Exercise-Induced Laryngeal Obstruction (EILO) while breathing cold air.

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Master’s thesis

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Preface

Through physical activity, I have made friendships and learned about my strengths and weaknesses, both physically, socially, and mentally. My life has in many ways been built and motivated around physical activity, helping me towards a healthy life. However, through my studies and work as a physical therapist, I have learned that a healthiest possible life is not only about being physically active, but a privilege influenced by multiple determinants. Determinants that must be explored in varies ways and nested together.

In this master thesis and the article that follows from page 40, aimed for publication in British Medical Journal (BMJ) Open Sport and Exercise Medicine, the theoretical grounding is based in the biomedical perspective. The aims were primarily thought to be investigated and evaluated in a thesis for two medical students, but due to the COVID-pandemic, the project was delayed. Through my work at the Vitality Center for Children and Youth, Haukeland University Hospital, I got insight into the project and was invited to do this as my master project. Planning and organizing the project started in the spring of 2021, and the project kept gradually evolve through the fall and winter. Eventually reaching a comprehensive and memorable spring, resulting in the thesis and article you are about to read.

I have many to thank for the opportunity to do this master project. First and foremost, all included patients, without them this project would have been impossible. Professor Ola Drange Røksund has since my bachelor's degree, been an inspiration, and are the main reason for me being able to write this. He has further motivated me through conversations, and with an encouraging and supportive approach, guided me into an arena to thrive in. I have been lucky and thankful for having the skilled and pedagogical supervisor Silje Mæland, guiding me through an inspirational and educational process. Thanks to the project leader professor Jon Hardie for letting me be a part of this project and many hours in "the lab" together. Thanks to Maria Vollsæter and Hege Clemm for guidance and inspiration. I will also thank my leader at the Vitality center Lars Peder Bovim for being supportive and helpful throughout this whole period. Finally, I will thank my family, friends, colleagues, and teachers for your support, feedback, and questions.

Content

Terms

CLE score: Continuous Laryngoscopy Exercise score. The scoring system for diagnosing EILO during a CLE test. Contains of four sub-groups, each graded from zero to three at glottic and supraglottic level at moderate and maximal effort. A higher CLE score indicates more laryngeal closure.

CLE test: Continuous laryngoscopy exercise test. Visualizing laryngeal movements during exercise, typically on a treadmill. The gold standard for diagnosing EILO.

Cold air: Air with a temperature ≤ minus 15°C.

EIB: Exercised-Induced Bronchoconstriction.

EILO: Exercised-Induced Laryngeal Obstruction

FEV1: Forced expiratory flow during the first second during a spirometry.

Glottic: The vocal cords.

Identical workload at moderate effort: The point in time a patient first reached moderate effort when looking at two CLE tests.

Identical workload at maximal effort: The point in time a patient first reached maximal effort when looking at two CLE tests.

Laryngoscopy: Visual evaluation of larynx with optic equipment leaded through the nostrils.

Maximal effort: The moment a patient ceased to run during the CLE test, due to the standardized treadmill protocol gradually increasing in workload.

Moderate effort: The moment a patient starts to run during the CLE test, due to the standardized treadmill protocol gradually increasing in workload.

Performance: A patient's test duration during a CLE test.

Rom air: Room air with a temperature of 20 to 22°C.

Spirometry: Lung function test, measuring flow and volume of air exhaled and inhaled.

Supraglottic: Structures above the vocal cords/glottic.

Young adult: Defined in this project as 18 to 40 years.

Summary

Exercise-Induced Laryngeal Obstruction (EILO) while breathing cold air.

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Introduction: Exercise-induced laryngeal obstruction (EILO) is important to consider when investigating patients with exercise-induced dyspnea. The gold standard in EILO-diagnostics is the Continuous Laryngoscopy Exercise test (CLE test), visualizing laryngeal response patterns during a standardized treadmill exercise test with increasing workload until maximal effort. Although literature reports more symptoms of EILO during exercise in cold air, compared to room air, no studies have systematically investigated a potential difference.

Aims: To evaluate a potential difference in laryngeal obstruction when breathing cold air compared to room air, among young adults (suspected of EILO.

Methods: In this randomized crossover design, nine patients (29.8 (21-38) years (mean (range)) suspected of having EILO were included. They performed, in random order, two standard CLE tests, one while breathing cold air (-15°C) and one while breathing room air (20-22°C). The degree of laryngeal closure was scored using standard CLE score at moderate and maximal effort on glottic, and supraglottic level. A higher CLE score indicates more laryngeal closure.

Results: There was no difference in the CLE sum score (0.1(-0.7-0.5) (mean difference (95%confidence interval (CI)). When breathing cold air during the CLE tests, the mean difference in test duration were 15 seconds shorter (2-28 (95%CI)), and the supraglottic closure at moderate effort was higher at an identical workload (0.6 (0.2-1) (mean difference (95%CI)), compared to when breathing room air.

Conclusion: Breathing cold air compared to room air did not influence the CLE sum score. The patients ran for a shorter period when breathing cold air, and supraglottic obstruction was more prominent at moderate effort. More extensive studies with more patients are needed.

