differentiate between thoracic and abdominal breathing and use a very small sample size of 13 patients with obstructive lung disease and 7 patients with restrictive lung disease[13].

To the best of our knowledge, the analysis of breathing patterns of asthmatic patients during exercise with the distinction between thoracic and abdominal breathing has not been reported. An exercise challenge test in combination with respiratory inductance plethysmography (RIP) bands will be used to determine the breathing pattern during exercise. These bands are used to continuously measure the circumference of the chest, which can be used to quantify the breathing

pattern by calculating the Bf and Vt[14]. To comfortably measure breathing patterns (among other parameters) during physical exercise, Carré technologies inc. (Montréal, Canada) developed a smart shirt called Hexoskin[15]. This vest uses RIP technology and will be used during the exercise challenge test to measure the breathing patterns of asthma patients during exercise.

Rationale

The breathing pattern during exercise of patients with asthma is currently unknown. Nevertheless, for a substantial number of patients with asthma (29% of patients with asthma suffer from DB) an atypical breathing pattern is expected to be an important factor that induces EID. A lack of knowledge about their breathing pattern and the effect of this on EID makes that these patients are probably not receiving optimal treatment.

Study Aim

This study aims to investigate the breathing patterns that occur in patients with asthma during exercise and whether these differ from the patterns observed in healthy subjects. Additionally, the effect of different breathing patterns of patients with asthma on the level of EID will be investigated. The research questions are:

"What is the breathing pattern of patients with asthma during exercise, how does this differ from the breathing pattern of healthy subjects during exercise and what is the influence of the breathing pattern on the level of exercise induced dyspnoea".

Chapter 2: Background

Anatomy of the respiratory system

The respiratory system consists of the upper respiratory tract and the lower respiratory tract. The upper respiratory tract is the part of the airways above the vocal cords, consisting of the nostrils, nasal cavity, oral cavity, and pharynx (Figure 1). At the level of the larynx the distinction between upper and lower respiratory tract is made. The lower respiratory tract consists of the trachea, bronchi, bronchioles, and alveoli. The trachea splits into the left and right main bronchus, which split into respectively two and three secondary bronchi (one for every lobe of the lungs). Going deeper into the lungs, the airways continue to bifurcate, creating new so-called generations with the trachea being the zeroth generation, the main bronchi the first generation, and so on. As depicted in Figure 2 humans have approximately 23 generations of airways. Until the sixteenth generation the airways only conduct air and are therefore called, together with the upper airways and pharynx, the conducting airways. The conducting airways together contain 100-150ml of air. This volume is not participating in gas exchange and is called the anatomical dead space. From the seventeenth generation onwards, alveoli are present making gas exchange with the blood possible. These generations are called the respiratory airways.

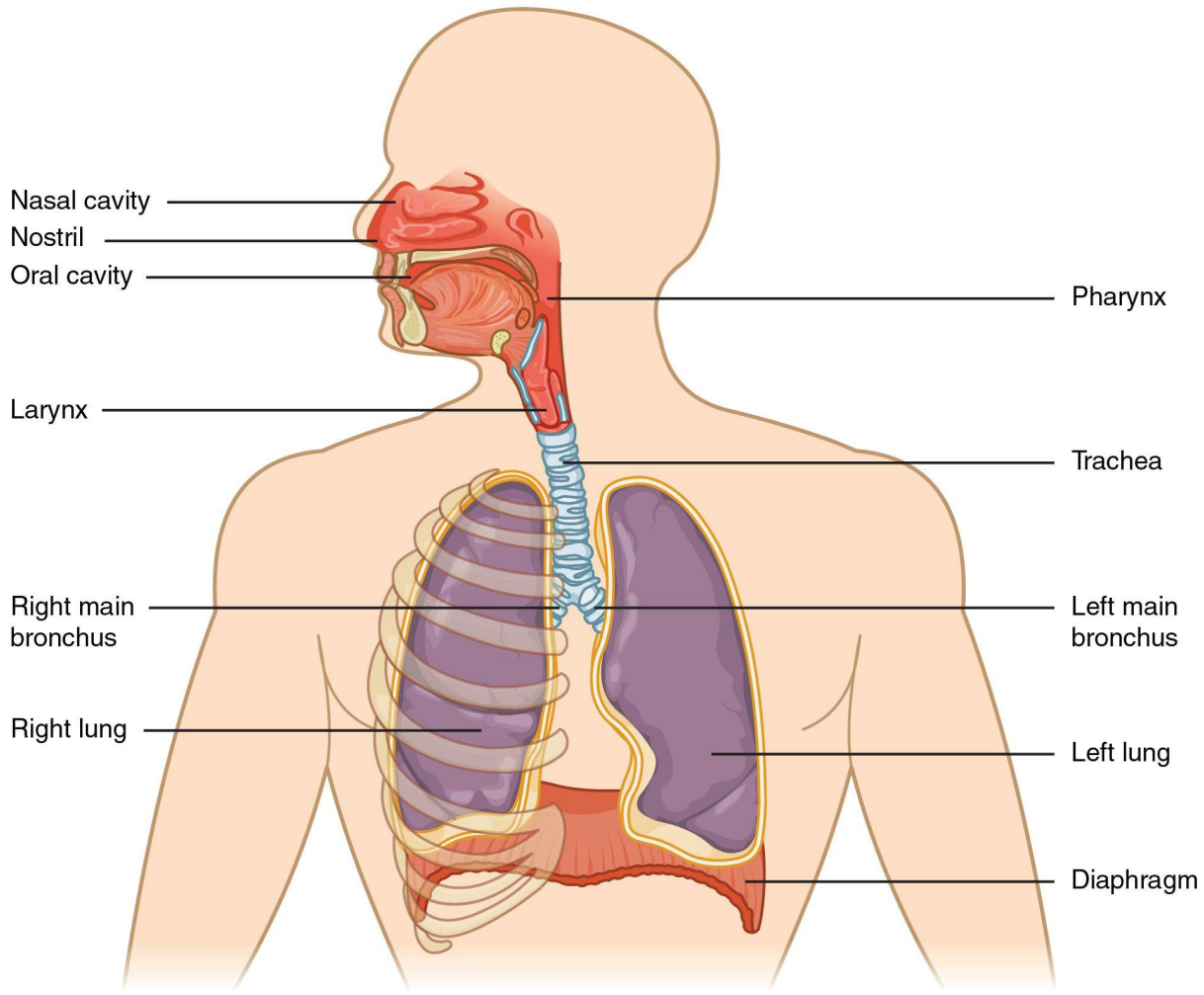

Figure 1: Schematic overview of the respiratory system[16]

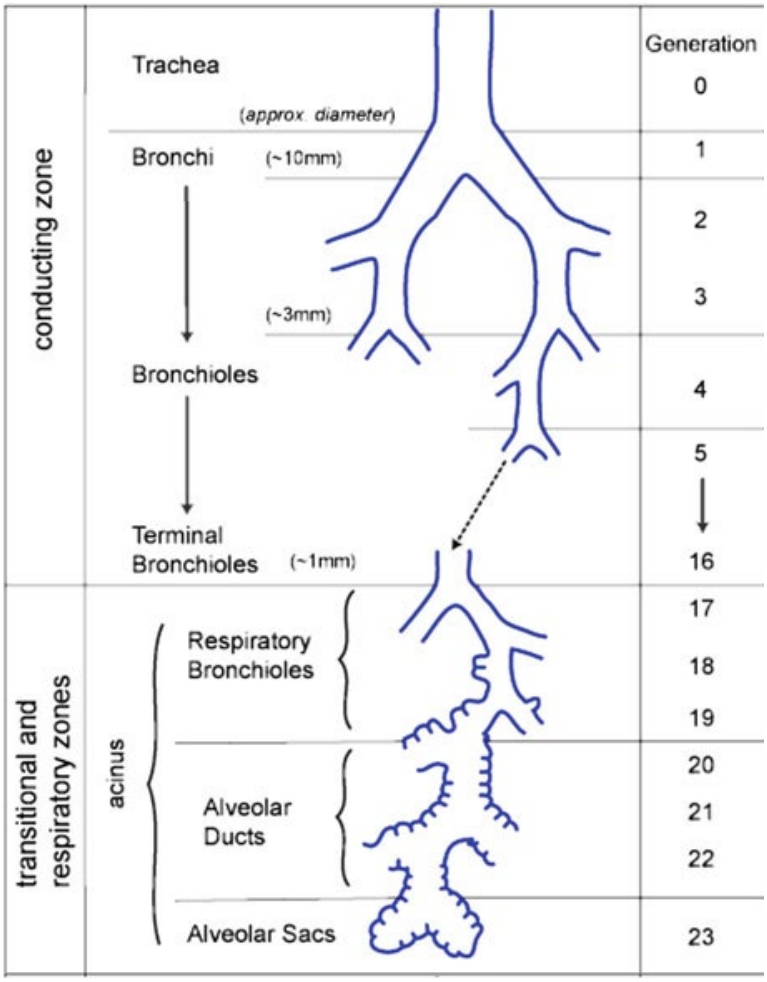

FIGURE 2: SCHEMATIC OVERVIEW OF THE AIRWAY GENERATIONS [17]

Breathing physiology

Air enters the lungs via the nose and mouth and is heated, humidified, and filtered in the nasal space. The air flows deeper into the lungs via the conductive airways until it enters the respiratory zones of the lungs were gas exchange with the blood takes place. This gas exchange is a passive process, where oxygen diffuses from the airways into the blood and carbon dioxide diffuses from the blood into the airways after which the carbon dioxide is exhaled.

Breathing is initiated by increasing the volume in the chest, which results in a negative pressure in the chest causing air to flow in from outside the body. In rest, the major mechanism to increase chest volume is the rise in the chest cavity's rostral-caudal diameter (vertical), which is caused by a downwards movement of the diaphragm as shown in Figure 3. When larger flows of air are required, for example during exercise, accessory muscles are used; the external and internal intercostal muscles increase thoracic volume by increasing the transverse diameter, using the so called bucket-handle and water-pump-handle effect as shown in Figure 4. Exhalation during rest in a healthy person is a passive process caused by the relaxation of the inspiratory muscles (mainly diaphragm, Figure 3) and the elastic recoil of the lungs. During forced expiration (for example during exercise) accessory expiratory muscles are used as shown in Figure 4. These muscles are often also used during rest by patients with an obstructive lung disease like asthma or COPD.

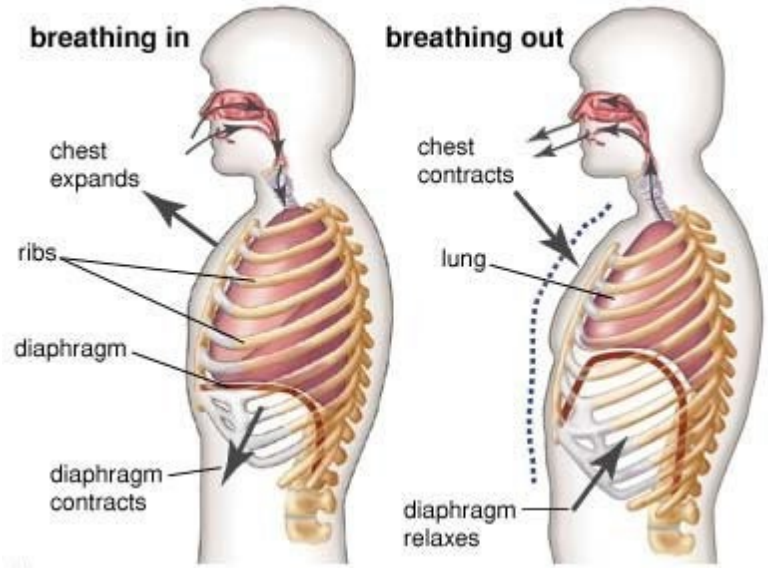

FIGURE 3: DURING INSPIRATION THE THORACIC VOLUME IS INCREASED BY CONTRACTION OF THE DIAPHRAGM AND CHEST EXPANSION. DURING EXPIRATION THE THORACIC VOLUME IS DECREASED DUE TO RELAXATION OF THE DIAPHRAGM AND CHEST CONTRACTION [18]

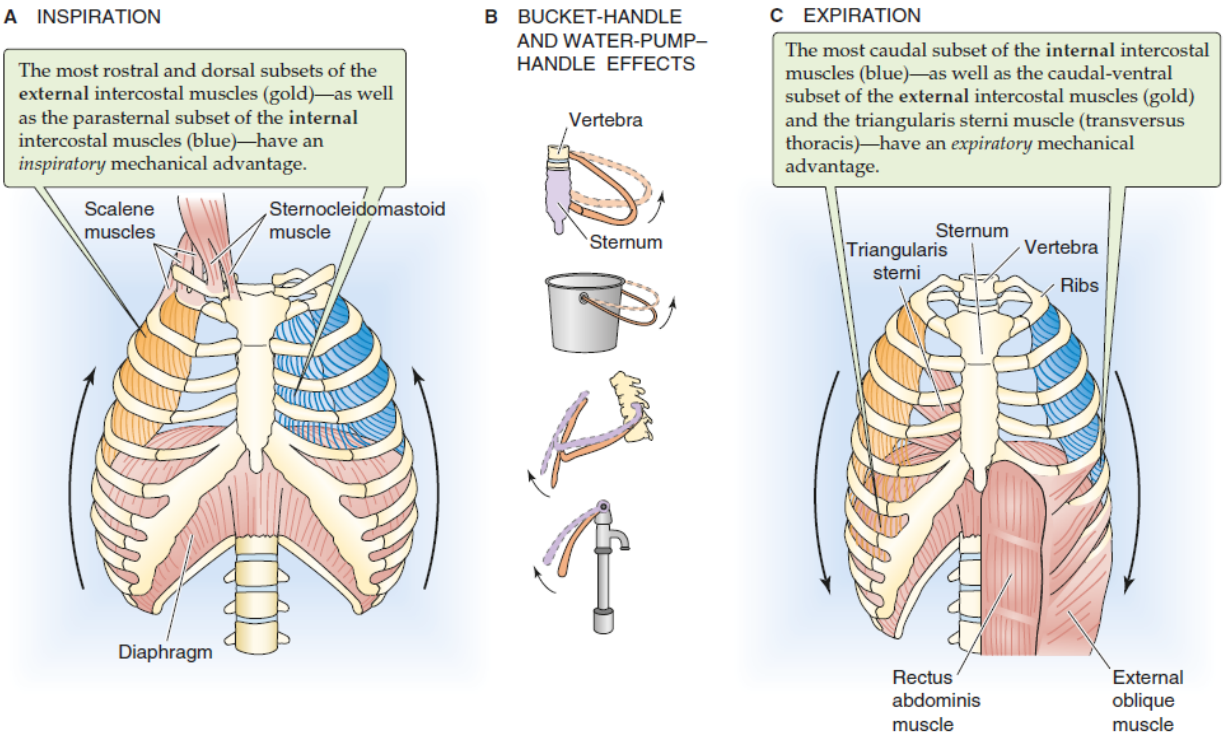

FIGURE 4: MUSCLE FUNCTION DURING INSPIRATION AND EXPIRATION. THE MIDDLE PICTURE SHOWS THE MECHANISMS OF THE THORAX TO INCREASE ITS VOLUME (THE SO-CALLED BUCKET-HANDLE AND WATER-PUMP-HANDLE EFFECT) [19]

Dysfunctional breathing (DB)

DB is an umbrella term for different chronic breathing pattern disorders causing complaints like dyspnoea and chest pains. The most common and well known form of DB is hyperventilation syndrome, characterized by high Bf, high Vt and often hypocapnia. However several other forms of DB exist but in literature there is no clear consensus on how to describe DB and its subgroups and sometimes different terms are used for the same condition which makes it even harder to objectify DB[20]. An attempt to create a consensus was done by Barker et al. and Boulding et al.[20], [21]. Barker et al. divides DB into four groups as shown in Figure 5. In all four of these groups a major component is pattern disordered breathing caused by alterations in respiratory muscle activity. For functional thoracic DB this is the main component, while for extra-thoracic DB the functioning of the upper airways also plays a role, like for example with vocal cord dysfunction. For structural DB (either thoracic or extra-thoracic) anatomical and neurological abnormalities play an additional role, while with functional DB these abnormalities do not exist.

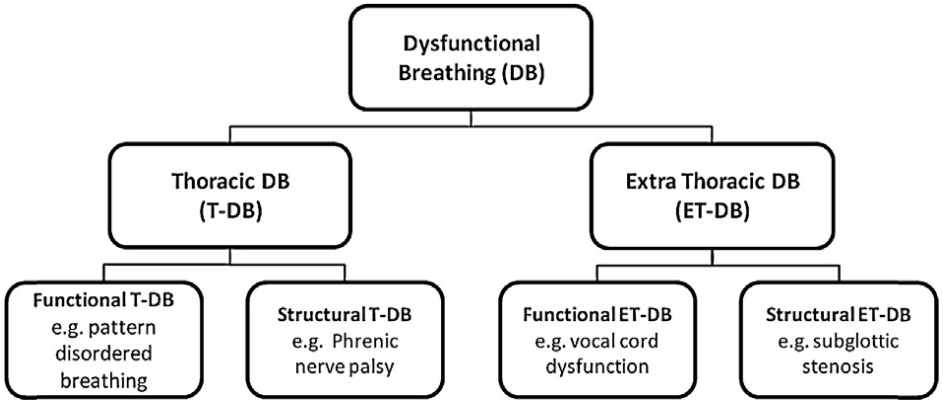

FIGURE 5: AN OVERVIEW OF DYSFUNCTIONAL BREATHING. ADAPTED FROM BARKER ET AL.[20]

Boulding et al. proposes several classes of DB, mainly focussing on the breathing pattern[21]. These classes are hyperventilation syndrome, periodic deep sighing, thoracic dominant breathing, forced abdominal expiration and thoraco-abdominal asynchrony. Sometimes these classes do overlap; meaning that patients can suffer from a breathing pattern that is not covered by only one class.