Key words: Exercise-induced laryngeal obstruction, Cold air, Continuous Laryngeal Exercise test, Exercise-induced dyspnea, Exercise, Physical activity, and Randomized crossover design.

Sammendrag

Introduksjon: Anstrengelses-utløst larynks obstruksjon (EILO) er viktig å ta hensyn til i utredningen av pasienter med anstrengelses-utløst pustebesvær. Gullstandarden for å undersøke pasienter med mistanke om EILO er en kontinuerlig laryngoskopi anstrengelses test (CLE test), da den visualiserer strupen underveis i en standardisert tredemølle protokoll som øker gradvis i belastning frem til maksimal anstrengelse. Det rapporteres i litteraturen om at symptomer på EILO er vanligere når det pustes inn kald luft sammenlignet med romluft, men ingen studier har systematisk undersøkt en mulig forskjell.

Hensikt: Å undersøke en mulig forskjell i larynks obstruksjon når det pustes inn kald luft sammenlignet med romluft, blant unge voksne med mistanke om EILO.

Metode: I denne randomiserte overkrysningsstudien ble ni pasienter (29.8 (21-38) år (gjennomsnitt (variasjonsbredde)) med mistanke om EILO inkludert. De gjennomførte i randomisert rekkefølge to CLE tester, en med kald luft (-15°C) og en med romluft (20-22°C), for å finne and skåre grad av larynks obstruksjon ved moderat og maksimal anstrengelse på stemmebåndsnivå og over stemmebåndsnivå. En høyere CLE skår indikerer større grad av obstruksjon.

Resultat: Det ble ikke funnet noen forskjell på total CLE skår (0.1 (-0.7-0.5) (gjennomsnittlig forskjell (95% konfidensintervall (KI)). Pasientene løp 15 sekunder kortere (2-28 (95%KI)) når de pustet kald luft, og hadde en høyere CLE skår over stemmebåndsnivå ved moderat anstrengelse ved identisk arbeidsbelastning (0.6 (0.2-1) (gjennomsnittlig forskjell (95%KI)), sammenlignet med når de pustet romluft underveis i CLE testen.

Konklusjon: Det å puste kald luft viste ingen forskjell på total larynks obstruksjon, sammenlignet med romluft. Pasientene løp kortere når de pustet kald luft og hadde mer lukking ved moderat anstrengelse. Mer forskning med flere pasienter er nødvendig.

Nøkkelord: Anstrengelses-utløst larynks obstruksjon, Kald luft, Kontinuerlig laryngoskopi anstrengelses test, Anstrengelses-utløst pustebesvær, Trening, Fysisk aktivitet og Randomisert overkrysningsstudie.

1. Introduction

Exercise-induced dyspnea, or shortness of breath, is a common complaint among adolescents and young adults.[1] Typical symptoms are wheezing, coughing, throat tightness and chest tightness,[2] which an obstruction in the airways can induce. This is typical referred to as asthma.[3] This thesis and the article will use the term exercise-induced bronchoconstriction (EIB) instead of asthma. It specifies the obstruction being exercise-induced and located in the bronchi in the lower airways (illustrated in red in figure 1). EIB is a well-known and studied problem in physically active persons.[4] The highest prevalence is fifty percent among high-performance athletes inhaling cold and dry air.[5] The prevalence is poorly understood in the general population due to most research focusing on children, adolescents, and athletes.[6] However, prevalence is estimated to be five to twenty percent in the general population,[6] and eight to ten in the Norwegian childhood population.[7] Breathing cold air is a well-known trigger for EIB [3, 8-11] and increases the ability to correctly identify a patient having EIB.[9, 12, 13] A study has also found a reduction in performance, with a lesser oxygen consumption (VO_2) among athletes with EIB exercising in the cold.[14] Reduced performance however, is also found among athletes with no lung diseases.[15] Medication to open the bronchi and reduce airway obstruction is the primary treatment for EIB.[3]

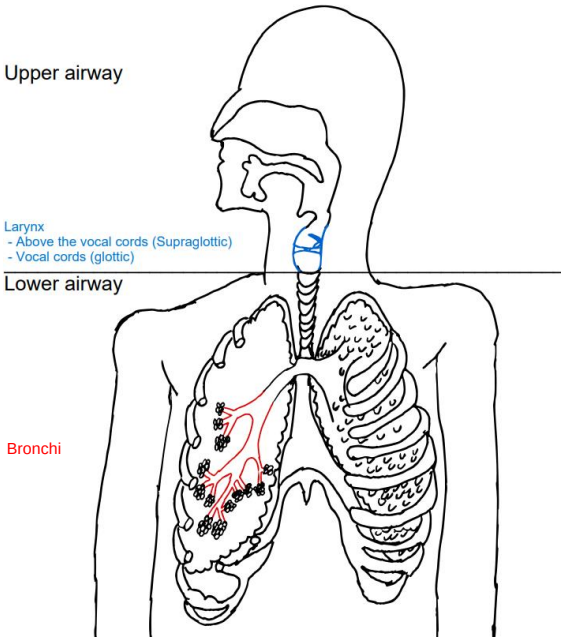

Figure 1. A schematic drawing of the airways highlighting the bronchi and larynx.