Respiratory inductance plethysmography (RIP) and its calibration

The principle behind RIP was first presented by Konno and Mead in 1967, who were able to measure lung volumes using the changes in abdominal and thoracic circumference[22]. The technology uses two elastic straps containing wire coils with an alternating current, one circumfering the thorax and one circumfering the abdomen. Due to breathing these circumferences change, resulting in a change of the self-inductance of the coils, which is measured by the RIP. However, several factors, like movement artefacts, strap displacement and temperature can influence the output value of the RIP. Because of this, the relation between inductance and RIP is not direct and therefore displayed in arbitrary units (AU).

To visualize asynchronous breathing Konno and Mead describe the use of Lissajous figures, in this context also called Konno and Mead plots[22]. To create these figures the thoracic volume is plotted against the abdominal volume. When a person breathes perfectly in synchrony, i.e., the

thoracic expands synchronously with the abdomen, this figures shows a clear line as shown in the middle plot in Figure 6. When asynchrony and thus phase difference increases the Lissajous plots will become wider as shown in the other graphs in Figure 6.

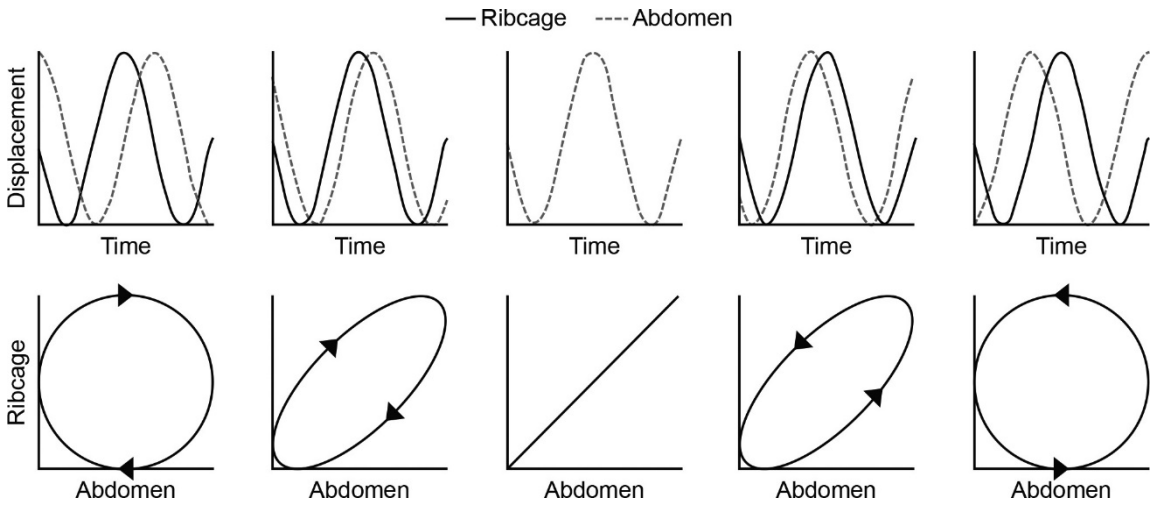

FIGURE 6: LISSAJOUS PLOTS FOR DIFFERENT LEVELS OF ASYNCHRONY. THE UPPER GRAPHS SHOW A PHASE DIFFERENCE BETWEEN THORACIC AND ABDOMINAL MOVEMENT . THE LOWER GRAPHS SHOW THE CORRESPONDING LISSAJOUS PLOTS. ADOPTED FROM HMEIDI ET AL.[23].

To obtain lung volumes (in ml) from these digital waveforms (in AU) the RIP bands have to be calibrated. Different methods can be used for this as presented by Sackner et al. in 1980 and 1989[24][25]. First, scaling factors have to be determined such that a numerical change in the output of both straps corresponds to the same change in lung volume. Thus, for example a volume change in the abdomen of 200ml can result in an increase of 100 AU in the output of the abdominal RIP-band and at the same time a volume change of 200ml in the thorax can result in an increase of 50 AU in the output of the thoracic RIP-band. In that case the scaling factor for the thoracic rip band should be twice as large (100/50=2), such that the calibrated output of both RIP bands is equal with an equal volume change. When this is done the sum of the change in both RIP bands has a linear relationship with the tidal volume, under the assumption that these scaling factors are constant for different volumes.

The theory presented by Konno and Mead can be used to calibrate the RIP bands. Namely, they state that the chest wall movement can be approximated by an open system with two moving parts (thoracic and abdominal expansion) and thus two degrees of freedom.

Using this theory Sackner et al. present equation 1 to model the two compartment model[25]. In this equation V_t equals the tidal volume and ΔuV_{th} and ΔuV_{abd} stand for the uncalibrated thoracic and abdominal volumes (i.e., the increase of the digital waveform in AU). The scaling factor M is the gain factor to scale the total sum of the RIP signals to tidal volumes.

$$V_t = M(K(\Delta \mathrm{uV}_{th}) + \Delta \mathrm{uV}_{\mathrm{abd}}) \qquad \text{Eq. 1}$$

The scaling factor K can be determined by letting a subject perform an isovolumetric manoeuvre as described by Konno and Mead[22], leading to a closed two-compartment model with one degree of freedom. During this isovolumetric manoeuvre the circumference of the thorax and abdomen change while the volume in the lungs remains constant. An increase of the thoracic circumference will result in an increase in the thoracic RIP signal ΔV_{th}. This increase will simultaneously lead to a decrease in the abdominal circumference and abdominal RIP signal ΔV_{abd} and vice versa. During an isovolumetric manoeuvre the ΔV_t in equation 1 equals zero and because $M \neq 0$, this results in equation 2. With this equation and the changes of both RIP signals during this manoeuvre, the K value can be determined.

$$K = -\frac{\Delta \mathrm{uV_{abd}}}{\Delta \mathrm{uV_{th}}} \qquad \text{Eq. 2}$$

Another method for calibration was described earlier by Sackner et al. using two different tidal volumes measured with a spirometer (Vt_1 and Vt_2) and the corresponding thoracic and abdominal RIP values (Th_1, Abd_1, Th_2, Abd_2) measured during the same breaths[24]. The scaling factors for respectively the thoracic and abdominal RIP bands are called X and Y as seen in equation 3 and 4, from which equation 1 is a small adjustion.

$$Vt_1 = X(Th_1) + Y(\mathrm{Abd_1}) \qquad \text{Eq. 3}$$
$$Vt_2 = X(Th_2) + Y(\mathrm{Abd_2}) \qquad \text{Eq. 4}$$

Rewriting these equations leads to equations 5 and 6, that can be used to calculate the scaling factors X and Y.

$$X = \frac{Abd_1 \cdot Vt_2 - Abd_2 \cdot Vt_1}{Th_2 \cdot Abd_1 - Th_1 \cdot Abd_2} \qquad \text{Eq. 5}$$
$$Y = \frac{Th_1 \cdot Vt_2 - Th_2 \cdot Vt_1}{Th_1 \cdot Abd_2 - Th_2 \cdot Abd_1} \qquad \text{Eq. 6}$$

A few years later, Sackner et al. presented another calibration method that does not require isovolumetric manoeuvres and can be used during quiet tidal breathing. This method is called Qualitative diagnostic calibration (QDC) and is also based on equations 1 and 2 [25]. Instead of using isovolumetric manoeuvres, this method uses natural variations in Vt. They state that the breath to breath variation of Vt fits a normal distribution curve. Based on this, if a subject could breathe with a constant Vt, equation 2 can be rewritten, using the breath to breath standard deviation, as:

$$K = -\frac{\mathrm{SD}(\Delta \mathrm{uV_{abd}})}{\mathrm{SD}(\Delta \mathrm{uV_{th}})} \qquad \text{Eq. 7}$$

In order to use this method constant tidal volumes are necessary, which is not possible for a human subject. Therefore, a large number of breaths need to be collected and any breaths with large deviations from the mean sum of the uncalibrated RIP-signals have to be excluded.

Chapter 3: Methods

This thesis describes the preliminary results of the HexAs study after the inclusion of 28 subjects; a study observing the breathing patterns of asthma patients and healthy subjects during an exercise challenge test (V'O2-max cycling ergometry test) using a Hexoskin smart shirt. The study (trial number NL9310) was approved by the Medical Ethics Committee United (MEC-U) in March 2021.

In the study the breathing patterns of both study groups were compared to investigate whether asthma patients use different breathing patterns during exercise. The breathing pattern was objectified by investigating the contribution of Bf, Vt, abdominal breathing, and thoracic breathing to the respiratory minute volume. Additionally, the relation between different breathing patterns and other secondary outcome parameters like level of dyspnoea (as measured with BORG + visual analogue scale (VAS)) was investigated. To objectify the influence of EIB on the level of dyspnoea, lung function tests were performed before and after the V'O2-max test.

Population (base)

In this study 12 asthma patients and 16 healthy subjects were included according to the following criteria:

Inclusion criteria

Asthma group:

- Confirmed asthma diagnosis by a health professional
- Patient using inhaled corticosteroids on daily basis
- Normal lung function under treatment (FEV1>80% predicted post salbutamol)
- Age 18-60
- Written informed consent prior to participation

Healthy group:

- No pulmonary disease
- No usage of lung related medication
- Age 18-60
- Written informed consent prior to participation

Exclusion criteria

Potential subjects who met any of the following criteria were excluded from participation in this study:

- Has had an asthma exacerbation within 6 weeks before inclusion (asthma group only)
- More than 10 pack-years
- Pulmonary disease other than asthma
- No neurological or muscular disorder
- Not able to perform cycling ergometry.
- Does not fit one of the available Hexoskin shirts.
- Meets a contra-indication of the cycling ergometry protocol from the pulmonary department at MST (document: K6 protocol for cycling ergometry MST)
- Has been tested positively for COVID-19 in the past 3 months or has not fully recovered from an earlier COVID-19 infection
- Inability to read and/or understand the Dutch language

Sample size calculation

Because little was known about the breathing patterns during exercise of patients with asthma, it was not possible to determine an exact sample size. Based on the experiences from an ongoing study into the breathing patterns of elite athletes by J.J. van Dobbenburgh (Trial NL8074), it was expected that 4 different breathing patterns will occur when looking at the dominance of either abdominal or thoracal breathing. For the contribution of Vt and Bf to the respiratory minute volume 3 different patterns were expected (Vt increases first when physical exercise intensifies, Bf increases first, or both increase simultaneously). Combining these leads to 12 (4x3) possible breathing patterns. Nevertheless, it was not known which breathing patterns would occur and in which frequency. To observe the effect of different breathing patterns, it is desired to have an average of 4 subjects per breathing pattern, such that it is possible to observe any trends in differences between different breathing patterns. With an expected drop-out of 10%, this would lead to the inclusion of 54 patients with asthma and 54 healthy subjects. In this thesis the breathing patterns of the inclusions till July 2021 were analysed.

Study Protocol

On the day of the measurements, prior of signing the informed consent form, an explanation about the study procedures was given to the subjects, they were asked whether they had read the PIF, whether they understood everything correctly and whether they had any questions. Potential questions were answered and if the subject declared to understand everything correctly and agreed to participate, the informed consent form was signed.

After signing the informed consent form, the age, sex, and race of the participant were noted. The subjects were asked about their smoking status, which medication they are using and how frequently they perform physical exercise. Their weight, height, fat percentage and abdominal circumference were measured and based on the circumference the proper Hexoskin shirt size was selected. The subject started wearing the Hexoskin shirt shortly after signing informed consent to give the Hexoskin device enough time to warmup to a constant temperature, such that the effect of temperature drift as described by Mannée et al. was minimal[26]. A detailed description of the Hexoskin shirt is given in Appendix A 'The Hexoskin Shirt'.

The measurements consisted of three phases. During the first phase, the pre-exercise evaluation, the subjects performed several lung function measurements: an Impulse Oscillometry (IOS) measurement, a standard spirometry test and a CO diffusion test. The IOS and spirometry tests were performed to detect the level of obstruction and whether this level changes after the cycling ergometry test. The CO diffusion test is part of the standard protocol of cycling ergometry. The asthma patients repeated the IOS measurement and spirometry test approximately 10-15 minutes after the admission of 200 microgram salbutamol. As mentioned, two factors are expected to influence the level of EID: EIB and the breathing pattern. This study will focus on the breathing pattern component. To reduce EIB, salbutamol will be given to the patients with asthma prior to the exercise challenge test. This is comparable to a real setting in which patients use salbutamol before exercise. Furthermore, this will equal the level of bronchoconstriction (minimal or none) in the different subjects. It is assumed that the natural breathing pattern (that they may or may not have taught themselves subconsciously) is not influenced by the administration of salbutamol. Most likely the patients would breathe differently when they experience EIB, but this is not the effect we want to investigate as this is not their natural breathing pattern.

In clinical practice, a dose of 400µg salbutamol is given for reversibility tests. However, this was considered to be an overdose because patients were still using their own medication. Therefore, a total dose of 200 microgram of salbutamol was given, which was expected to be sufficient for the purposes of this study.

In the waiting time after the administration of salbutamol, the subjects were asked to fill in three questionnaires: The asthma control questionnaire (ACQ), mini–Asthma Quality of Life Questionnaire (mini-AQLQ) and the Nijmegen Questionnaire[27]–[29]. The subjects in the healthy group only answered the Nijmegen Questionnaire.

The second phase is the cardiopulmonary exercise test (CPET). The subjects performed a standard maximal cycling ergometry test using a steep ramp protocol as done in clinical practice according to the protocol used in Medisch Spectrum Twente. The only difference with this protocol was that the subjects were wearing a Hexoskin shirt. Before and after the cycling ergometry the subjects were asked to give BORG scores for dyspnoea and leg fatigue[30]. Additionally, the level of dyspnoea in the past 7 days and before and after the CPET was scored with a 100mm visual analogue scale (VAS)[31]–[33].

The third phase is the after-exercise evaluation. This consisted of performing another IOS measurement and spirometry 10-15 minutes after the end of the CPET. After these final measurements the subject was allowed to take off the Hexoskin shirt. An overview of the study procedures is shown in Table 1.

TABLE 1: STUDY PROCEDURES

Study groups	Procedure	Duration
Healthy + Asthma	Explanation procedures, informed consent	5 min
Healthy + Asthma	Put on Hexoskin Shirt and other sports clothes, measure/ask demographic data	10 min
Healthy + Asthma	IOS (start at least 5 minutes after putting on Hexoskin shirt)	2 min
Healthy + Asthma	Spirometry	10 min
Healthy + Asthma	CO diffusion test	10 min
Asthma	Admission of 200 microgram salbutamol	1 min
Healthy + Asthma	Questionnaires (Nijmegen Questionnaire and for asthma also ACQ and mini-AQLQ)	5-10 min
Asthma	IOS	2 min
Asthma	Spirometry	10 min
Healthy + Asthma	BORG + VAS	1 min
Healthy + Asthma	Cycling Ergometry	35 min
Healthy + Asthma	BORG + VAS	1 min
Healthy + Asthma	IOS (10-15 min after cycling ergometry)	2 min
Healthy + Asthma	Spirometry (10-15 min after cycling ergometry)	5 min

Study Measurements

Primary measurements

The primary outcome parameter is the breathing pattern. In this study the breathing pattern is defined as follows: the breathing pattern describes the relative contribution of the breathing frequency (Bf), tidal volume (Vt), abdominal breathing and thoracic breathing to the respiratory minute volume and how these contributions change with different levels of physical exercise.

These four parameters were all measured during the CPET. The Bf and Vt were measured with the Vyntus CPX metabolic cart (Vyaire Medical, Metawa, Illinois). The relative contribution of abdominal and thoracic breathing can be obtained from the RIP-signals for thoracic and abdominal expansion obtained from the Hexoskin shirt.

Additional measurements

To study the causes and effects of different breathing patterns the subjects were asked about their smoking status, medication use, frequency of physical exercise, whether they experience dyspnoea during physical exercise and whether they are familiar with DB. Additionally, several parameters were measured. The height, weight and 4-site skinfold thickness as described by Durnin et al. were measured[34]. The measured lung function parameters were FEV1, FVC, FEV1/FVC, MEF50% as measured with standard spirometry and airway impedance (Zrs), airway resistance (R5, R20, R5-20) and airway reactance (X5, Ax, Fres) measured with respectively the Vyntus body plethysmograph (Vyaire Medial, Metawa, Illinois) and the Vyntus IOS (Vyaire Medial, Metawa, Illinois). The asthma control was assessed using the ACQ7 and mini-AQLQ and to screen for hyperventilation and DB the Nijmegen Questionnaire was assessed. Level of dyspnoea and leg fatigue were assessed using the BORG and VAS scores. Additional parameters obtained from the CPET were V'E, oxygen consumption (V'O2), carbon dioxide production (V'CO2), Respiratory exchange ratio (RER), Heart rate (HR), Heart rate reserve (HRR) and Breathing Reserve Index (BRI=V'E/FEV1*40).

Pre-processing of the Data

As earlier described, the raw signals acquired from the RIP-bands in the Hexoskin shirt need to be pre-processed to be able to analyse the breathing patterns of the subjects. First, to remove the offset, any drift and fast perturbations a Butterworth bandpass filter with cut-off frequencies of 0.01Hz and 45Hz was used. For the calculation of the total expansion of the RIP bands and after calibration the tidal volumes, the peaks in the RIP signals were identified. In the situation of perfect synchronous breathing (If there is no time difference between the peaks in the abdominal and thoracic signal) the value at the positive peak and the subsequent negative peak can be added to calculate the total expansion of each band. However, in the case of asynchronous breathing this would give an overestimation of the total expansion. Namely, during asynchronous breathing, air is moved to other parts inside the thorax, causing a small shift from abdominal expansion to thoracic expansion or vice versa. This is the same phenomenon that occurs during an isovolumetric manoeuvre, although in a lesser amount.

Because of this phenomenon the total expansion of each RIP band is calculated by a summation of the value at the positive peak of the signal that is lagging behind (either the thoracic or abdominal) and the value of this same signal at the time instance of the negative peak of the other signal. For the other RIP band, the opposite is done, using the value at the negative peak and the value at the time instance of the foregoing positive peak of the other RIP band. These calculated values are an estimation of the total expansion of the RIP bands and represent the uncalibrated tidal volumes. How to calculate these is depicted in Figure 7.

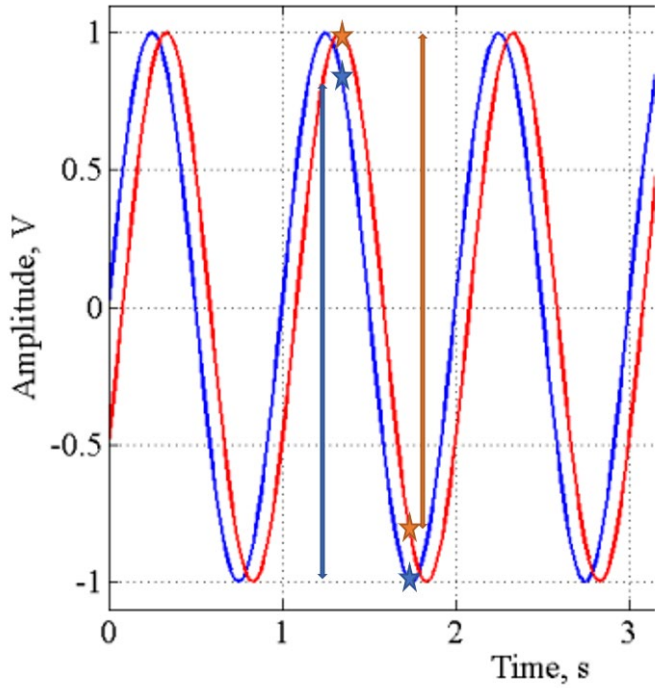

FIGURE 7: A VISUAL EXPLANATION OF THE CALCULATION OF THE UNCALIBRATED VOLUME EXPANSION OF THE RIP BANDS. THE RED LINE REPRESENTS THE THORACIC SIGNAL AND THE BLUE LINE REPRESENTS THE ABDOMINAL SIGNAL. TO CALCULATE THE UNCALIBRATED VOLUME OF THE THORAX THE HEIGHT BETWEEN THE POSITIVE PEAK AND THE VALUE OF THE THORAX AT THE TIME INSTANCE OF THE SUBSEQUENT NEGATIVE PEAK OF THE ABDOMEN IS USED (INDICATED BY THE RED STARS). FOR THE ABDOMEN THE OPPOSITE IS DONE.

To convert the total expansions to Vt in ml the calibration factors need to be determined. In this study six different calibration methods were used based on the methods presented by Sackner et al. in 1980 and 1989[16][17]. To use this method constant Vt's are required. This was expected to be the case during the rest phase and the end of the warmup phase of cycling ergometry. However, for several subjects this appeared to be not true. Therefore, three other comparable methods were used to calculate the K factor. For the first method the timeframe with the most stable Vt's is used. To determine this timeframe, the standard deviation across the 10 previous breaths, the current breath and the 10 future breaths was calculated at every time instance. Next, at the time frame where this standard deviation was the lowest, the K factor was calculated. The second method uses the timeframe with the most stable sum of the normalized RIP-signals for thorax and abdomen. The third method calculates the K factor for every timeframe across the whole cycling ergometry recording and the mean of these K factors is then used as the K factor.

Because of variations in the signal, the value of K was determined as the median value of 8 previous, the current and 8 future values of K (median filter with order 17). With this value for K, at the same timeframe the value of M was calculated using equation 1 and the mean of the same 21 Vt's that were used to calculate the standard deviation.

To test the validity of these calibration factors, two subjects performed an isovolumetric manoeuvre to calculate the calibration factors using the method presented by Konno and Mead, which can be considered as the golden standard. These subjects also performed the cycling ergometry test according to the protocol of the study. The calculated value for K using the isovolumetric

manoeuvre was compared to the calculated K-values with the methods based on Sackner et al. 1980 and 1989, as described above.

A second method to validate the calibration that was used is a comparison between the tidal volume calculated with the RIP bands and the tidal volume measured with the spirometer during the cycling ergometry test. This was done by visual analysis of the graph and by calculating the mean difference between both tidal volumes.

Breathing pattern analysis

The breathing patterns were analysed using two different methods, each looking at different parameters to describe the breathing pattern. For each method the subjects will be grouped based on their pattern and the differences between these groups will be analysed to explain the cause and/or results of these different breathing patterns.

With the first method the subjects will be grouped based on the trends of Vt and Bf during the cycling ergometry test, these groups will be called BPgroups. To quantify this a new parameter was designed. This parameter, called the BPratio, is defined as the ratio between Vt and Bf and to be able to compare this ratio between subjects, a correction is done by dividing this ratio by the predicted vital capacity (VC_{pred}) of a subject. VC_{pred} was chosen instead of predicted body weight, which is often used for Vt prediction, because the correlation of VC_{pred} to Vt is expected to be higher based on the study by Hoftman et al.[35]. This results in the breathing pattern ratio (BPratio) as shown in equation 8. To analyse the trend of the BPratio during the exercise test, the mean BPratio was calculated during different phases of the test (during rest, warmup, 0-20%, 20-40%, 40%-60%, 60%-80% , 80%-100% of max load and during recovery).

$$BPratio = \frac{V_t}{B_f * VC_{pred}} \qquad \text{Eq. 8}$$

The second method categorizes the subjects based on their level of asynchronous breathing using Lissajous figures as presented by Konno and Mead[22], these groups will be called LISgroups. The Lissajous figures used in this study are slightly different than the ones presented by Konno and Mead. In their study these figures were made by plotting the calibrated volumes of the abdomen and thorax against each other. In our study the normalized uncalibrated volumes were used because the calibrated volumes appeared to be unreliable. Additionally, instead of plotting these volumes, the derivatives of these volumes were plotted against each other. The goal of these Lissajous figures is to detect asynchronous breathing, which can be defined as an opposite volume movement of thorax and abdomen. Which is a change that is better represented by volume change than by absolute volume/expansion of the thoracic or abdominal part.

The Lissajous figures were made for the same phases of the cycling ergometry test as the BPratio. As described in the background information, the level of distribution in these figures tells us something about the synchrony of breathing of a subject. To calculate this distribution a linear fit was done to calculate the best fit straight line through the datapoints in these graphs. For every point the shortest distance to this line was calculated and the mean of these distances was calculated for every phase of the exercise to quantify the level of asynchrony. This results in a value for the width or distribution of the Lissajous figures during these phases. This new parameter will be called the Ldis. A higher Ldis will indicate a higher level of ineffective breathing (i.e. a volume shift causing opposite displacement of thorax and abdomen).

Statistics

Descriptive statistics were used to examine the outcome measures. The outcome measures were expressed in means (+- standard deviation) or numbers (%). Univariate analyses were performed with SPSS statistics (IBM Corp. Released 2013, Version 22.0) to test the differences of physical activity measures, asthma control and demographic variables between the study groups. Homogeneity of variances was verified with the Levene's test. Continuous parameters were visually inspected for normality of the distribution using histograms. The variables that did not have a normal distribution were tested with the non-parametric Kruskal-Wallis test followed by multiple pairwise comparisons. Normally distributed variables were tested with Analysis of Variance (ANOVA) followed by Tukey's Honestly Significant Difference test for the post-hoc comparisons. P-values below or equal to 0.05 were considered as significantly different. A p-value between 0.05 and 0.15 was considered to be a trend. Categorical variables were analysed using the Chi-square test or the fisher's exact test in the case of an expected count per group lower than 5.

To analyse which breathing patterns occur more often in which study group (healthy or asthma), the fisher's exact test was used because of the low expected count per group. This was done for all three categorization methods that were described in the previous section separately.

For these breathing patterns (again for all three separately) an ANOVA test was done to test for differences in the normally distributed continuous variables) followed by Tukey's Honestly Significant Difference test for the post-hoc comparisons. This analysis for normality of the distribution was also done based on visual inspection using histograms. The variables that were not normally distributed were tested analysed with the Kruskal-Wallis test. The categorical variables were tested using the fisher's exact test.

Chapter 4: Results

In this study 28 subjects were included in the period from April 2021 until July 2021. Of these subjects 12 were diagnosed with asthma and 16 were healthy. Of the healthy subjects 1 subject was not able to finish the cycling ergometry test due to chest pains. Therefore, the data of 12 patients with asthma and 15 healthy subjects was analysed. The characteristics of these subjects are shown in Table 2.

TABLE 2: SUBJECT CHARACTERISTICS. THE CONTINUOUS VARIABLES ARE PRESENTED WITH THE MEAN VALUE ± STANDARD DEVIATION, THEY WERE NORMALLY DISTRIBUTED AND THEIR P-VALUES ARE CALCULATED USING AN INDEPENDENT SAMPLES T-TEST. THE DISTRIBUTION OF SEX WAS COMPARED BETWEEN GROUPS USING A FISHER'S EXACT TEST.

	Healthy (n=15) Mean ±SD	Asthma (n=12)	p-value
Age (years)	37.2 ± 14.4	30.7 ± 15.2	0.264
Sex (N Male (%))	6 (40%)	5 (41.7%)	1.000
Height (cm)	179 ± 6	176 ± 9	0.217
Weight (kg)	76.9 ± 9.4	84.7 ± 15.5	*0.120*
BMI (kg/m²)	23.9 ± 2.4	27.4 ± 4.5	**0.013**
Fat percentage (%)	27.3 ± 8.6*	30.2 ± 6.7*	0.387
Abdominal circumference (cm)	84.7 ± 9.1	89.2 ± 13.5	0.312
Nijmegen Questionnaire	8.1 ± 5.4	9.1 ± 5.9	0.647
FEV1 %pred at baseline (%)	94.3 ± 11.5	97.6 ± 12.9	0.486
FEV1/FVC at baseline (%)	77.2 ± 4.5	78.6 ± 10.5	0.671

*only measured for 11 healthy subjects and 11 asthma patients
Bold indicates significance (p<0.05), *italic* indicates a trend towards significance (0.05 < p < 0.15)

TABLE 3: SUBJECT CYCLING ERGOMETRY RESULTS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING AN INDEPENDENT SAMPLES T-TEST OR THE MAN-WHITNEY U TEST (DEPENDING ON NORMAL DISTRIBUTION).

	Healthy (n=15)	Asthma (n=12)	p-value
%pred max load	159 (133 - 190)	151 (115 - 182)	0.373*
%pred V'O2 at max	85 (74 - 103)	92 (77 - 92)	0.554⁺
Heart rate reserve at max (=(220-age)-HR)	4 (-7 - 13)	7 (2 - 16)	0.438*
%pred of max V'E at max (=40*FEV1)	79 (66 - 83)	72 (55 - 79)	0.269⁺
VAS for dyspnoea over past 7 days (mm)	0.0 (0.0 - 2.0)	13 (2.3 - 27.5)	**<0.001***
VAS for dyspnoea before exercise test (mm)	1.0 (0.0 - 3.0)	2.5 (0.3 - 14.3)	0.152*
VAS for dyspnoea after exercise test (mm)	33.0 (23.0 - 81.0)	68.0 (56.5 - 84.8)	0.167*

BORG for dyspnoea before exercise test	0.0 (0.0 - 0.5)	0.3 (0.0 - 0.5)	0.347*
BORG for heavy legs before exercise test	0.0 (0.0 - 0.5)	0.0 (0.0 - 0.4)	0.516*
BORG for dyspnoea after exercise test	4.0 (3.0 - 6.0)	4.5 (4.0 - 7.0)	0.614*
BORG for heavy legs after exercise test	8.0 (7.0 - 8.0)	6.0 (4.0 - 7.8)	*0.103**

* Significance was tested using a Mann-Whitney U test
⁺ Significance was tested using an independent sample's t-test
Bold indicates significance ($p<0.05$), *italic* indicates a trend towards significance ($0.05 < p < 0.15$)

Validity of the calculated K-values

Two subjects that also performed the cycling ergometry test while wearing the Hexoskin shirt, performed an isovolumetric manoeuvre to calculate the K-value using the method described by Konno and Mead. These results were compared to the calculated K-values using the methods based on the QDC method from Sackner et al. 1989. These results are shown in Table 4.

TABLE 4: COMPARISON OF CALCULATED K-VALUES BASED ON THE AUC METHODS FROM SACKNER ET AL. 1989 (KVT, KRIP AND KMEAN) AND BASED ON THREE SETS (K1, K2 AND K3) OF ISOVOLUMETRIC MANOEUVRES OF TWO SUBJECTS.

Subj. nr.	Kvt	Krip	Kmean	K1	K2	K3
HexAs104	0.931	1.105	1.086	1.051	0.856	0.975
HexAs212	0.470	0.925	0.592	1.276	1.271	1.768

The X and Y values calculated with the methods based on Sackner 1980 were very aberrant from the expectation (sometimes either the calculated X or Y showed a negative value, which is not possible, as a negative volume is also not possible). Therefore, the methods based on Sackner et al. 1980 were not further analysed for validity.

An example of the second method to validate the calibration by comparing the Vt's calculated with the RIP bands and the Vt's with the spirometer is shown in Figure 8. When calibration is perfect the blue lines in this figure (representing both Vt's) would be exactly the same. The further these lines are apart from each other, the worse the calibration of the RIP-bands is. Hence, at the beginning of the exercise phase the calibration seems to be good, but at the beginning of the recovery phase, the calibration is much worse.

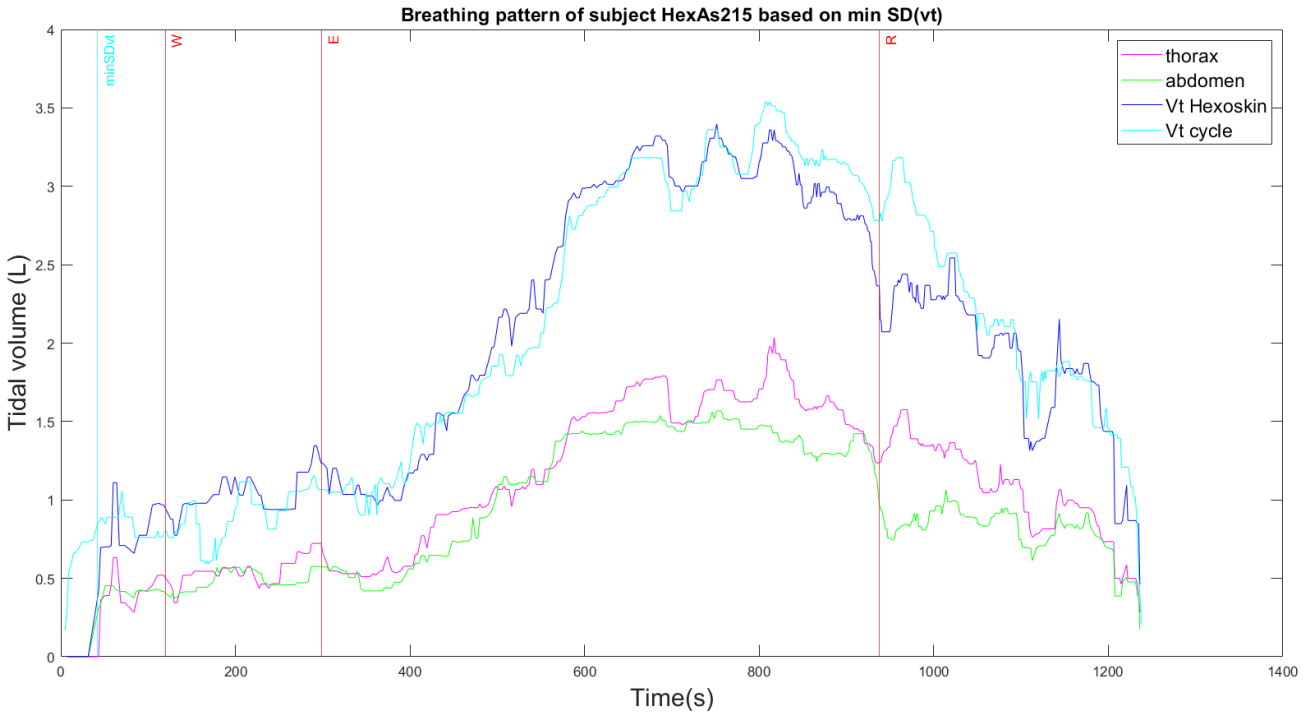

FIGURE 8: THIS FIGURE SHOWS A COMPARISON BETWEEN THE TIDAL VOLUME CALCULATED FROM THE RIP SIGNALS AND THE TIDAL VOLUME MEASURED BY THE SPIROMETER DURING THE CYCLING ERGOMETRY TEST. THE BLUE LINES SHOW THE TOTAL TIDAL VOLUMES. THE PINK AND GREEN LINE RESPECTIVELY SHOW THE CALIBRATED THORACIC AND ABDOMINAL SIGNAL. (W=START WARMUP, E=START EXERCISE, R=START RECOVERY)

Breathing pattern groups based on Vt, Bf and BPratio (BPgroups)

Three breathing pattern groups were made based on the trends observed in Vt, Bf and the BPratio during the cycling ergometry test. A description of the patterns observed in each group is given in Table 5. Examples of the different patterns of Vt, Bf and BPratio for every BPgroup is shown in Figure 9, Figure 10 and Figure 11.

TABLE 5: DESCRIPTION OF GROUPS BASED ON THE VT, BF AND BPRATIO

BPgroup	Description
1	As the load increases the Vt increases, roughly at 75% of maximal load the Bf increases, sometimes leading to a decrease of Vt. The BPratio shows an increasing trend until the final 20%, after which often a decrease is observed.
2	As the load increases the Vt increases, roughly at 30% of maximal load the Bf starts to increase as well, with a steeper increase near the end of the exercise test. The BPratio increases until halfway, then stays equal or slowly decreases with a faster decrease near the end of the test.
3	As the load increases both Vt and Bf increase with equal steepness, with a faster increase of Bf near the end of the test. The BPratio remains roughly the same during the test, with a decrease near the end of the test.