As a common airway obstruction, misdiagnosing of EIB has resulted in many patients being wrongly diagnosed and medicated.[16] In retrospect, patients have been diagnosed and treated too late for a less-known cause of airway obstruction, termed Exercise-Induced Laryngeal Obstruction (EILO). EILO is an inspiratory airway obstruction in the larynx in the upper airways (illustrated in blue in figure 1).[1] Patients with EILO are a large, heterogeneous, and vastly understudied group,[2] despite the estimated prevalence of five to seven percent

in the general population.[17, 18] EILO-treatment aims to reduce the perceived exercise-induced dyspnea, but the optimal approach remains to be determined.[19]

Note that the pathophysiology of EIB is beyond the aim of this master project and therefore not discussed. However, when evaluating EILO, mentioning EIB due to overlapping clinical features is appropriate, despite a different pathophysiological background.

1.1 EILO and cold air

The larynx's primary role during ventilation is to transport air in and out of the lungs.[20] The vocal cords, further referred to as glottic, are placed in the larynx (illustrated in figure 2). Glottic consist of voluntary muscles.[21] It is the narrowest part of the upper airways,[22] making it vulnerable to causing turbulent air and high airways resistance during inspiration.[23] The airway resistance should be as low as possible during exercise, and glottic movements are supposed to open up to reduce the airway resistance.[1] As the glottic closes, the tract gets smaller. If enough inwards movement is observed during inspiration when exercising, it is called EILO on the glottic level.[24] It is also described, among other things, as Vocal Cord Disorder,[25] but in this thesis denoted only as EILO on the glottic level. Obstruction above the glottic level can also induce EILO, specified as EILO on the supraglottic level (illustrated in figure 2). The supraglottic level consists of cartilage called Cuneiform tubercles, further called supraglottic. An inspiratory obstruction occurs if supraglottic falls enough inwards over the respiratory tract during inspiration.[24]

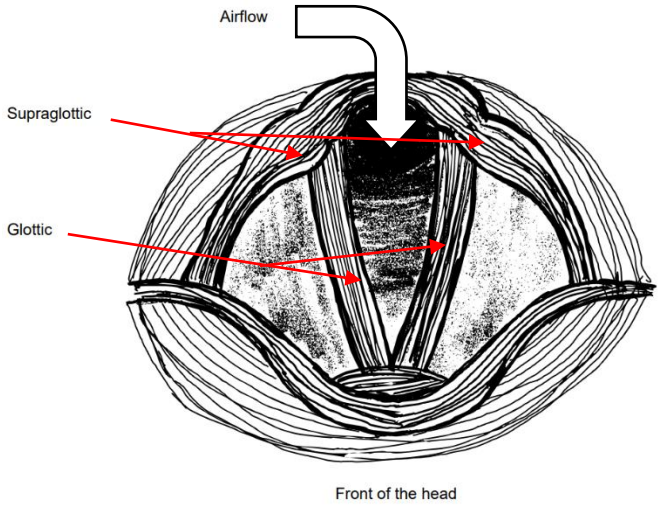

Figure 2. Illustration of larynx seen from above. The arrow illustrating air entering the lower airways through larynx and the respiratory tract.

Symptoms for both glottic and supraglottic EILO occurs during or immediately after exercise.[2] Symptoms not mentioned previously and closely associated with EILO are inspiratory stridor (abnormal breathing sounds during inspiration), panic, abnormal and hyperventilation.[25] Symptoms for EILO worsen with increased exercise due to

increased respiratory work.[2] The demand for respiratory work depends upon how physically active and relatively fit a person is, plausibly explanation why EILO is most commonly seen among adolescents and young physically active adults.[25] Being physically active might also make patients more attentive to minor airflow disturbances during exercise than sedentary people.[26] However, knowledge regarding important epidemiological data such as prevalence, age, and gender distribution is lacking. On that note, research and awareness regarding EILO are increasing.

The first publication on EILO on the glottic level came in 1983.[27] Since then, research to better understand EILO has gradually evolved. The first publication on the current gold standard for diagnosing EILO, further referred to as the Continuous Laryngeal Exercise test (CLE test), was published in 2006.[28] In 2021, EILO became an official diagnosis in Norway.[29] Despite EILO being gradually more understood and known, patients with EILO are still often wrongly diagnosed.[18, 30, 31] Also, patients with EILO tends to be diagnosed too late and are given ineffective medical EIB treatment.[16] It is important to note that EILO can co-exist with EIB.[17, 18, 31] However, to distinguish and better understand different kinds of exercise-induced dyspnea, a better understanding of EILO is needed.

In Nordic countries, breathing cold air while exercising is common, and among winter athletes the prevalence of EILO is 27 percent.[32] Typical symptoms of EILO on the glottic level, like inspiratory stridor, are most commonly observed in outdoor sports athletes,[33] proposing that environmental factors can induce and affect patients with EILO. At the same time, researchers express that patients with EILO have a reduced performance when training in the cold,[2] and studies discuss whether breathing cold air might induce EILO.[2, 34, 35] A study also found that patients' breathing difficulties, related to cold air exposure, are no predictor to distinguish EILO from other lung diseases well known for being triggered by cold air.[36] These findings indicates that EILO might be triggered by cold air. However, no studies have systematically investigated if breathing cold air affects patients with EILO. To secure proper diagnostic and treatment of patients suspected of EILO, it is therefore necessary to investigate if breathing cold air causes and affects EILO.

Cold air is further defined as dry air with a temperature of ≤ -15°C, room air as 20 to 22°C, and young adults as 18 to 40 years.

1.2 Breathing cold air

From studies done on athletes with no lung diseases, a significant shorter test duration has been found when breathing cold air compared to room air.[14, 15] A possible reason for a reduced performance might be loss of water. At rest, air usually enters through the nose, where the air gets moist and warm, giving adequate air before entering the throat.[37] Breathing through the mouth, during vigorous work, affects the temperature in the upper airways faster than when breathing through the nose.[37] If simultaneously breathing cold air, the temperature of the air will be lower, resulting in the need to heat the air up even more. In order to heat up the air, the throat has an increased demand for water, resulting in cells shrinking.[37] However, how and if this affects EILO in cold air is unknown.

A high prevalence of EIB among winter athletes is found.[14] As early as 1984, a research team found that breathing cold air is a reliable and reproducible method to investigate EIB.[12] When establishing the method for breathing cold air as an examination during exercise, among patients suspected of EIB, the researchers let the participants perform lung function tests periodically during a running session. They also used well-established bronchial provocation tests to compare the participants and a control group. Due to a lack of knowledge regarding EILO, and the CLE test being the only established test for investigating EILO, a similar approach is not possible when examining EILO and breathing cold air.

1.3 The continuous laryngeal exercise test (CLE test)

The CLE test is a progressive exercise test on a treadmill.[28] The test is standardized to start at rest and gradually increase the workload until the patient reaches maximal effort, with a preset protocol. A flexible fiberoptic laryngoscope is inserted via the nostril to give an optimal view of the larynx throughout the test (See figure 3). The footage is used to examine a possible EILO on glottic, supraglottic, or both levels. The degree of inwards movement is scored, resulting in a CLE sub- and sum score. The CLE sum score goes from zero to twelve, and a higher score indicates more closure. By using a special device, evaluating patients on a treadmill while breathing cold air is possible,[38] enabling CLE tests to be performed

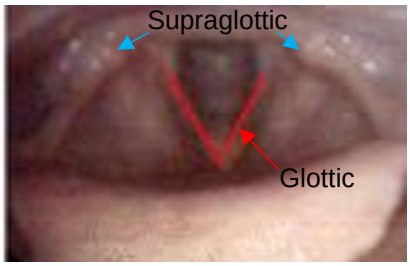

Figure 3. Real picture from a laryngoscopy filming larynx during a CLE test. Obtained from Maat et al., approved for use by the authors in this project.[24]

while breathing cold air. Due to the device however, oxygen consumption, the gold standard for evaluating physical performance, cannot be measured during a CLE test while breathing cold air. [39] Despite oxygen consumption being measured during a CLE test in room air, performance is further defined as a patients test duration.

1.4 The theoretical grounding

The theoretical grounding from previous research on EILO is dominantly a biomedical perspective. The biomedical perspective focuses on measurable biological factors.[40] Examples of this are using measurable provocation tests to investigate biological response in patients with EILO,[41] and gathering medical history in patients' journals, exclusively looking for measurable medical data.[33]

The biomedical approach to disease has been successful beyond all expectation.[40] However, biological factors are not sufficient to describe anyone's health and challenges. The biopsychosocial model illustrates how health consists of biological, psychological, and social factors (illustrated in figure 4).[40] To assume that it's sufficient only to investigate biological factors is, in the light of this, counterproductive, since this will result in a limited understanding. At the same time, when investigating a field not clearly defined, as for this project, an explorative study from a biomedical perspective can be used.

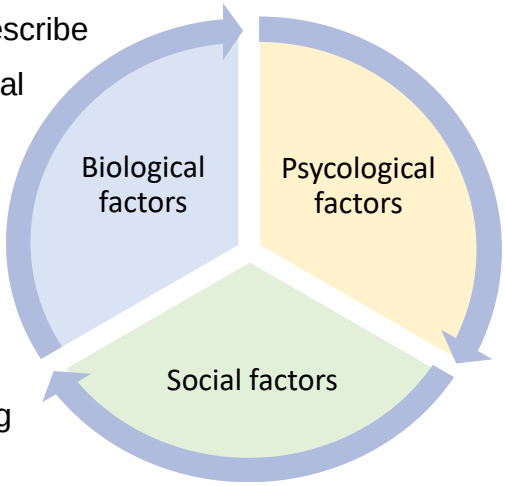

Figure 4. The biopsycosocial model illustrated. Illustrating how health consists of the three equally important factors: biological, psycological and social.

2. Purpose

The purpose of this master project is to give a better biological understanding of whether breathing cold air might affect or induce EILO and/or performance among young adults suspected of EILO.