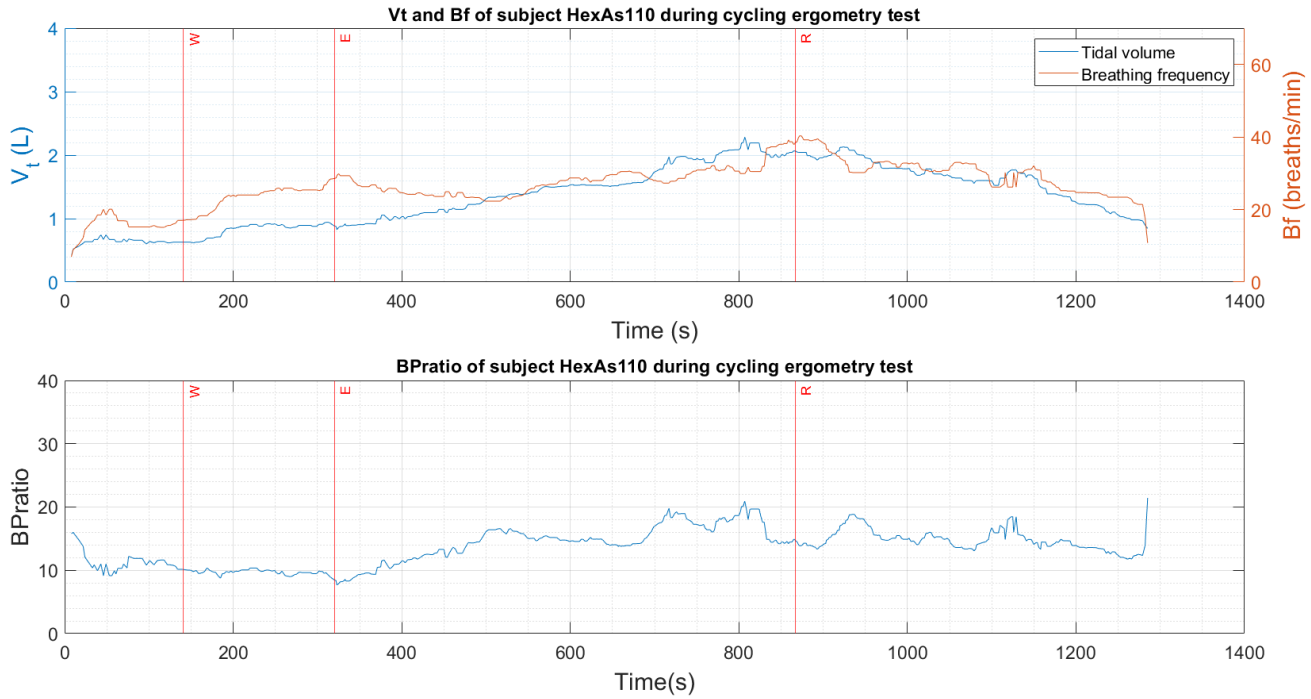

FIGURE 9: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN BPGROUP 1, THE UPPER GRAPH SHOWS THE TREND OF VT AND BF, THE LOWER GRAPH SHOWS THE TREND OF THE BPRATIO DURING THE CYCLING ERGOMETRY TEST (W=START WARMUP, E=START EXERCISE, R=START RECOVERY)

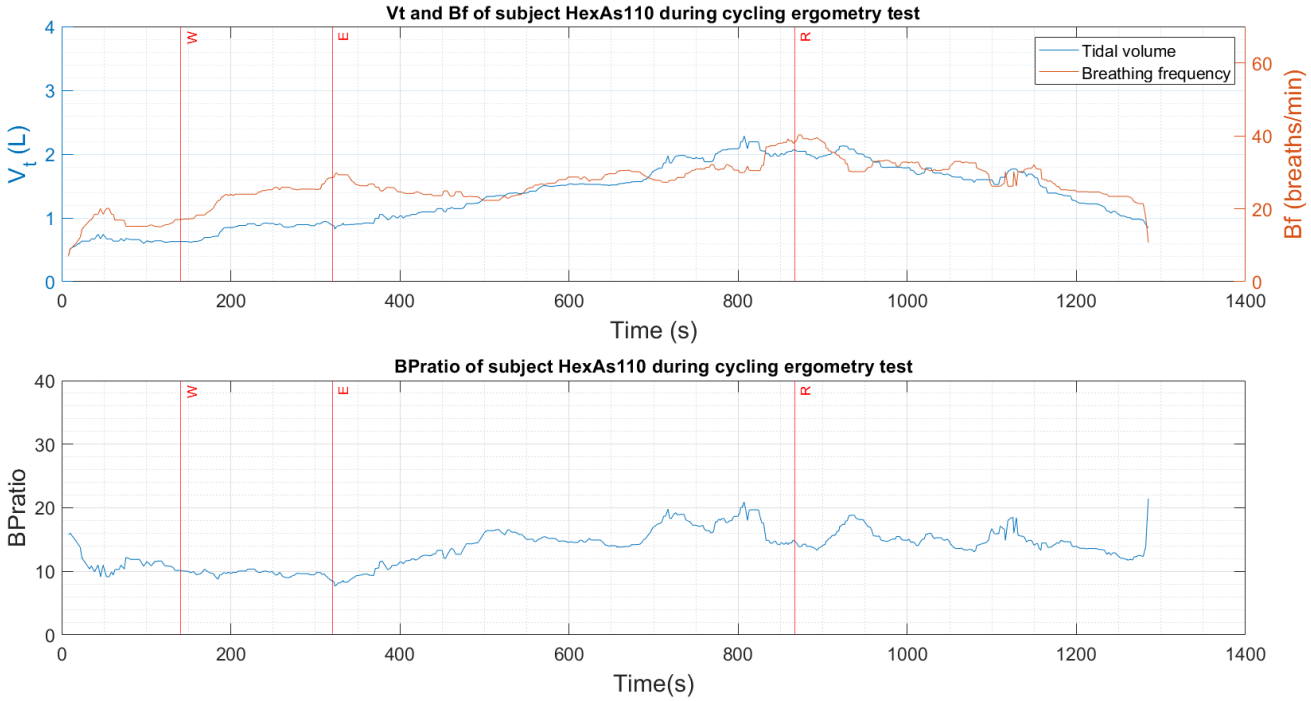

FIGURE 10: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN BPGROUP 2, THE UPPER GRAPH SHOWS THE TREND OF VT AND BF, THE LOWER GRAPH SHOWS THE TREND OF THE BPRATIO DURING THE CYCLING ERGOMETRY TEST (W=START WARMUP, E=START EXERCISE, R=START RECOVERY)

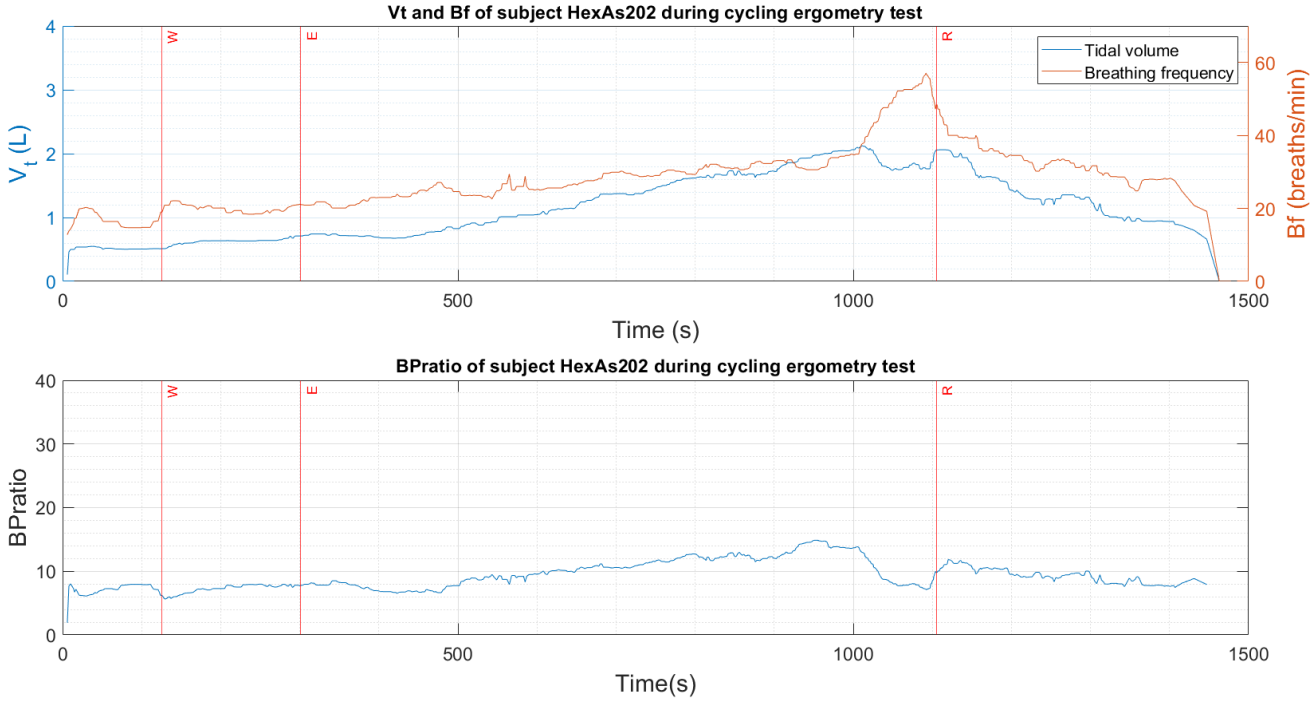

FIGURE 11: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN BPGROUP 3, THE UPPER GRAPH SHOWS THE TREND OF VT AND BF, THE LOWER GRAPH SHOWS THE TREND OF THE BPRATIO DURING THE CYCLING ERGOMETRY TEST (W=START WARMUP, E=START EXERCISE, R=START RECOVERY)

The distribution of subjects among the three groups (called BPgroup 1, 2 and 3) is shown in Table 6. The Fisher-Freeman-Halton exact test shows a significant difference in the distribution of the subjects among the groups (p=0.043). Notable is that BPgroup 2 only contains asthma patients and BPgroup 3 mainly consists of healthy subjects. This also indicated by the Fisher's exact test that shows a significant difference between BPgroup2 and BPgroup3 (p=0.048) and a trend towards significance between BPgroup1 and BPgroup2 (p=0.090).

TABLE 6: CROSSTAB SHOWING THE DISTRIBUTION OF THE SUBJECTS AMONG THE BPGROUPS

			BPgroup			
			1	2	3	Total
Asthma	No	Count	10	0	5	15
		%	66.7%	0.0%	33.3%	100.0%
	Yes	Count	7	4	1	12
		%	58.3%	33.3%	8.3%	100.0%
Total		Count	17	4	6	27
		%	63.0%	14.8%	22.2%	100.0%

The subject characteristics, the BPratio during different phases of the exercise test, the cycling ergometry test results, spirometry results and the results from the questionnaires for the subjects in the different BPgroups are shown in Table 7. Additional parameters can be found in appendix B. For the comparison between two groups the p-values (which were adjusted by the Holm/Bonferroni

correction for multiple tests) are not shown, because this would result in large amounts of pointless data. However, some of these surely are relevant, which are: the difference in ACQ between BPgroup 1 and BPgroup 2 (0.14 vs 1.6 (p=0.137)), the difference in VAS for dyspnoea over past 7 days between BPgroup 2 and BPgroup 3 (35.5 vs 0 (p=0.041)), the difference in mini-AQLQ score for exercise impairment between BPgroup 1 and BPgroup 2 (7.0 vs 4.4(p=0.022)), and the difference in total mini-AQLQ between BPgroup 1 and BPgroup 2 (6.67 vs 4.65 (p=0.111)).

TABLE 7: COMPARISON OF SUBJECT CHARACTERISTICS AND STUDY PARAMETERS BETWEEN THE DIFFERENT BPGROUPS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST. THE P-VALUE OF SEX WAS DETERMINED USING THE FISHER-FREEMAN-HALTON EXACT TEST. IN APPENDIX D A COMPARABLE TABLE IS SHOWN FOR THE PATIENTS WITH ASTHMA ONLY.

	BPgroup 1	BPgroup 2	BPgroup 3	p-value
Age (years)	36.0 (23.0 - 51.5)	34.0 (21.0 - 48.5)	25.5 (23.3 - 40.0)	0.571
Sex (N Male (%))	10 (91)	0 (0)	1 (9)	0.057[d,e]
BPratio rest	9.9 (8.5 - 13.5)	8.3 (7.6 - 10.2)	7.5 (6.4 - 9.2)	0.108[e]
BPratio warmup	11.3 (8.9 - 14.4)	8.6 (7.4 - 12.7)	8.6 (7.1 - 9.7)	0.082[e]
BPratio 0-20% of max	10.9 (8.8 - 15.1)	10.1 (9.8 - 11.0)	9.1 (7.2 - 13.5)	0.357
BPratio 20-40% of max	14.5 (12.2 - 19.9)	13.5 (12.3 - 17.0)	9.9 (8.8 - 11.8)	**0.023**[b,f]
BPratio 40-60% of max	18.1 (15.5 - 23.2)	16.4 (11.9 - 22.0)	11.9 (10.8 - 12.7)	**0.003**[b]
BPratio 60-80% of max	22.1 (19.9 - 25.2)	16.3 (12.1 - 17.9)	13.5 (12.0 - 14.7)	**<0.001**[a,b]
BPratio 80-100% of max	16.9 (14.4 - 18.1)	15.9 (11.8 - 17.2)	11.4 (9.9 - 14.2)	0.015[b]
BPratio recovery	16.5 (14.2 - 18.9)	13.9 (10.0 - 15.4)	11.2 (9.6 - 12.9)	**0.004**[b]
Height (cm)	181.0 (177.3 - 183.5)	168.5 (167.4 - 175.9)	173.5 (169.2 - 183.8)	**0.036**[a]
Weight (kg)	80.3 (74.4 - 90.5)	73.3 (63.2 - 89.1)	73.0 (65.7 - 81.1)	0.163
BMI (kg/m²)	25.4 (23.5 - 27.1)	25.8 (20.5 - 31.8)	22.3 (21.1 - 28.5)	0.471
%pred max load	159 (122 - 192)	144 (115 - 180)	152 (134 - 181)	0.743
%pred V'O2 at max	84.9 (68.9 - 100.5)	96.2 (81.3 - 111.5)	93.3 (76.9 - 106.9)	0.489
Heart rate reserve at max (=(220-age)-HR)	3 (-1 - 7)	15 (7.0 - 19)	11 (-7 - 16)	0.079[d]
%pred of max V'E at max (=40*FEV1)	79.1 (66.6 - 84.9)	53.9 (42.4 - 72.8)	74.9 (62.3 - 82.0)	0.064[d]
Vtex at max (L)	2.8 (2.5 - 3.3)	2.0 (1.9 - 2.1)	2.1 (1.8 - 2.3)	**<0.001**[a,b]
Bf at max (breaths/min)	40.8 (37.0 - 49.3)	38.6 (31.9 - 42.8)	51.2 (37.6 - 58.7)	0.15

VAS for dyspnoea over past 7 days (mm)	2 (0 - 8)	36 (6 - 56)	0 (0 - 5)	**0.048**[c]
VAS for dyspnoea before exercise test (mm)	2 (0 - 6)	17 (1 - 62)	1 (1 - 3)	0.380
VAS for dyspnoea after exercise test (mm)	64 (27 - 76)	84 (39 - 89)	49 (5 - 76)	0.350
BORG for dyspnoea before exercise test	0.0 (0.0 - 0.5)	0.3 (0.0 - 0.9)	0.0 (0.0 - 0.1)	0.420
BORG for heavy legs before exercise test	0.0 (0.0 - 0.5)	0.0 (0.0 - 0.4)	0.0 (0.0 - 0.6)	0.885
BORG for dyspnoea after exercise test	4.0 (3.5 - 6.0)	6.0 (3.3 - 8.8)	5.5 (3.5 - 7.3)	0.585
BORG for heavy legs after exercise test	7.0 (5.0 - 8.0)	6.0 (4.3 - 8.5)	8.0 (6.3 - 9.3)	0.397
Asthma control Questionnaire-7	0.1 (0.1 - 0.6)	1.6 (0.5 - 2.1)	1.1*	0.114[d]
Mini-AQLQ: activity	7 (6.8 - 7)	4.4 (3.5 - 5.8)	6.3*	**0.026**[a]
Mini-Asthma quality of life questionnaire	6.7 (6.5 - 6.9)	4.6 (4.4 - 6.2)	5.7*	0.087[d]
Nijmegen Questionnaire	7.0 (2.5 - 12.0)	10.0 (7.8 - 16.8)	9.0 (7.3 - 13.5)	0.382
%pred FEV1 pre exercise	99.0 (90.0 - 106.5)	101.5 (89.4 - 114.8)	90.5 (82.6 - 98.8)	0.228
FEV1/FVC pre exercise (%)	77.6 (71.8 - 82.7)	87.7 (76.5 - 90.3)	80.5 (76.3 - 83.8)	0.135
%pred FEV1 post exercise	99.2 (89.0 - 106.3)	101.5 (87.5 - 118.6)	91.3 (83.3 - 98.1)	0.398
FEV1/FVC post exercise (%)	76.9 (70.5 - 83.2)	90.3 (75.7 - 94.0)	81.2 (79.7 - 84.7)	0.083

***** BPgroup 3 only contains one asthma patient, therefore the value of this subjects was given for the asthma control questionnaire and mini-AQLQ (only asthma patients filled this in), as a median and interquartile range could not be calculated.
[a] Significant difference between group 1 and 2
[b] Significant difference between group 1 and 3
[c] Significant difference between group 2 and 3
[d] Trend towards a significant difference between group 1 and 2
[e] Trend towards a significant difference between group 1 and 3
[f] Trend towards a significant difference between group 2 and 3
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests.
Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)

Breathing pattern groups based on Lissajous plots of the thoracic and abdominal flow (LISgroups)

Four breathing pattern groups were made based on the trend of the distribution of points in the Lissajous plots during the cycling ergometry test. A description of the patterns observed in each group is given in Table 8. Examples of the different Lissajous plots for every LISgroup are shown in Figure 12, Figure 13, Figure 14 and Figure 15.

TABLE 8: DESCRIPTION OF GROUPS BASED ON LDIS

LISgroup	Description
1	This group starts the exercise phase with relative synchronous breathing ($Ldis_{0\text{-}20}$<0.15) and during the test the asynchrony increases until a $Ldis_{80\text{-}100}$>0.35 at maximum load
2	This group starts the exercise phase with relative synchronous breathing ($Ldis_{0\text{-}20}$<0.15), and during the test the asynchrony increases until a $Ldis_{80\text{-}100}$<0.35 at maximum load
3	This group starts the exercise phase with moderate asynchronous breathing (0.25<$Ldis_{0\text{-}20}$<0.30) and during the test the asynchrony increases until a $Ldis_{80\text{-}100}$>0.60 at maximum load
4	This group starts the exercise phase with moderate asynchronous breathing (0.15<$Ldis_{0\text{-}20}$<0.20) and during the test the asynchrony increases only little until 0.20<$Ldis_{80\text{-}}$100<0.30 at maximum load.

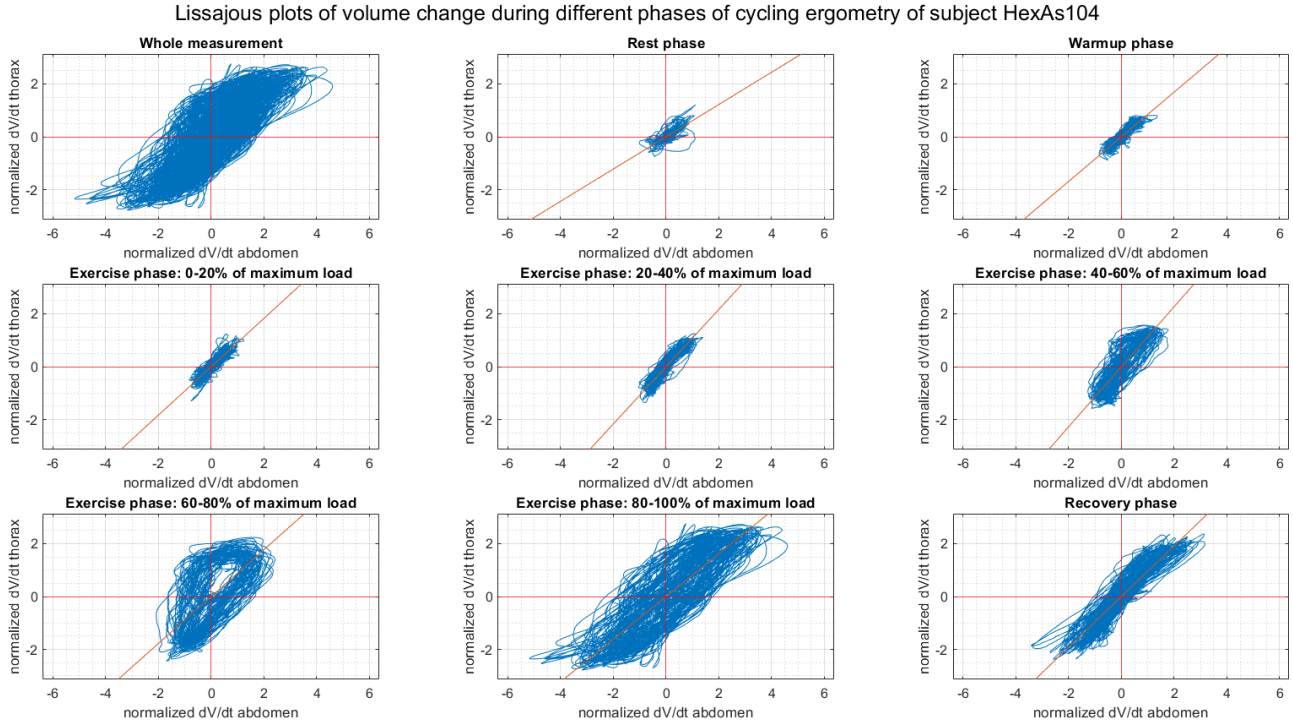

FIGURE 12: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN LISGROUP 1, THE UPPER LEFT GRAPH SHOWS THE LISSAJOUS PLOT OF THE WHOLE MEASUREMENT AND EACH SUBSEQUENT GRAPH SHOWS A DIFFERENT PHASE OF THE CYCLING ERGOMETRY TEST.

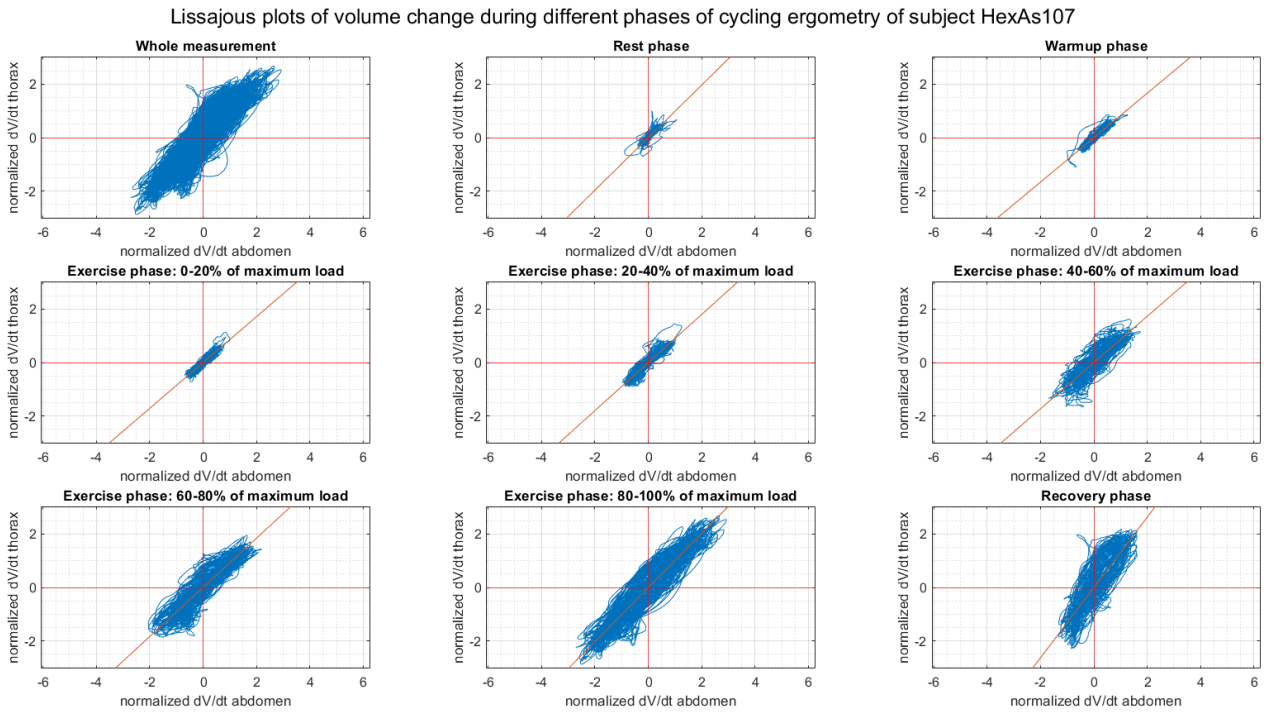

FIGURE 13: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN LISGROUP 2, THE UPPER LEFT GRAPH SHOWS THE LISSAJOUS PLOT OF THE WHOLE MEASUREMENT AND EACH SUBSEQUENT GRAPH SHOWS A DIFFERENT PHASE OF THE CYCLING ERGOMETRY TEST.

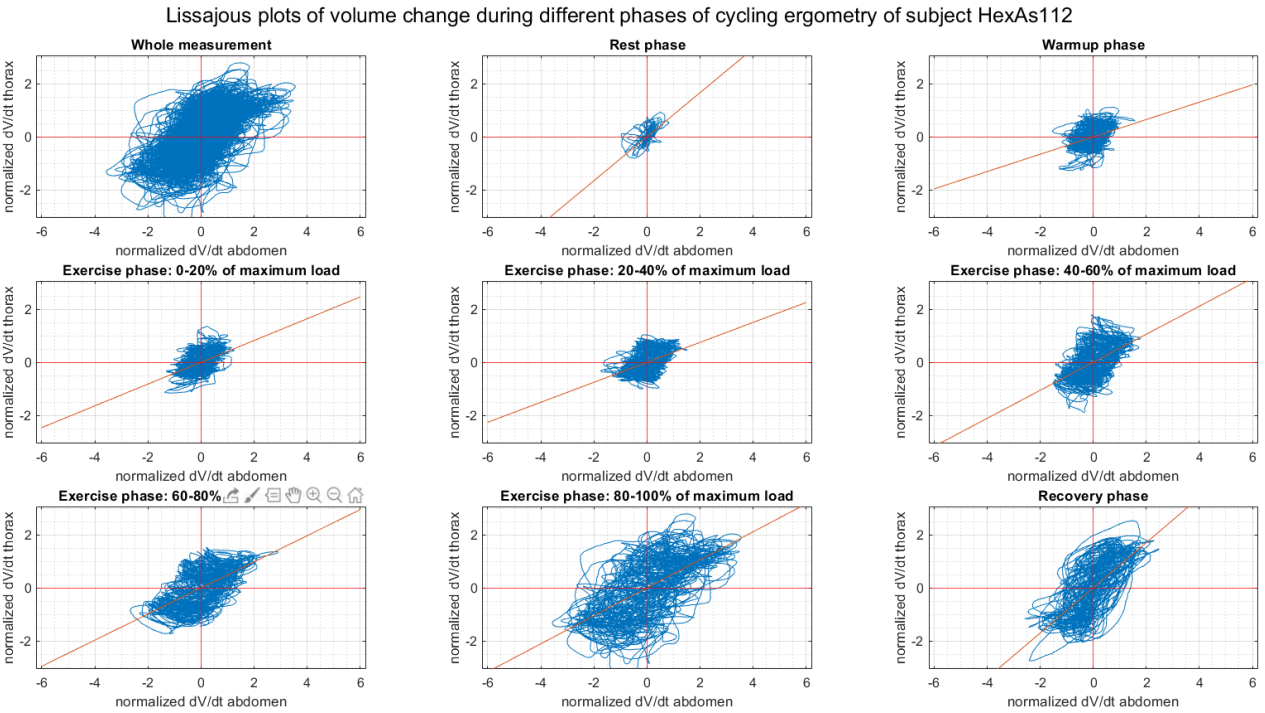

FIGURE 14: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN LISGROUP 3, THE UPPER LEFT GRAPH SHOWS THE LISSAJOUS PLOT OF THE WHOLE MEASUREMENT AND EACH SUBSEQUENT GRAPH SHOWS A DIFFERENT PHASE OF THE CYCLING ERGOMETRY TEST.

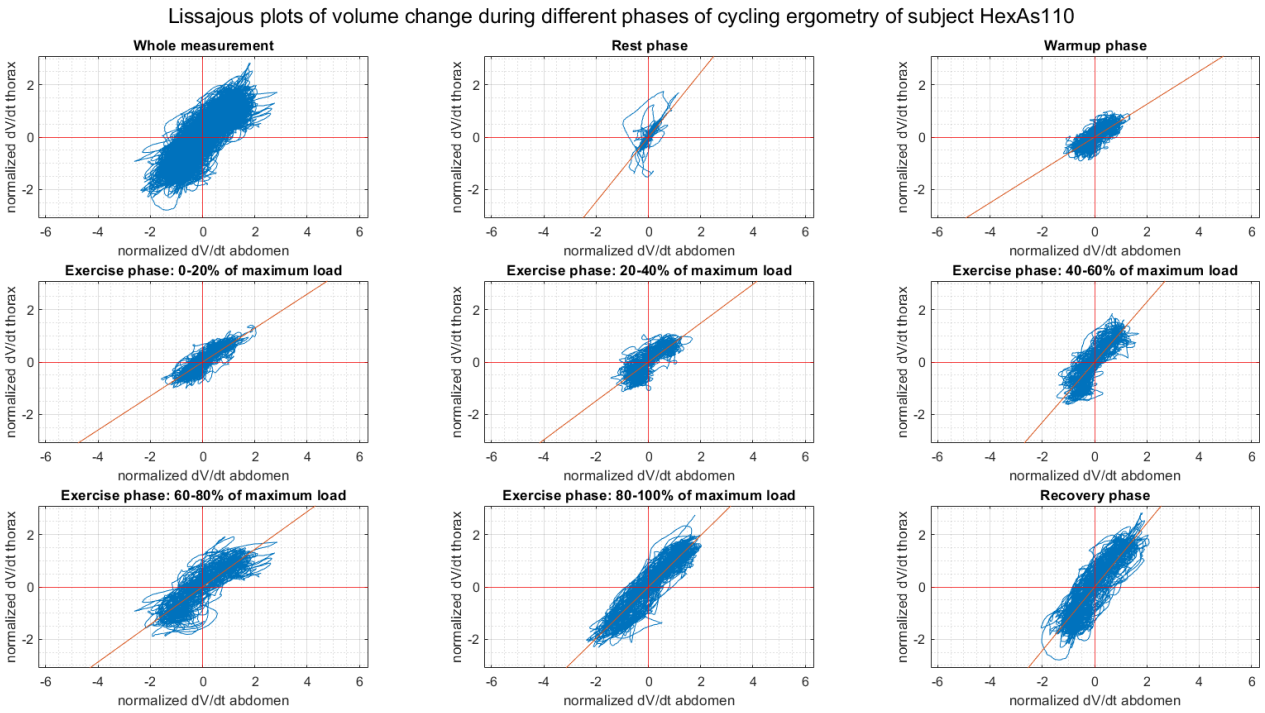

FIGURE 15: AN EXAMPLE OF THE BREATHING PATTERN OF A SUBJECT CLASSIFIED IN LISGROUP 4, THE UPPER LEFT GRAPH SHOWS THE LISSAJOUS PLOT OF THE WHOLE MEASUREMENT AND EACH SUBSEQUENT GRAPH SHOWS A DIFFERENT PHASE OF THE CYCLING ERGOMETRY TEST.

The distribution of subjects among the four groups (called LISgroup 1, 2, 3 and 4) is shown in Table 9. The Fisher-Freeman-Halton exact test shows a non-significant difference in the distribution of the subjects among the groups (p=0.271).

TABLE 9: CROSSTAB SHOWING THE DISTRIBUTION OF THE SUBJECTS AMONG THE LISGROUPS

			LisGroup				
			1.00	2.00	3.00	4.00	Total
Asthma	No	Count	8	5	1	0	14
		%	57.1%	35.7%	7.1%	0.0%	100.0%
	Yes	Count	3	5	2	2	12
		%	25.0%	41.7%	16.7%	16.7%	100.0%
Total		Count	11	10	3	2	26
		%	42.3%	38.5%	11.5%	7.7%	100.0%

The subject characteristics, the Ldis during different phases of the exercise test, the cycling ergometry test results, the forced spirometry results, and the results from the questionnaires for the subjects in the different LISgroups are shown in Table 10. Additional parameters can be found in appendix C. As mentioned before, for the comparison between two groups the p-values (which were adjusted by the Holm/Bonferroni correction for multiple tests) are not shown, because this would result in large amounts of pointless data. However, for the difference of the VAS after exercise between LISgroup 1 and LISgroup 4 (52.9mm vs 91.5mm (p=0.058)) the p-value is surely relevant.

TABLE 10: COMPARISON OF SUBJECT CHARACTERISTICS AND STUDY PARAMETERS BETWEEN THE DIFFERENT LISGROUPS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST.

	LISgroup 1	LISgroup 2	LISgroup 3*	LISgroup 4*	p-value
Age (years)	25 (23 - 45)	39 (27 - 51)	(19 - 26)	(43 - 51)	0.078[j]
Sex (N Male (%))	8 (73)	2 (18)	1 (9)	0 (0)	**0.041[a]**
Ldis rest	0.05 (0.05 - 0.09)	0.05 (0.03 - 0.06)	(0.11 - 0.27)	(0.02 - 0.08)	**0.052[d,h]**
Ldis warmup	0.09 (0.07 - 0.10)	0.08 (0.06 - 0.09)	(0.27 - 0.30)	(0.16 - 0.19)	**0.006[b,d,k]**
Ldis 0-20% of max	0.09 (0.08 - 0.11)	0.08 (0.07 - 0.09)	(0.26 - 0.28)	(0.15 - 0.19)	**0.006[b,d,k]**
Ldis 20-40% of max	0.13 (0.10 - 0.17)	0.10 (0.09 - 0.12)	(0.28 - 0.30)	(0.18 - 0.19)	**0.003[d,h,k]**
Ldis 40-60% of max	0.24 (0.16 - 0.28)	0.15 (0.14 - 0.17)	(0.32 - 0.39)	(0.22 - 0.22)	**0.004[a,d]**
Ldis 60-80% of max	0.40 (0.30 - 0.47)	0.21 (0.20 - 0.23)	(0.37 - 0.46)	(0.27 - 0.29)	**0.001[a,d]**
Ldis 80-100% of max	0.54 (0.44 - 0.56)	0.27 (0.25 - 0.3)	(0.60 - 0.64)	(0.23 - 0.23)	**<0.001[a,c,d,f]**
Ldis ratio recovery	0.26 (0.23 - 0.29)	0.20 (0.17 - 0.23)	(0.39 - 0.53)	(0.23 - 0.27)	**0.004[d,g]**
Height (cm)	182.2 (180.9 - 184)	172.1 (169.1 - 179.6)	(172.0 - 190.5)	(160.7 - 167.5)	**0.006[a,c]**
Weight (kg)	79.7 (73.4 - 87.8)	77.6 (66.7 - 86.8)	(61.8 - 123.8)	(86.4 - 92.4)	0.464
BMI (kg/m²)	23.7 (22.7 - 26.1)	25.4 (23 - 27.7)	(19.5 - 34.1)	(32.9 - 33.5)	0.121[i]
%pred max load	147 (119 - 185)	183 (134 - 194)	(94 - 145)	(178 - 184)	0.079[j]
%pred V'O2 at max	92.4 (77.4 - 103.8)	83.2 (68.6 - 95.4)	(101.0 - 126.7)	(75.6 - 80.2)	0.061[j]
Heart rate reserve at max (=(220-age)-HR)	3 (-7 - 6)	9 (1 - 16)	(5 - 13)	(8 - 19)	0.235
%pred of max V'E at max (=40*FEV1)	78.7 (68.0 - 83.3)	72.5 (58.8 - 83.0)	(45.5 - 76.0)	(76.2 - 77.9)	0.367
Vtex at max (L)	2.7 (2.3 - 3.2)	2.4 (2.1 - 2.7)	(1.8 - 3.5)	(1.7 - 2.0)	0.083[c]
Bf at max (breaths/min)	45.9 (34.8 - 50.6)	42.5 (36.3 - 53.4)	(35.6 - 38.9)	(38.3 - 48.1)	0.814
VAS for dyspnoea over past 7 days (mm)	2 (0 - 7)	2 (0- 5)	(0 - 51)	(14 - 57)	0.129
VAS for dyspnoea before exercise test (mm)	2 (0 - 8)	1 (0 - 2)	(1 - 30)	(1 - 3)	0.210
VAS for dyspnoea after exercise test (mm)	55 (19 - 69)	64 (27 - 82)	(67 - 86)	(90 - 93)	**0.045[i]**
BORG for dyspnoea before exercise test	0.0 (0.0 - 0.5)	0 (0 - 0.1)	(0.0 - 1.0)	(0.0 - 0.0)	0.223