2.1 Research question

Are CLE score and/or performance among young adults suspected of EILO affected by breathing cold air?

2.2 Hypothesis

2.2.1 Alternative hypothesis

i) When breathing cold air, young adults suspected of EILO have a different CLE score than when breathing room air.

ii) When breathing cold air, young adults suspected of EILO acquire the debut of EILO at a different time than when breathing room air.

iii) When breathing cold air, young adults suspected of EILO have a different test duration than when breathing room air.

2.2.2 Null hypothesis

i) When breathing cold air, young adults suspected of EILO have no different CLE score than when breathing room air.

ii) When breathing cold air, young adults suspected of EILO acquire no debut of EILO at a different time than when breathing room air.

iii) When breathing cold air, young adults suspected of EILO have no different test duration than when breathing room air.

3. Method

3.1 Research design

This project was an explorative study with a randomized crossover design. By using the crossover design, a patient's CLE score during a CLE test while breathing room air was compared with a CLE test while breathing cold air. The order for the first test was randomized, and each patient worked as its own control. The crossover design has previously been used when investigating the effect of physical activity and possible differences in lung function when breathing room versus cold air.[38, 42-44] Figure 5 illustrates how the research design was used.

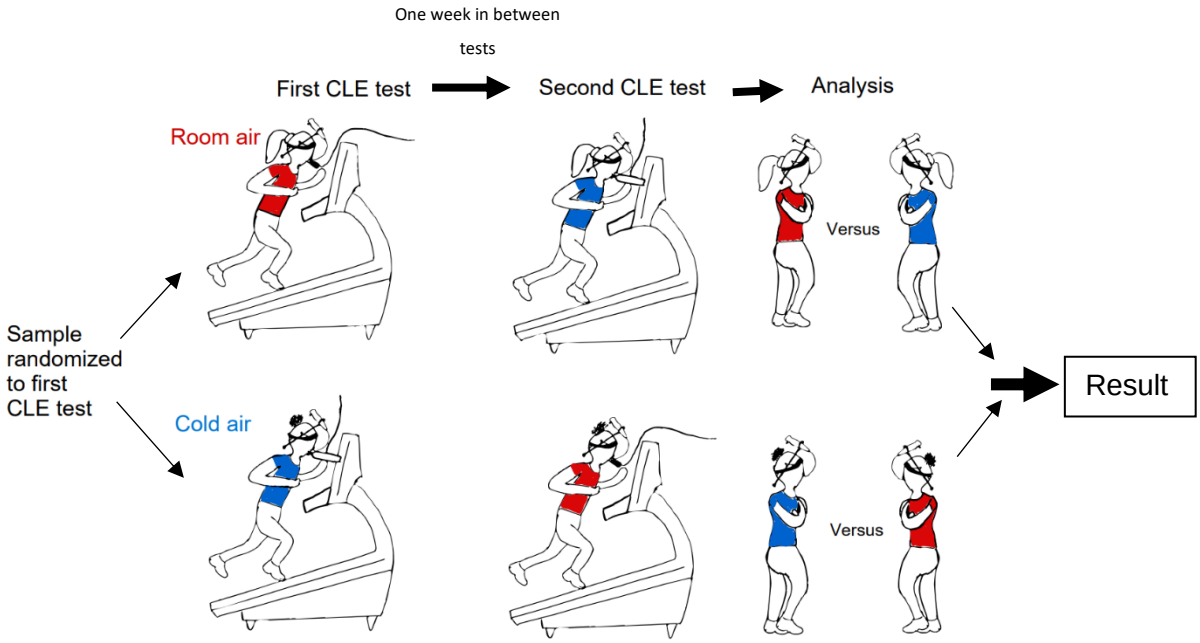

Figure 5. Illustrating the crossover design used in this project. CLE test = Continuous Laryngoscopy Exercise test.

3.2 Inclusion and exclusion criteria

3.2.1 Inclusion criteria

All participants included in this study were suspected of EILO after visiting a specialist clinic for pulmonary disease. They were defined as young adults, meaning between 18 and 40 years. Since the participants were selected through a clinic they are further described as patients.

3.2.2 Exclusion criteria

- Patients evaluated for exercise-induced dyspnea were excluded if having:
    - Asthma or EIB
    - Other lung diseases
    - More than ten years of smoking
    - Smoking more than one cigarette per week the last year
    - Lung infection
    - Airway allergy

3.3 The study subjects

Nine patients suspected of EILO were screened for inclusion and exclusion criteria, invited, and selected for the study through the specialist clinic. There is no data when it comes to how many patients that were evaluated for exercise-induced dyspnea attending the clinic during the inclusion process, from august 2021 to January 2022. Nor if any patients eligible for inclusion declined an invitation. Still, a flow chart is illustrated in figure 6 to give a better overview of the inclusion process.