BORG for heavy legs before exercise test	0.0 (0.0 - 0.5)	0 (0 - 0.5)	(0.0 - 0.0)	(0.0 - 0.0)	0.322
BORG for dyspnoea after exercise test	5.0 (3.0 - 6.0)	4.0 (3.0 - 5.5)	(4.0 - 8.0)	(7.0 - 9.0)	0.247
BORG for heavy legs after exercise test	8.0 (6.0 - 9.0)	7.0 (5.0 - 8.0)	(3.0 - 7.0)	(4.0 - 9.0)	0.622
Asthma control Questionnaire-7	(0.1 - 1.1) *	0.1 (0.1 - 1.1)	(0.7 - 2.3)	(1.1 - 1.6)	0.261
Mini-AQLQ: activity	(6.0 - 7.0) *	6.8 (4.8 - 7.0)	(4.3 - 7.0)	(4.5 - 6.3)	0.692
Mini-Asthma quality of life questionnaire	(5.9 - 6.9) *	6.6 (5.6 - 6.7)	(4.6 - 6.7)	(4.3 - 5.7)	0.206
Nijmegen Questionnaire	10.0 (7.0 - 13.0)	7.5 (2.8 - 10.0)	(2.0 - 19.0)	(7.0 - 9.0)	0.551
%pred FEV1 pre exercise	92.6 (89.7 - 100.4)	103.8 (96.1 - 116.7)	(69.7 - 104.4)	(86.6 - 90.9)	<u>0.142</u>
FEV1/FVC pre exercise (%)	76.7 (71.6 - 80.4)	82.2 (77.8 - 86.3)	(73.0 - 87.8)	(72.8 - 82.8)	0.253
%pred FEV1 post exercise	91.9 (90.5 - 103.6)	104.3 (91.0 - 117.6)	(70.5 - 106.6)	(84.5 - 87.5)	<u>0.116</u>
FEV1/FVC post exercise (%)	79.8 (72.7 - 82.6)	80.2 (76.9 - 86.1)	(74.4 - 94.3)	(71.7 - 83.1)	0.497

***** This value was not available for enough subjects to give median and interquartile range; therefore, the minimum and maximum value are given (min - max), LISgroup 3 and 4 did only contain 3 and 2 subjects, LISgroup 1 did only contain 3 asthma patients, therefore there were not enough cases for the ACQ and mini-AQLQ (only filled in by asthma group).
[a] Significant difference between group 1 and 2
[b] Significant difference between group 1 and 3
[c] Significant difference between group 1 and 4
[d] Significant difference between group 2 and 3
[e] Significant difference between group 2 and 4
[f] Significant difference between group 3 and 3
[g] Trend towards a significant difference between group 1 and 2
[h] Trend towards a significant difference between group 1 and 3
[i] Trend towards a significant difference between group 1 and 4
[j] Trend towards a significant difference between group 2 and 3
[k] Trend towards a significant difference between group 2 and 4
[l] Trend towards a significant difference between group 3 and 4
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests.
Bold indicates significance ($p<0.05$), <u>*underscore*</u> indicates a trend towards significance ($0.05 < p < 0.15$)

Chapter 5: Discussion

This study shows that the new parameters BPratio and Ldis can be used to describe the breathing pattern of subjects during a cycling ergometry test. Based on the BPratio and Ldis respectively three and four groups can be distinguished based on the change in contribution of Vt and Bf, and the change in level of asynchronous breathing as a result of increasing load. The aim of the study was to investigate whether asthma patients follow different breathing patterns than healthy subjects. From the results in Table 7 can be derived that asthma patients with worse asthma control, based on ACQ, VAS and mini-AQLQ, increase their Bf earlier than those with better asthma control. The difference with healthy subjects who increase their Bf earlier is that asthma patients tend to do this at 30-40% of maximal load and healthy subjects do this from the start. Table 10 shows that subjects with very asynchronous breathing during maximal exercise showed a lowered performance level, while subjects with an almost stable and only mild asynchronous breathing showed elevated levels of dyspnoea at the end of exercise (VAS 91.5mm vs 52.9mm, p=0.058). Notably is that 33% of asthma patients were categorized in LISgroup 3 and 4, while only 7% of healthy subjects was categorized in these groups, indicating a difference in breathing pattern between asthma patients and healthy subjects.

Comparison between both study groups

From the results in Table 2 can be derived that based on the subject characteristics the healthy group is comparable to the asthma group. The only parameter that shows a significant difference between the groups is the BMI, probably caused by a difference in weight (which shows a trend towards significance). According to Chlif et al. the Bf/Vt ratio is higher in obese patients due to a higher Bf [36]. Despite the fact that the BMI of the subjects in their study was much higher (BMI=39±6 kg/m^2) than that of the subjects investigated in this study, the higher BMI in the asthma group might still influence the breathing patterns measured in this study. Therefore, when analysing differences between breathing patterns this has to be considered.

Validation of the calculated K-values

Observation of Table 4 tells us that the validity of the calculated values based on the QDC method of Sackner et al. 1989 is very low. Certainly, the calculated K values for HexAs104 are similar to the values obtained from the isovolumetric manoeuvres, but this is certainly not the case for HexAs112. From this can be concluded that the methods used in this study to calculate the K value are not reliable. An explanation for this is that the methods used require stable tidal volumes and therefore, the timeframe with the most stable tidal volumes was searched. At this point a contradiction occurs. The shorter the timeframe used, the more stable the tidal volumes will be. On the other hand, the method is based on the fact that the distribution of inhaled air between thoracic and abdominal parts is normally distributed[25]. To be certain that this distribution applies a longer period of measuring is required (Sackner et al. even uses 5-10 min of quiet breathing). Therefore, because both requirements cannot be met sufficiently at the same time, this calibration method is not reliable to use for the purposes of this study.

As stated in the results, the methods derived from Sackner et al. 1980 neither proved to be reliable for the purposes of this study. Jayasekera et al. also used a calibration method based on this method[37]. They describe a method that searches for the most ideal pair of the calibration factors X and Y that produces the smallest error compared to the tidal volume measured with capnography. The problem they encountered was that several very different pairs of calibration factors could be found with a similar error. As a result, it was impossible to determine the real values

of the calibration factors. This can be explained using the findings of Sartene et al. and Banzett et al. that the variability of thoracoabdominal partitioning during quiet breathing is very small[38], [39]. Based on this de Groote et al. explain that when the rib cage signal is constantly proportional to the abdominal signal a correct Vt will always be calculated by appropriate scaling[14]. However, this correct Vt does not indicate that the scaling between thorax and abdomen is correct. Namely, when the proportion between both signals changes (i.e., when someone starts with more thoracal breathing), the error between the calculated tidal volume from RIP and the measured tidal volume will vastly increase. This is exactly what was observed in this study when looking at Figure 8, where a similar comparison is depicted. During the start of the exercise test both rip bands increase synchronously and thus the proportion between both signals stays the same and the value of both tidal volumes is roughly as high. However, at the end of the test and especially at the start of the recovery phase the proportion between both signals changes and as a result the calculated tidal volume from the RIP bands is much more different from the measured tidal volume than at the start of the exercise test, indicating bad calibration.

Because the methods used for the determination of the calibration factors of RIP could not be validated it is not possible to say something about the absolute contribution of thoracic and abdominal breathing to the tidal volumes, nor about the relative contribution of both RIP bands. Despite of this, it is possible to determine when the circumference of the thorax or abdomen is increasing or decreasing and thus it is possible to detect asynchrony in breathing, which is used for the Lissajous plots.

Breathing pattern groups based on Vt, Bf and BPratio (BPgroups)

Observation and interpretation of the results

A very interesting distribution between BPgroups can be observed when looking at Table 6. The majority of subjects follow the pattern described by BPgroup 1 (constantly increasing Vt and only increasing Bf at the end). Only 5 patients with asthma and 5 healthy subjects follow a different pattern. Remarkably, these asthma patients and healthy subjects are almost perfectly separated into BPgroup 2 (4 asthma patients, Bf increases from 30-40% of maximal load) and BPgroup 3 (5 healthy subjects and 1 asthma patient, Bf increases from the beginning of the exercise phase). This indicates that, when deviating from the "normal" pattern (BPgroup 1), asthma patients follow a different pattern than healthy subjects.

Another notable fact is that 90% of the subjects with a deviant breathing pattern are female. This means that 56% of female subjects have deviant breathing pattern and that a deviant breathing pattern was only observed in 9% of the male subjects. This is an indication for the fact that the female sex is a risk factor for a deviant breathing pattern.

Other than that, a significant difference, or a trend towards one, is observed between BPgroups looking at V'O2 at max, heart rate reserve at max, V'E at max, %pred V'E at max, Vtex at max, Bf at max, ACQ, mini-AQLQ and FVC. The difference in Vtex and Bf at max is of course a result of the classification of the subjects among the groups and supports that these groups are really different. The difference in V'O2 at max, FVC and V'E at max is most likely a result from the difference in height and thus a lower predicted value for these parameters. However, %pred V'E at max shows a trend towards significance, which indicates that the subjects in BPgroup 2 did not reach their maximal V'E, together with a significantly higher heart rate reserve, this might indicate that these subjects did not reach their physiological maximum. However, when looking at the data of these specific subjects, only one subject shows a high heart rate reserve and low %pred V'E with a respiratory exchange rate of 1.06, and thus might not have reached her physiological maximum.

The others only showed either a high heart rate serve or low %pred V'E, not both and additionally showed a respiratory exchange rate≥1.15.

Explanation of the results based on the literature

As mentioned, when deviating from the "normal" pattern (BPgroup1), it appears that asthma patients follow a different pattern than healthy subjects. To explain this, first, an explanation for deviating from the normal pattern has to be found. After this, an explanation for the different patterns of asthma patients and healthy subjects can be given.

Most likely sex plays a role in developing a deviant breathing pattern, mostly indicated by an earlier increase of the Bf. An explanation for this is the fact that it was observed that women tend to have a higher resting Bf[40] and rely more on breathing frequency to increase their V'E[41]. Certainly, as LoMauro et al. state, this difference between sexes can also be attributed to a difference in height[42]. On the contrary, Mead compared females and males of comparable age and found that the relationship between lung size and VC differs between both sexes[43]. This difference suggests that sex, independent of height, may alter the BPratio, which agrees with our results.

Another explanation for a deviant breathing pattern is the difference in neurological regulation of Vt and Bf. Vt is mostly regulated by metabolic stimuli like V'CO2, while Bf is mostly regulated by physical stress perception, i.e. the experience of stressors like leg fatigue, dyspnoea or getting tired[44], [45]. Furthermore, Boiten et al. showed that experiencing stressors can induce changes in breathing pattern in asthma patients[46]. This implies that the perception of the load is larger for the subjects in BPgroup 3 and BPgroup 2. For BPgroup 3 these parameters for dyspnoea, and thus level of stress perception does not show a difference with another group and thus does not explain the different breathing pattern. For BPgroup 2 a higher level of stress perception is indicated by a higher VAS among the past 7 days and a lower mini-AQLQ score overall and for the subdomains activity and environment. However, the VAS and BORG scores before and after the test are not significantly different, which would be expected when the higher Bf is a result of stress perception. A hypothesis is that these subjects are used to a certain level of dyspnoea or stress during exercise and are used to increasing their Bf as a result of stress perception, which slowly developed into their natural breathing pattern. The results from this study underwrite this hypothesis but the study population is too small to confirm this. In literature, patients with DB are typically described as people with high levels of internal stress, which underwrites the hypothesis[47][48]. Additionally, results from Arnold et al. show that Bf is elevated in patients developing an asthma exacerbation, which might explain the elevated VAS and mini-AQLQ in the subjects with an earlier increase of Bf[49].

Admittedly, as the above does not give a closing explanation for the earlier increase of Bf, this deviant breathing pattern might also be physiological. The cycling ergometry test did not show a lowered ability to exercise in BPgroup 2 and BPgroup 3, which indicates that the observed breathing pattern does not form an impediment to perform physical exercise.

The above gives a reasoning for the deviant breathing patterns of the subjects in BPgroup 2 and BPgroup 3. However, the difference between the asthma patients in BPgroup 2 and healthy subjects in BPgroup 3 is hard to explain. The only parameters that clearly differ between BPgroup2 and Bgroup3 are %pred of V'E at max, Bf at max and VAS scores. The mini-AQLQ and ACQ were only performed by asthma patients, from which only one is classified in BPgroup 3, which is therefore not reliable for comparison. The V'E at max and Bf are clearly higher (most likely not significant due to the small groups) for the subjects in BPgroup3, which indicates (as Vtex at max is almost equal) an inability of the subjects in BPgroup2 to increase their Bf to high levels. This is most

likely due to their asthma, however their FEV1 and FER did not decrease as a result of the exercise (as expected due to the administration of salbutamol). During exercise, it is more efficient to increase Vt instead of Bf, because increasing Bf also increases the amount of dead space ventilation. Additionally, the work rate of breathing is elevated in patients with obstructive disease and thus asthma[50]. As the work rate is increased it becomes even more important to breath efficiently, which means increasing Vt. This contradicts with the earlier findings that the subjects in BPgroup 2 have learned themselves to breath with high Bf. An explanation might be that the observed breathing pattern is a result of both phenomena and therefore shows a mixed breathing pattern, but with the small number of subjects in this study this could not be proved.

The final observation that needs to be explained is the fact that the subjects in BPgroup 3 increase their Bf earlier. The earlier explanation that they developed this pattern due to stress perception can still stand. However, from the data in this study it does not become clear what causes this stress perception. The ACQ and mini-AQLQ were not taken by the healthy subjects and the Nijmegen Questionnaire did not show evidence for DB. Therefore, it remains unclear what causes this earlier elevation of Bf. The earlier given explanation that the deviant breathing pattern is not pathological but only a rarer breathing pattern might also be true.

Breathing pattern groups based on Ldis (LISgroups)

Observation and interpretation of the results

The classification of subjects among the LISgroups was based on Ldis. As described in the methods a higher Ldis indicates a higher level of ineffective breathing. Whether asynchronous breathing is an indication of dysfunctional must appear from the cycling ergometry parameters and questionnaires. The parameters in Table 10 that show a significant difference or a trend towards one, are %pred max load, %pred V'O2 at max, Vtex at max, VAS for past 7 days and for dyspnoea after exercise test and the subdomain of mini-AQLQ for environment. Additionally, the patient characteristics age, height, BMI, fat percentage also show a significant difference or a trend towards one.

The difference in age, height, BMI, and fat percentage is most notable for LISgroup 4. These subjects are older and shorter, with a higher BMI and fat percentage. These results suggests that this would lead to more synchronous breathing. LISgroup 4 also has a lower Vt at max and a higher VAS for dyspnoea after the exercise, indicating an impediment to use their full breathing potential. Together this suggests that a synchronous breathing indicates a breathing disorder.

The %pred for max load and V'O2 at max for LISgroup3 are surprising when looking at the differences between groups. %pred for max load was lower, while the %pred V'O2 at max was higher compared to the other LISgroups. This might be an indication of an insufficient breathing pattern, because when the breathing would be sufficient these subjects would either reach a higher load or would not need such a high V'O2 level to reach this load. This would indicate that asynchronous breathing is an indication of DB.

Explanation of the results based on the literature

Thus, both LISgroup 3 and LISgroup 4 give an implication of DB, which is not the case for LISgroup 1 and LISgroup 2, despite a certain level of asynchrony. This indicates that the asynchrony observed in LISgroup 1 and 2 is not dysfunctional and thus that a certain level of asynchrony is normal and does not cause any complaints or impediment. Notable is that 33% of asthma patients were categorized in LISgroup 3 and LISgroup4 implicating DB, versus only 7% of healthy subjects that were categorized in these groups. These prevalences agree with the percentages for DB found by Thomas et al. in asthma patients versus the general population(29% vs 8%)[11].

The statement that a certain level of asynchrony is functional is further supported by Tomich et al., who found that during different exercises asynchrony increases in healthy subjects[51]. Additionally, Tobin et al. describes that asynchronous breathing results from an increased respiratory work rate, which logically happens during exercise[52], and thus indicating that increasing asynchronous breathing is a physiological response to physical exercise.

As mentioned, the Ldis of the subjects in LISgroup 3 suggest that when asynchrony increases this is no longer functional. At the same time the subjects in LISgroup4 suggest that constant synchronous breathing also indicates DB, which seems to contradict the previous statement. Nevertheless, both statements might be true.

The subjects in LISgroup4 breath synchronously during the whole measurement but show an impediment to increase their Vt and also an elevated level of dyspnoea after the exercise test. Striking is that the subjects in LISgroup1 and LISgroup2 show an increase of asynchrony during the exercise test, but do not show any signs of DB. Together with the studies by Tomich et al. and Tobin et al. and the fact that the subjects in LISgroup4 do show signs of DB (inability to increase Vt and a high VAS for dyspnoea) but not an elevated level of asynchrony, suggests that they suffer from any kind of DB[51], [52]. If this is true, this means that an inability to increase asynchrony is a sign of DB.