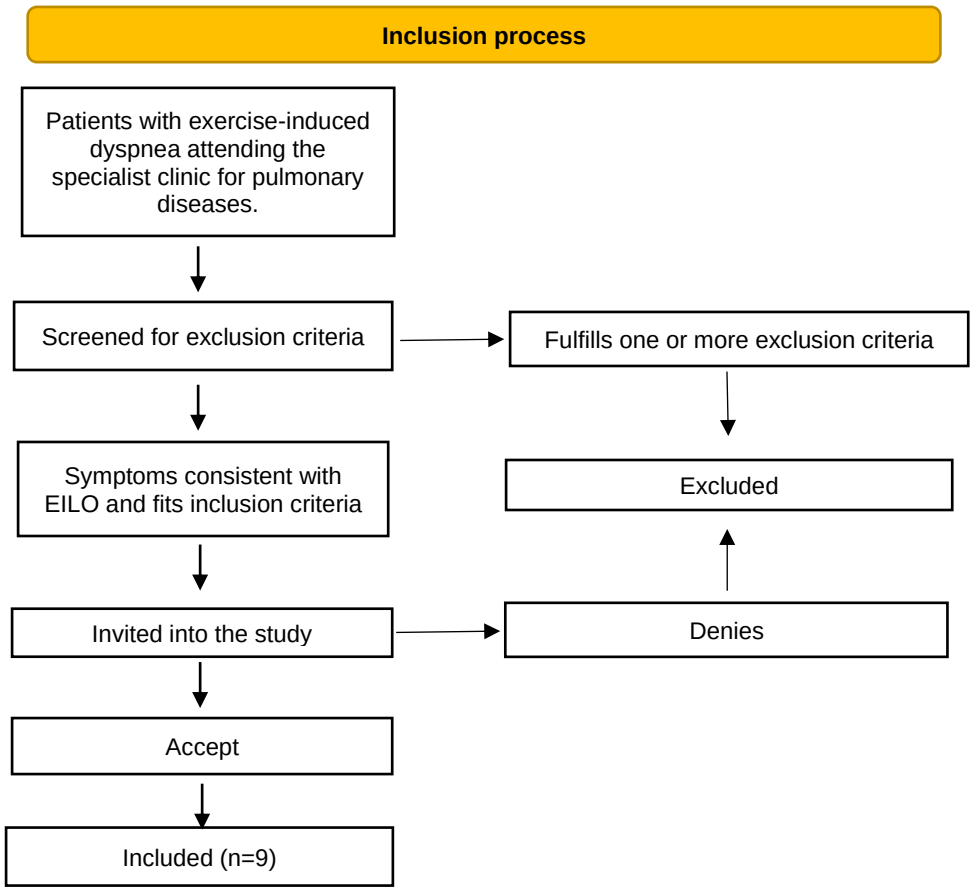

Figure 6. The inclusion process

3.4 Sample size

Adequate sample size was essential to avoid random errors, biases, also called systematic errors, and confounding variables.[45] Confounding variables are factors related to both the independent and dependent variable, and occurs when a third known or unknown variable are mixed in with the findings.[46] A power analysis to secure knowledge regarding a proper sample size was not performed. This was due to lack of knowledge regarding essential parameters such as effect size and distribution. However, for this explorative project, a sample size of 20 was assumed appropriate.

3.5 The randomizing procedure

Prior to testing, the order for a patient's first CLE test was randomized to either:

- A CLE test while breathing cold air or,
- A CLE test while breathing room air.

An independent person, otherwise not involved in this project, randomized the order in blocks of four. The randomization was done by drawing a number "1" or "2", where "1" represented starting with the CLE test breathing room air,s and "2" represented starting with the CLE test breathing cold air. After the test-number was chosen, another number representing the ID number for one of the patients in a respected block was drawn. Blocks of four secured that half the sample started breathing cold air and the other half with room air at relatively short intervals.

A note with information regarding the patients' order for the two tests was put into a non-transparent envelope. Further, the envelope was sealed and locked in a cabinet in the laboratory. Right before a patient's first CLE test, the note with the respected ID number was read.

3.6 Variables

Table 1 shows variables measured during the different phases of each visit.

Table 1. Variables measured during the different phases of the visit.

Pre-test	**CLE test**	**Post-test**
Lung function: • Spirometry	CLE score	Lung function: • Spirometry
Questionnaire	Test duration (min)	
Weight in kg	Perceived breathlessness: Borg CR10 scale	
Height in cm	Heartrate (BPM)	
	Oxygen consumption (mL/min/kg)	

CLE test = Continuous Laryngeal Exercise test. BPM = beats per minute. Oxygen consumption is written in cursive and was only measured during the CLE test while breathing room air.

3.6.1 Questionnaire

A questionnaire was filled out, to give background data for each patient and knowledge regarding their exercise-induced dyspnea, shown in attachment 1. The questionnaire was filled out prior to the first CLE test. The questions used in this master project to describe the sample were 7.1, 7.2, 7.3.2, 7.3.3, and 7.4 (see attachment 1). After filling out the questionnaire, weight and height were measured with clothes on, but without shoes.

3.6.2 Spirometry

Following height and weight and before the CLE test, the lung function was evaluated by preforming a spirometry. Spirometry measured airflow and air volume during inspiration and expiration, with forced expiratory volume in the first second (FEV1) being a particularly relevant measure.[47] FEV1 was the amount of air in liters a patient managed to exhale in the first second during maximal expiratory effort, both in duration and power.