On the contrary, research by Fregonezi et al. states that asynchronous breathing is adopted by asthma patients resulting in exercise limitation[53]. This, together with the discrepancy between the maximal workload and maximal O_2 consumption of the subjects in LISgroup 3 and their large increase of asynchrony, suggests that large asynchrony is a sign of DB. It has to be noted that this kind of DB is different than the kind that of DB that is indicated by synchronous breathing. This form of dysfunctional, with increased Ldis, can be associated with thoraco-abdominal asynchrony as described by Boulding et al.[21]. This is also associated by Upton et al. with decreased perceived asthma control, but this could not be confirmed by this study (ACQ-7 and mini-AQLQ not significantly different between groups ($p=0.261$ and $p=0.206$)).

Notable is that Ashutosh et al. describe that patients with obstructive disease (in their case COPD) often have an asynchronous breathing pattern. These patients also showed significantly lower FEV1 in their study. However, the patients in our study did not show decreased FEV1 before and after the exercise, as intended by the administration of a bronchodilator. This makes it unlikely that bronchial obstruction caused the asynchronous breathing patterns of the subjects in our study because EIB, and thus a lowered FEV1, would then still be present after the exercise test.

Strengths, shortcomings, and future recommendations

The major strength of this study is the innovative character. As far as we know, this study is the first to analyse the breathing pattern of subjects using Vt, Bf, and the thoracic and abdominal expansion during different phases of exercise and studied the "natural" breathing pattern of asthma patients by cancelling out the effect of EIB with the administration of bronchodilators before the exercise test. Earlier studies described the general effect of exercise on Vt and Bf but did not describe the change of their relative contribution during increasing exercise load. This study describes the change in relative contribution of Vt and Bf during different phases of the exercise test by introducing a new parameter: the BPratio. This parameter can be compared between different subjects due to scaling with the predicted VC. Scaling diminishes any possible unwanted differences caused by a different resting Vt as a result of height differences.

Next to this, a new way to use Lissajous plots was introduced by plotting the volume changes of thorax and abdomen against each other instead of the absolute volumes. We believe that this better represents asynchronous breathing because asynchronous breathing will cause paradoxical change of circumference, which is better represented by plotting volume changes (derivative of the volumes) against each other than by plotting absolute volumes against each other.

Another strength is the large amount of data that was collected to explain any differences between breathing patterns. Many cycling ergometry parameters were collected during the exercise test, many lung function parameters were measured before and after the exercise test and several questionnaires were taken by the subjects. Altogether, this allowed us to give an extensive insight in the differences between the subjects with different breathing patterns. The time did not allow to analyse all this data, for example the results from the IOS tests were not further analysed in this study, but the data is available to analyse in follow up studies.

A shortcoming of this study is the lack of a reliable calibration of the RIP bands. Calibration of these RIP bands appeared to be harder than expected which resulted in the inability to analyse whether certain subjects use either their thorax or abdomen more during breathing. As for example extensive thoracic breathing is known to be a kind of DB it would be very interesting to analyse whether this was the case in certain subjects and whether this would lead to or result from certain BPratios or levels of Ldis. As earlier mentioned, the major issue that was encountered is that normal distribution is required while a stable Vt is required, resulting in conflicting requirements for the number of breaths used for the calibration. Therefore, it should be tested whether there is an optimal number of breaths that results in a reliable calibration. If this is not possible, another method for calibration is required. For this the most reliable option is a 5 min period with quiet breathing as used for the QDC method presented by Sackner et al. or letting the subjects perform isovolumetric manoeuvres as described by Konno and Mead[22], [25]. Both would lead to an addition to the study protocol for future measurements. The disadvantage of using these methods is that the subjects in this study did not perform these calibration procedures, which means that their data cannot be used for analysis of thoracic or abdominal dominance. If it is desired to use their data as well, the calibration methods presented in this study have to be further developed or another new calibration method has to be designed.

Another point of discussion is that Ldis will increase proportional to the Vt, with equal phase difference. After all, when the amplitude of two sine waves increases, the width of the Lissajous figure they create will also increase. This means that during the exercise test Ldis will always increase when Vt increases, which might give a false indication of dysfunctional breathing. To correct for this, it is possible to divide Ldis by the Vt, but it is not sure whether this would lead to more reliable results. Namely, the amount of volume that is ineffectively moved surely increases. Due to time limitations the correction for Vt was not investigated in this study.

The inclusion of more subjects in this study would allow for a more in depth analysis of the differences between breathing patterns. Based on the breathing patterns of the subjects in this study it was possible to make subgroups for the different breathing patterns, both for the BPgroups and the LISgroups. However, these were not further analysed because the number of subjects in certain subgroups was very small (sometimes only 1), which does not allow for a reliable analysis. Even with the current groups it was sometimes hard to evaluate the differences. A larger study population would also allow to analyse whether subjects in a certain BPgroup are more prone to be classified in a certain LISgroup or vice versa.

Other interesting topics to research are the effects of changing certain conditions. For example, by testing the effect of the reliever medication on the breathing pattern by letting asthma patients perform the same tests without the use of medication. Based on this study it can be hypothesized that this would increase dyspnoea and thus increase stress perception, which would trigger earlier increase of Bf, like seen in BPgroup 2 and 3. Other possibilities are testing whether seasonal allergies affect the breathing pattern by letting patients perform the same tests during different periods of the year or testing whether the breathing pattern can predict exacerbations by following the patients for a certain period and check whether they develop an asthma exacerbation in this period. When experiencing more complaints, it is again expected that this would lead to an earlier increase of Bf.
To check the reliability of the repeated tests and whether any differences are not caused by coincidence, the reproducibility of a breathing pattern should be researched as well. This can easily be done by letting subjects perform the same test one week later under the same circumstances and analyse whether they show the same breathing patterns as the week before.

At last, when the mentioned additional research is performed and if it is confirmed that certain breathing patterns are dysfunctional it has to be researched whether these can be treated. The most likely treatment is breathing retraining by a physiotherapist. This has shown to be useful by Courtney et al. and Denton et al.[47][54]. However, whether this is also useful for every deviant breathing pattern observed in this study has to be researched.

Chapter 6: Conclusion

To conclude, asthma patients more often show a deviant breathing pattern than healthy subjects based on the level of asynchronous breathing. This was quantified by two different novel methods to analyse the breathing patterns of subjects during physical exercise. Based on the new parameter BPratio subjects could be distributed in groups according to the change in contribution of Vt and Bf over time. This showed that some subjects increase their Bf much earlier than expected. It was hypothesized that these subjects developed this breathing pattern as their natural breathing pattern as a response to elevated stress perception during exercise in the past, which was based on high VAS and ACQ scores and a low mini-AQLQ score. With another new parameter Ldis patients were grouped based on their level of asynchronous breathing. This showed that both lowered and increased levels of asynchrony might be a sign of DB, which was based on lowered performance level and increased sense of dyspnoea at the end of maximal exercise and was more common in patients with asthma. Due to the small number of patients with these deviant breathing patterns it was hard to confirm the theories presented in this study and therefore these must be interpreted with caution. Nevertheless, this study resulted in defining new promising parameters to analyse breathing patterns for the diagnosis of DB in patients with asthma, which might resulted in more optimal treatment of patients with asthma suffering from EID due to an easier diagnosis of co-existing DB.

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Appendix A: The Hexoskin shirt

An adaption of the description of the Hexoskin shirt as described in the METC-protocol for the Hexoskin study by Mannée et al.

During this study, the Hexoskin shirt (Carré Technologies Inc, Montreal, Canada) was used to measure the breathing pattern during exercise. In a previous study, Mannée et al. demonstrated the usability of the Hexoskin shirt to reliably measure dynamic hyperinflation and lung volumes[26]. In this study, the main purpose of the Hexoskin is measuring the breathing patterns.

Measurement parameters and principles

The Hexoskin shirt is a commercially available shirt, in which several sensors are integrated. The Hexoskin shirt contains sensors for a 3 leads electrocardiogram (ECG), RIP-sensors and 3-axial accelerometer. The main parameters measured by these sensors are:

- HR: The ECG-sensors are placed on the thorax and abdomen, see Figure 16 (left). The sensors are made of special conducting fabrics incorporated in the shirt. It has to be ensured that proper skin contact is present, and the sensors should be humidified before measurement.

FIGURE 16: PLACEMENT OF (FROM LEFT TO RIGHT) ECG SENSORS, RIP SENSORS, AND 3-AXIAL ACCELEROMETERS

- RR: The RIP-sensors are two straps, one around the thorax and one around the abdomen, see Figure 16(middle). How these sensors work is further explained below.
- Acceleration (A): The accelerometer is placed in a pocket at the side of the shirt, see Figure 16 (right). It detects acceleration in 3 directions.

From the three main parameters (HR, RR, and A) either parameter can be derived:

- Heart rate variability: this parameter describes the increase and decrease in heart rate and can be useful, amongst eithers, to investigate recovery of a subject after exercise.
- Tidal volume: by means of the stretching of the RIP bands a measure of tidal volume is derived. Together with RR this leads to a measure for Vt.

- Step count or cadence and calories: an enumeration of the three parameters is derived from the accelerometer data and that can be useful to describe and quantify a person's daily activity level.

Respiratory inductance plethysmography

RIP is based on Lenz' law, and Faraday's law, which describe the induction of a perpendicular magnetic field relative to the coil to which an alternating current is applied. RIP consists of two bands respectively placed at the rib cage and abdomen[55]. Each band contains wires through which an alternating current is running (with use of a direct current and an oscillator), inducing self-inductance. The surface enclosed by the wires changes due to breathing. This changes the self-inductance, therefore the change in self-inductance reflects the respiration of a subject [56]. Changes in circumference of the chest wall can be related to actual volumes in- or expired [14]. In 1967, Konno and Mead [22], were the first to measure volumes with only the change in circumference of the rib cage and abdomen. The RIP technique is the most common used 'circumference' technique, and is used in multiple studies to measure lung volumes based on the two-degree of freedom theory of Konno and Mead [20–23]. In the past years, the main techniques for calibration of RIP (from arbitrary units (from current output) to volume) are qualitative diagnostic calibration [25] and least squares method[58].

The Hexoskin shirt

All data measured with the Hexoskin shirt is transmitted to a smart phone application via a secured Bluetooth connection. Data is anonymously synchronized to a server of Carré Technologies. Carré Technologies is only connected to the 'owner' (the coordinating researcher) of the shirt, they cannot relate the data to subjects performing in the study. Any specifics in the data storage will only lead back to the coordinating researcher. The synchronization is only necessary to provide the coordinating researcher with the necessary raw data. Nevertheless, the server (based in Canada) provides an extensive fire wall and access to the data is secured through a user's account which credentials are only known by the coordinating researcher and the principal investigator. For the researchers, the data is coded.

The Hexoskin shirt is provided with an electromagnetic compatibility CE certification (see attachment D4 'Hexoskin CE Certification'). In the CHOPIN study, the Hexoskin shirt was approved by the medical technology department of the Medisch Spectrum Twente. For additional information on the Hexoskin shirt, see attachment D2 'Risicoanalyse'.

Summary of findings from non-clinical studies

RIP technology has been tested in non-clinical studies, for example in animal studies. In the study of Su et al.[59]. RIP was used to measure tidal volume (after calibration) and was compared to flow meter data. They concluded that it was possible to measure tidal volumes with RIP, with use of a simple linear regression calibration. However, the data were obtained from sedated and anesthetized swine, and therefore cannot be applied to humans in spontaneous breathing [55], [57], [58], [60]–[62].

A recent study of Villar et al. has investigated the validity and reproducibility of the parameters measured by the Hexoskin shirt[63]. In their study, 20 healthy subject performed tests with the Hexoskin shirt and a reference measurement device. In the tests, posture and activity level changed. The study shows that the Hexoskin shirt has a moderate to good reproducibility.

Moreover, correlation was good between the Hexoskin shirt and the reference device. Tidal volume and V'E were measured in arbitrary units and not in litres.

The earlier mentioned study by Mannée et al. showed that a linear relationship exist between output data (in arbitrary units) and varying circumference and ambient temperature[26]. This makes the Hexoskin shirt a feasible tool to measure lung volumes.

Summary of findings from clinical studies

In the past years, the Hexoskin shirt is tested in numerous studies, which can be found on the research page of Hexoskin[15]. 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 [64]–[67].

Phillips et al.[68] studied the reliability and validity of the Hexoskin shirt in low to vigorous exercise levels. Six healthy subjects were studied. However, only RR was compared to a reference method. Moreover, Elliot et al. [69] studied the reliability and validity in 10 elite cyclists. HR and RR were measured adequately by the Hexoskin shirt. The calculated V'E (as calculated by the algorithm of Hexoskin) showed large errors with the V'E measured by lab equipment, especially at higher intensity exercise. The authors mention that an appropriate calibration needs to be applied to obtain better V'E. Therefore, Mannée et al. studied the feasibility of the Hexoskin shirt to measure Hyperinflation and describe reliable calibration methods for temperature and volume[26].

Appendix B: additional measurements BPgroups

TABLE 11: COMPARISON OF SUBJECT CHARACTERISTICS AND STUDY PARAMETERS BETWEEN THE DIFFERENT BPGROUPS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST. THE P-VALUE OF SEX WAS DETERMINED USING THE FISHER-FREEMAN-HALTON EXACT TEST. IN APPENDIX B A COMPARABLE TABLE IS SHOWN FOR THE PATIENTS WITH ASTHMA ONLY.

	BPgroup 1	BPgroup 2	BPgroup 3	p-value
Fat percentage (%)	29.5 (23.6 - 34.0)	(28.9 - 41.3)[+]	28.3 (16.3 - 37.9)	0.406
Abdominal circumference (cm)	90 (79 - 96)	83 (73 - 98)	77 (74 - 95)	0.441
V'O2 at max (L)	3.2 (2.4 - 3.4)	2.0 (1.9 - 2.1)	2.3 (2.1 - 2.8)	**0.011**[a,e]
Respiratory exchange rate at max	1.24 (1.20 - 1.28)	1.16 (1.08 - 1.26)	1.17 (1.20 - 1.29)	0.414
Heart rate at max (bpm)	181 (169 - 193)	171 (154 - 191)	181 (167 - 204)	0.678
V'E at max (L/min)	125 (111 - 143)	74 (63 - 91)	89 (78 - 132)	**0.014**[a]
Mini-AQLQ: symptoms	6.8 (6.0 - 6.8)	4.8 (4.3 - 6.4)	5.6*	0.134
Mini-AQLQ: emotions	7.0 (6.7 - 7.0)	6.2 (4.5 - 6.8)	6.0*	0.183
Mini-AQLQ: environment	6.7 (6.0 - 7.0)	4.3 (3.8 - 6.1)	4.7*	0.070[d]

[+] fat percentage was only measured for 3 subjects in BPgroup2, therefore only the minimal and maximal value is shown, as a median and interquartile range could not be calculated.
***** BPgroup 3 only contains one asthma patient, therefore the value of this subjects was given for the asthma control questionnaire and mini-AQLQ (only asthma patients filled this in), as a median and interquartile range could not be calculated.
[a] Significant difference between group 1 and 2
[b] Significant difference between group 1 and 3
[c] Significant difference between group 2 and 3
[d] Trend towards a significant difference between group 1 and 2
[e] Trend towards a significant difference between group 1 and 3
[f] Trend towards a significant difference between group 2 and 3
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests
Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)

TABLE 12: COMPLETE RESULTS OF THE FORCED SPIROMETRY TESTS FOR THE DIFFERENT BPGROUPS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST. IN APPENDIX B A COMPARABLE TABLE IS SHOWN FOR THE PATIENTS WITH ASTHMA ONLY.

	BPgroup 1	BPgroup 2	BPgroup 3	p-value
FVC pre exercise (L)	5.26 (4.36 - 5.81)	4.19 (3.63 - 4.39)	4.52 (3.12 - 5.18)	**0.040[d]**
%pred FVC pre exercise	104.7 (96.8 - 109.2)	99.4 (95.2 - 104.3)	96.8 (84.3 - 103.2)	0.172
FEV1 pre exercise (L)	3.91 (3.35 - 4.90)	3.74 (2.80 - 3.89)	3.57 (2.61 - 3.99)	0.219
%pred FEV1 pre exercise	99.0 (90.0 - 106.6)	101.5 (89.4 - 114.8)	90.5 (82.6 - 98.8)	0.228
FEV1/FVC pre exercise (%)	77.6 (71.8 - 82.7)	87.7 (76.5 - 90.3)	80.5 (76.3 - 83.8)	0.135
%pred FEV1/FVC pre exercise	95.9 (87.2 - 98.8)	101.3 (93.2 - 109.3)	95.45 (91.8 - 100.9)	0.260
MEF50 pre exercise (L/s)	3.72 (3.15 - 5.51)	4.94 (2.72 - 6.60)	3.69 (3.27 - 4.19)	0.652
%pred MEF50 pre exercise	78.3 (64.2 - 98.8)	105.0 (63.2 - 151.9)	76.5 (68.5 - 87.8)	0.495
FVC post exercise (L)	5.24 (4.32 - 5.70)	4.14 (3.58 - 4.28)	4.47 (3.00 - 5.12)	0.052[d]
%pred FVC post exercise	100.0 (94.5 - 110.5)	98.6 (90.1 - 106.0)	95.7 (80.9 - 102.5)	0.254
FEV1 after exercise (L)	3.91 (3.35 - 4.61)	3.76 (2.76 - 3.96)	3.58 (2.59 - 4.12)	0.453
%pred FEV1 post exercise	99.2 (89.0 - 106.3)	101.5 (87.5 - 118.6)	91.3 (83.3 - 98.1)	0.398
FEV1/FVC post exercise (%)	76.9 (70.5 - 83.2)	90.3 (75.7 - 94.0)	81.2 (79.7 - 84.7)	0.083
%pred FEV1/FVC post exercise	94.0 (87.1 - 99.9)	105.00 (92.8 - 112.3)	97.8 (92.8 - 102.4)	0.134
MEF50 post exercise (L/s)	3.79 (2.90 - 5.68)	5.21 (2.91 - 6.68)	3.78 (3.31 - 4.34)	0.780
%pred MEF50 post exercise	80.8 (61.2 - 106.2)	110.3 (67.1 - 153.7)	83.1 (73.3 - 86.8)	0.466
FVC pre reliever (L)	5.75 (4.91 - 5.86)	4.17 (3.62 - 4.43)	3.01*	**0.015[a,e]**
%pred FVC pre reliever	100.9 (97.6 - 117.1)	98.9 (95.7 - 104.6)	85.5*	0.245
FEV1 pre reliever (L)	3.96 (3.75 - 5.30)	3.70 (2.60 - 3.91)	2.50*	0.139
% pred FEV1 pre reliever	96.5 (91.7 - 110.4)	99.5 (83.2 - 115.9)	87.2*	0.584
FEV1/FVC pre reliever (%)	79.7 (68.6 - 82.7)	86.3 (70.9 - 90.8)	83.2*	0.385
%pred FER pre reliever	92.5 (86.5 - 97.4)	99.7 (86.2 - 110.0)	101.7*	0.426
MEF50 pre reliever (L/s)	4.75 (3.18 - 5.46)	4.71 (2.35 - 6.23)	3.54*	0.760

%pred MEF50 pre reliever	97.0 (63.0 - 116.0)	105 (63.0 - 152.0)	87.9*	0.878

***** BPgroup 3 only contains one asthma patient, therefore the value of this subjects was given for the asthma control questionnaire and mini-AQLQ (only asthma patients filled this in), as a median and interquartile range could not be calculated.
[a] Significant difference between group 1 and 2
[d] Trend towards a significant difference between group 1 and 2
[e] Trend towards a significant difference between group 1 and 3
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests
Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)

Appendix C: additional measurements LISgroups

TABLE 13: ADDITIONAL PARAMETERS FOR THE COMPARISON OF SUBJECT CHARACTERISTICS AND STUDY PARAMETERS BETWEEN THE DIFFERENT LISGROUPS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST.

	LISgroup 1	LISgroup 2	LISgroup 3*	LISgroup 4*	p-value
Fat percentage (%)	23.9 (17 - 28.7)	32.2 (24.5 - 34.6)	(28.9 - 38.0)	(37.7 - 41.3)	**0.018**[c]
Abdominal circumference (cm)	87 (77 - 93)	83 (75 - 90)	(70 - 114)	(98 - 103)	0.221
V'O2 at max (L)	3.2 (2.2 - 3.7)	2.5 (1.9 - 3.1)	(1.9 - 3.2)	(2.1 - 2.3)	0.180
Respiratory exchange rate at max	1.30 (1.20 - 1.30)	1.20 (1.10 - 1.30)	(1.15 - 1.28)	(1.15 - 1.17)	0.251
Heart rate at max (bpm)	190 (169 - 193)	171 (166 - 181)	(181 - 196)	(150 - 169)	*0.064*
V'E at max (L/min)	130 (115 - 159)	101.5 (78 - 135)	(71 - 125)	(77 - 81)	*0.122*
Mini-AQLQ: symptoms	(5.4 - 6.8) *	6.8 (5.6 - 6.9)	(4.3 - 6.0)	(4.6 - 5.6)	0.226
Mini-AQLQ: emotions	(6.0 - 7.0) *	7.0 (6.5 - 7.0)	(6.0 - 7.0)	(4.0 - 6.0)	0.189
Mini-AQLQ: environment	(6.66 - 7.0) *	6 (4.7 - 6.3)	(4.33 - 7.0)	(3.66 - 4.67)	*0.116*[c]

***** This value was not available for enough subjects to give median and interquartile range; therefore, the minimum and maximum value are given (min - max), LISgroup 3 and 4 did only contain 3 and 2 subjects, LISgroup 1 did only contain 3 asthma patients, therefore there were not enough cases for the ACQ and mini-AQLQ (only filled in by asthma group).
[c] Significant difference between group 1 and 4
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests.
Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)

TABLE 14: RESULTS OF THE FORCED SPIROMETRY TESTS FOR THE DIFFERENT LISGROUPS. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST.

	LISgroup 1	LISgroup 2	LISgroup 3*	LISgroup 4*	p-value
FVC pre exercise (L)	5.72 (4.95 - 5.88)	4.43 (4.21 - 5.02)	(3.02 - 7.78)	(3.15 - 3.46)	**0.020**[c,g]
%pred FVC pre exercise (%)	100.3 (96.3 - 105.8)	103.6 (96.9 - 110.4)	(68.8 - 119.4)	(89.4 - 94.6)	0.244
FEV1 pre exercise (L)	3.99 (3.79 - 4.96)	3.65 (3.37 - 3.94)	(2.60 - 5.68)	(2.52 - 2.61)	0.067[i]
%pred FEV1 pre exercise	92.6 (89.7 - 100.4)	103.8 (96.1 - 116.7)	(69.7 - 104.4)	(86.6 - 90.9)	0.142
FEV1/FVC pre exercise (%)	76.7 (71.6 - 80.4)	82.2 (77.8 - 86.3)	(73.0 - 87.8)	(72.8 - 82.8)	0.253
%pred FEV1/FVC pre exercise	92.7 (87.3 - 95.4)	98.2 (96.4 - 103.2)	(86.4 - 100.7)	(90.9 - 101.3)	0.229
MEF50 pre exercise (L/s)	3.71 (3.16 - 4.87)	4.40 (3.42 - 5.47)	(3.31 - 5.91)	(2.14 - 3.67)	0.437
%pred MEF50 pre exercise	73.2 (63.2 - 83.6)	93.7 (77.8 - 117.9)	(69.7 - 96.9)	(53.8 - 91.2)	0.205
FVC post exercise (L)	5.68 (4.87 - 5.88)	4.40 (4.25 - 5.07)	(2.93 - 7.79)	(3.02 - 3.42)	**0.014**[c,g]
%pred FVC post exercise	98.6 (94.0 - 104.7)	105.1 (98.6 - 110.4)	(66.9 - 119.6)	(85.6 - 93.8)	0.129
FEV1 after exercise (L)	4.03 (3.85 - 4.97)	3.59 (3.39 - 4.07)	(2.62 - 5.80)	(2.45 - 2.51)	0.059[i]
%pred FEV1 post exercise	91.9 (90.5 - 103.6)	104.3 (91.0 - 117.6)	(70.5 - 106.6)	(84.5 - 87.5)	0.116
FEV1/FVC post exercise (%)	79.8 (72.7 - 82.6)	80.2 (76.9 - 86.1)	(74.4 - 94.3)	(71.7 - 83.1)	0.497
%pred FEV1/FVC post exercise	97.1 (89.4 - 98.1)	98.75 (92.2 - 103.08)	(88.0 - 107.3)	(89.5 - 101.7)	0.643
MEF50 post exercise (L/s)	4.05 (3.05 - 5.22)	4.55 (3.46 - 5.72)	(3.04 - 5.72)	(2.20 - 3.40)	0.376
%pred MEF50 post exercise	85.1 (63.6 - 89.1)	102.4 (75.3 - 122.4)	(64.1 - 103.2)	(55.1 - 84.4)	0.357
FVC pre reliever (L)	(5.75 - 5.86) *	4.54 (4.17 - 5.00)	(4.48 - 7.63)	(3.01 - 3.47)	0.055[i]
%pred FVC pre reliever	(97.6 - 105.5) *	100.1 (96.2 - 112.9)	(97.7 - 117.1)	(85.4 - 95.0)	0.280
FEV1 pre reliever (L)	(3.96 - 5.30) *	3.75 (3.43 - 3.92)	(3.85 - 5.61)	(2.29 - 2.50)	**0.044**[i]
% pred FEV1 pre reliever	(91.7 - 110.4) *	102.4 (87.3 - 115.9)	(96.5 - 103.2)	(78.7 - 87.2)	0.351
FEV1/FVC pre reliever (%)	(68.6 - 92.1) *	82.7 (72.4 - 89.5)	(73.6 - 85.9)	(65.9 - 83.2)	0.930
%pred FER pre reliever	(86.5 - 108.3) *	97.4 (87.6 - 107.3)	(87.1 - 97.7)	(82.3 - 101.7)	0.935
MEF50 pre reliever (L/s)	(3.18 - 6.82) *	4.81 (3.27 - 5.87)	(4.26 - 5.46)	(1.71 - 3.54)	0.397

%pred MEF50 pre reliever	(61.3 - 119.7) *	99.7 (68.4 - 133.00)	(87.5 - 89.6)	(42.9 - 87.9)	0.589

***** This value was not available for enough subjects to give median and interquartile range; therefore, the minimum and maximum value are given (min - max), LISgroup 3 and 4 did only contain 2 subjects, LISgroup 1 did only contain 3 asthma patients, therefore there were not enough cases for the pre reliever values (only for asthma group).
[c] Significant difference between group 1 and 4
[g] Trend towards a significant difference between group 1 and 2
[i] Trend towards a significant difference between group 1 and 4
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests.
Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)

Appendix D: results for asthma patients only

TABLE 15: COMPARISON OF SUBJECT CHARACTERISTICS AND STUDY PARAMETERS BETWEEN THE DIFFERENT BPGROUPS FOR THE ASTHMA PATIENTS ONLY. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST. THE P-VALUE OF SEX WAS DETERMINED USING THE FISHER-FREEMAN-HALTON EXACT TEST.

	BPgroup 1	BPgroup 2	BPgroup 3*	p-value
Age (years)	25 (21 - 45)	34 (21 - 48.5)	43	0.703
Sex (N Male (%))	25 (21 - 45)	34 (21 - 48.5)	43.0	**0.037**[a]
BPratio rest	10.17 (9.68 - 22.51)	8.33 (7.58 - 10.23)	8.8	0.090[d]
BPratio warmup	12.06 (8.5 - 16.6)	8.61 (7.45 - 12.65)	8.4	0.454
BPratio 0-20% of max	10.75 (8.32 - 16.51)	10.12 (9.84 - 11.03)	21.9	0.277
BPratio 20-40% of max	12.85 (9.97 - 20.97)	13.52 (12.28 - 16.99)	13.6	0.989
BPratio 40-60% of max	15.42 (13.35 - 26.59)	16.43 (11.93 - 22.02)	12.6	0.553
BPratio 60-80% of max	22.72 (18.09 - 25.71)	16.34 (12.11 - 17.92)	13.0	0.077
BPratio 80-100% of max	16.98 (13.79 - 18)	15.94 (11.83 - 17.24)	11.7	0.454
BPratio recovery	17.02 (14.12 - 18.18)	13.85 (10 - 15.41)	11.4	0.177
Height (cm)	182.4 (177.0 - 184.0)	168.5 (167.4 - 175.9)	160.7	**0.045**[e,f]
Weight (kg)	87.5 (79.7 - 91.7)	73.3 (63.2 - 89.1)	86.4	0.393
BMI (kg/m²)	26.9 (24.3 - 28.9)	25.8 (20.5 - 31.8)	33.5	0.360
Fat percentage (%)	28.0 (22.7 - 31.9)	(28.9 - 41.0) *	37.7	0.146
Abdominal circumference (cm)	90 (75 - 102)	83 (73 - 98)	98	0.597
%pred max load (%)	146.7 (108.7 - 193.1)	144.4 (115.2 - 179.6)	177.8	0.769
V'O2 at max (L)	3.2 (3.0 - 3.3)	2.0 (1.9 - 2.1)	2.3	**0.015**[a]
%pred V'O2 at max (%)	92.4 (68.9 - 103.8)	96.2 (81.3 - 111.5)	75.6	0.535
Respiratory exchange rate at max	1.26 (1.21 - 1.30)	1.16 (1.08 - 1.26)	1.15	0.354
Heart rate at max (bpm)	193 (169 - 193)	171 (154 - 191)	169	0.483

Heart rate reserve at max (=(220-age)-HR)	3 (2 - 7)	15 (7 - 19)	8	0.225
V'E at max (L/min)	125 (122 - 146)	74 (62 - 91)	81	**0.017**[a]
%pred of max V'E at max (=40*FEV1) (%)	78.5 (56.7 - 86.4)	53.9 (42.4 - 72.8)	77.9	0.174
Vtex at max (L)	2.8 (2.6 - 3.2)	2.0 (1.9 - 2.1)	1.7	**0.015**[a,e]
Bf at max (breaths/min)	38.8 (35.6 - 50.6)	38.6 (31.9 - 42.8)	48.1	0.455
VAS for dyspnoea over past 7 days (mm)	8 (2 - 23)	36 (6 - 56)	14	0.554
VAS for dyspnoea before exercise test (mm)	2 (0 - 12)	17 (0.8 - 62)	1	0.514
VAS for dyspnoea after exercise test (mm)	67 (55 - 69)	84 (39 - 89)	93	0.106
BORG for dyspnoea before exercise test	0.5 (0.0 - 0.5)	0.25 (0 - 0.9)	0.0	0.617
BORG for heavy legs before exercise test	0.0 (0.0 - 0.5)	0.0 (0 - 0.4)	0.0	0.825
BORG for dyspnoea after exercise test	4.0 (4.0 - 5.0)	6.0 (3.25 - 8.8)	7.0	0.484
BORG for heavy legs after exercise test	7.0 (4.0 - 8.0)	6.0 (4.3 - 8.5)	4.0	0.582
Asthma control Questionnaire-7	0.1 (0.1 - 0.6)	1.6 (0.5 - 2.1)	1.1	0.114[d]
Mini-AQLQ: symptoms	6.8 (6.0 - 6.8)	4.8 (4.3 - 6.4)	5.6	0.134
Mini-AQLQ: activity	7.0 (6.8 - 7.0)	4.4 (3.5 - 5.8)	6.3	**0.026**[a]
Mini-AQLQ: emotions	7 (6.7 - 7)	6.2 (4.5 - 6.8)	6.0	0.183
Mini-AQLQ: environment	6.7 (6 - 7)	4.3 (3.8 - 6.1)	4.7	**0.070**[d]
Mini-Asthma quality of life questionnaire	6.7 (6.5 - 6.9)	4.7 (4.4 - 6.2)	5.7	**0.087**[d]
Nijmegen Questionnaire	8 (2 - 11)	10 (8 - 17)	9	0.578

***** BPgroup 3 only contains one asthma patients, therefore the value of this subjects is shown as an median and interquartile range could not be calculated.

[a] Significant difference between group 1 and 2

[d] Trend towards a significant difference between group 1 and 2

[e] Trend towards a significant difference between group 1 and 3

[f] Trend towards a significant difference between group 2 and 3

For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests

Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)

TABLE 16: RESULTS OF THE FORCED SPIROMETRY TESTS FOR THE DIFFERENT BPGROUPS FOR THE ASTHMA PATIENTS ONLY. THE VARIABLES ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). P-VALUES WERE CALCULATED USING A KRUSKAL-WALLIS TEST.

	BPgroup 1	BPgroup 2	BPgroup 3*	p-value
FVC pre exercise (L)	5.26 (4.36 - 5.81)	4.19 (3.63 - 4.39)	3.15	**0.040**[d]
%pred FVC pre exercise	105.1 (96.75 - 109.15)	99.4 (95.15 - 104.33)	89.40	0.163
FEV1 pre exercise (L)	3.91 (3.35 - 4.9)	3.74 (2.8 - 3.89)	2.61	0.219
%pred FEV1 pre exercise	99 (90 - 106.55)	101.45 (89.38 - 114.8)	90.90	0.228
FEV1/FVC pre exercise (%)	77.6 (71.8 - 82)	87.66 (76.49 - 90.27)	82.80	0.135
%pred FEV1/FVC pre exercise	95.4 (87.2 - 98.75)	101.25 (93.15 - 109.28)	101.30	0.252
MEF50 pre exercise (L/s)	3.72 (3.15 - 5.51)	4.94 (2.72 - 6.6)	3.67	0.652
%pred MEF50 pre exercise	78.3 (64.2 - 98.8)	104.95 (63.15 - 151.93)	91.20	0.505
FVC post exercise (L)	5.26 (4.44 - 5.76)	4.14 (3.58 - 4.28)	3.02	**0.015**[a]
%pred FVC post exercise	104.4 (95.8 - 110.5)	98.55 (90.05 - 106)	85.60	0.110[e]
FEV1 after exercise (L)	3.94 (3.42 - 4.96)	3.76 (2.76 - 3.96)	2.51	0.213
%pred FEV1 post exercise	101.3 (90.45 - 106.25)	101.45 (87.45 - 118.6)	87.50	0.264
FEV1/FVC post exercise (%)	78 (72.86 - 83.92)	90.34 (75.65 - 94.01)	83.14	0.132
%pred FEV1/FVC post exercise	94.9 (88.7 - 99.9)	104.95 (92.78 - 112.25)	101.70	0.181
MEF50 post exercise (L/s)	3.83 (2.98 - 5.68)	5.21 (2.91 - 6.68)	3.40	0.759
%pred MEF50 post exercise	85.1 (66.85 - 106.15)	110.25 (67.13 - 153.68)	84.40	0.447
FVC pre reliever (L)	5.75 (4.91 - 5.86)	4.17 (3.62 - 4.43)	3.01	**0.015**[a,e]
%pred FVC pre reliever	100.9 (97.6 - 117.1)	98.9 (95.68 - 104.6)	85.40	0.245
FEV1 pre reliever (L)	3.96 (3.75 - 5.3)	3.7 (2.6 - 3.91)	2.50	0.139
% pred FEV1 pre reliever	96.5 (91.7 - 110.4)	99.45 (83.15 - 115.9)	87.20	0.584
FEV1/FVC pre reliever (%)	79.69 (68.63 - 82.72)	86.34 (70.91 - 90.79)	83.18	0.385
%pred FER pre reliever	92.5 (86.5 - 97.4)	99.7 (86.15 - 110.03)	101.70	0.426
MEF50 pre reliever (L/s)	4.75 (3.18 - 5.46)	4.71 (2.35 - 6.23)	3.54	0.760

%pred MEF50 pre reliever	89.6 (61.3 - 99.7)	99.85 (54.05 - 143.33)	87.90	0.878

***** BPgroup 3 only contains one asthma patients, therefore the value of this subjects is shown as an median and interquartile range could not be calculated.
[a] Significant difference between group 1 and 2
[d] Trend towards a significant difference between group 1 and 2
[e] Trend towards a significant difference between group 1 and 3
For the comparison between two groups significance values have been adjusted by the Holm/Bonferroni correction for multiple tests
Bold indicates significance ($p<0.05$), *underscore* indicates a trend towards significance ($0.05 < p < 0.15$)