Spirometry was measured sitting in a chair. The patients were relaxed, with a straight posture and knees and hips at an approximately 80–90-degree angle. The procedure was performed with a nose clip and the mouth covering a mouthpiece connected to a Vyntus Pneumo (Vyaire Medical GmbH, Leibnizstrasse, Hoechberg, Germany), and evaluated according to standardized criteria for spirometry.[47] The data obtained were

transferred to SentrySuite Software (Vyaire Medical GmbH, Hoechberg, Germany). The procedure consisted of four major parts:

- First, a resting breathing frequency for around 30 seconds
- Second, a full inspiration
- Third, a forced maximal expiration was performed until the airflow was 25 mL/sec.
- Fourth and last, a full inspiration.

The spirometry was also performed five minutes after the CLE test. To rule out EIB, a change ≥10% between a pre and post-exercise test was assumed to be compatible with untreated EIB.[48]

3.6.3 The CLE Test treadmill protocol

The preset protocol was used on a treadmill (Woodway PPS 55Med, Wil am Rhein, Germany). The protocol is described in table 2 and shown in figure 7. As shown, the CLE test started slowly and gradually increased in both speed and incline. The test was stopped when the patient ceased to run, due to fatigue, respiratory distress, or a combination. Note that since the protocol is standardized, a given point in time during the CLE test will always be associated with a given workload.

Table 2 Detailed description of the test-protocol on the treadmill.

Step	km/h	% Incline	Duration (min)
1	1,5	0	1:00
2	1,5	5	1:00
3	2,1	9	1:00
4	2,7	10	1:00
5	3,3	11	1:00
6	4,0	12	1:00
7	4,7	13	1:00
8	5,4	14	1:00
9	6,1	15	1:00
10	6,7	16	1:00
11	7,4	17	1:00
12	8,0	18	1:00
13	8,6	19	1:00
14	9,0	20	1:00
15	10	20	1:00

16	11	20	1:00
17	12	20	1:00
18	13	20	1:00
19	14	20	1:00
20	15	20	1:00

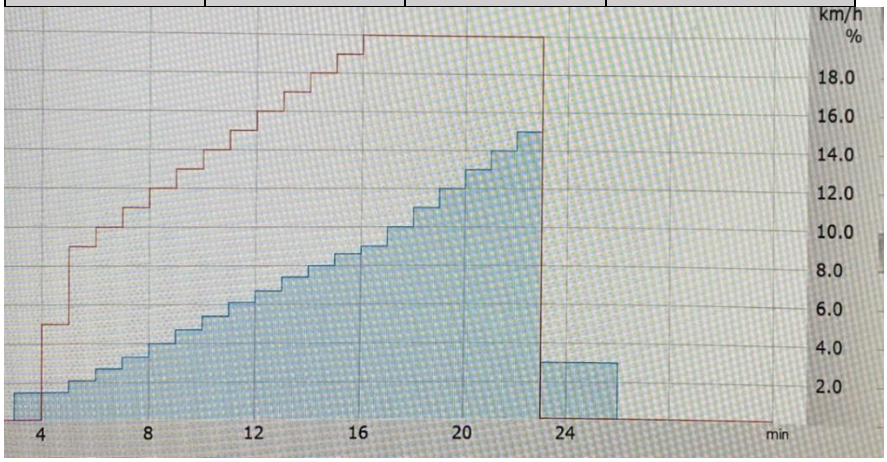

Figure 7. Schematic representation of the treadmill protocol.

3.6.4 The CLE test

The CLE test was performed according to the setup described by Heimdal et al.[28] The main objective of the test was to evaluate glottic and supraglottic movements during exercise and give a CLE score based on the movements observed. A laryngoscopy attached to the patient's head with a custom-made headgear allowed for video and audio recording of the laryngeal inlet during the test. Figure 8 shows the placement of the laryngoscope in the throat and how the recording was viewed on the monitor.

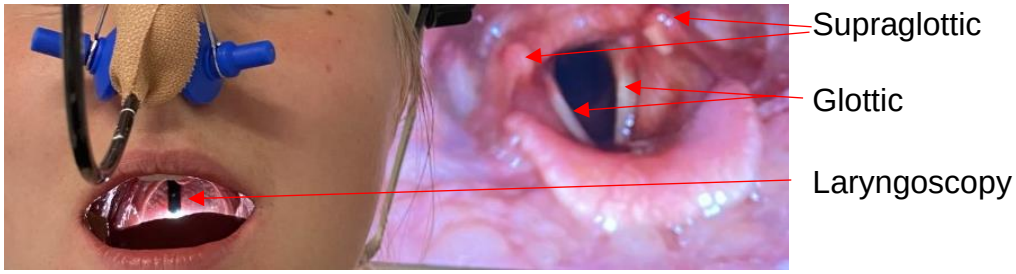

Figure 8. Footage showing placement of the laryngoscope in the throat and how the picture was shown on a monitor. Picture: private.

The CLE test consisted of three phases. Walking was the first phase, followed by running, and the last phase was after aborting the test. Moderate effort was the moment each patient started to run. In other words, the transition between phases one and two. The maximal effort was when each patient aborted the test, meaning the transition between phases two and three. Figure 9 illustrates the phases of the CLE test with moderate and maximal effort.

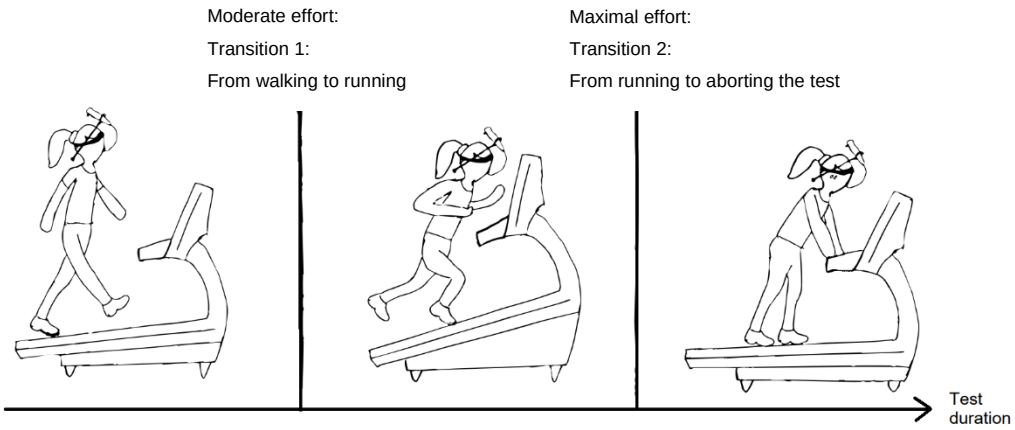

Figure 9. Illustrating the CLE test with moderate and maximal effort.

During the CLE test, the patients' heartrate was monitored with four electrodes on the chest connected to CUSTOMED (Custo Diagnostics, Germany). Also, immediately after reaching maximal effort, the patients estimated their perceived breathlessness using the Borg CR10 Scale,[49] denoted further as Borg Scale. The scale goes from 0 (no breathlessness) to 10 (extreme breathlessness).

3.6.5 The CLE test while breathing cold air

A Turbo Aire Challenger™ (Equilibrated BioSystems, Ind., Melville, NY) was used to evaluate glottic and supraglottic movements when breathing cold air (See attachment 2 for the manual). The device generated cold and dry high-pressured air into the airways and has been used in studies to generate cold air when investigating patients with EIB.[38, 43] See figure 10 for an illustration of the device.

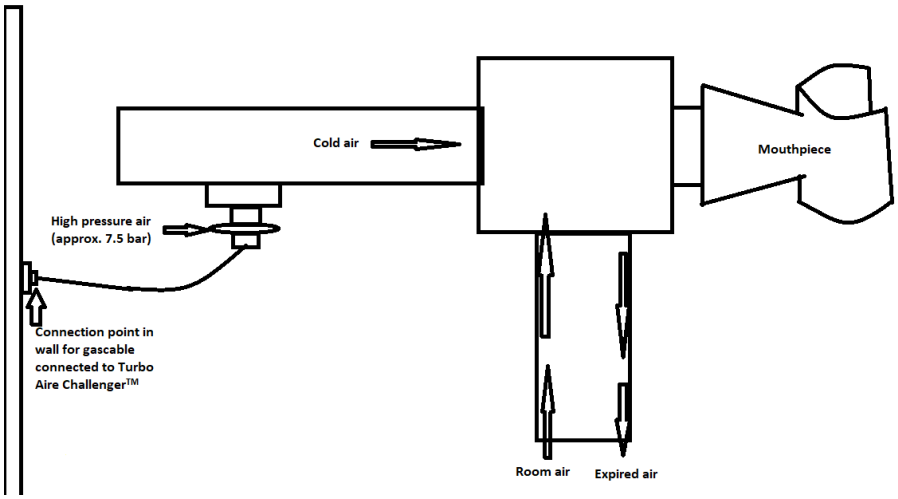

Figure 10. Illustration of Turbo Aire Challenger™ connected to a connection point for gas.

A comparison of the CLE test while breathing room air with the CLE test while breathing cold air are shown in table 3.

Table 3. Comparison of CLE test breathing room air versus cold air.

	CLE test breathing room air	**CLE test breathing cold air**
High-pressure air		X
Cold and dry air		X
Normal room air with no additional resistance	X	
Standardized treadmill test protocol	X	X
Breathing through a mouthpiece	X	X
Measuring ventilatory data	X	

Table describing the most essential similarities and differences between the CLE test breathing cold air and the CLE test breathing room air. CLE = Continuous Laryngoscopy Exercise test.

Figure 11 shows the complete setup before a CLE test while breathing cold air. When breathing room air, the white mouthpiece was replaced with a similar blue. On the back of the blue mouthpiece a turbine was placed and connected to Vyntus CPX (Vyaire Medical GmbH, Leibnizstrasse, Hoechberg, Germany) to measure oxygen consumption.

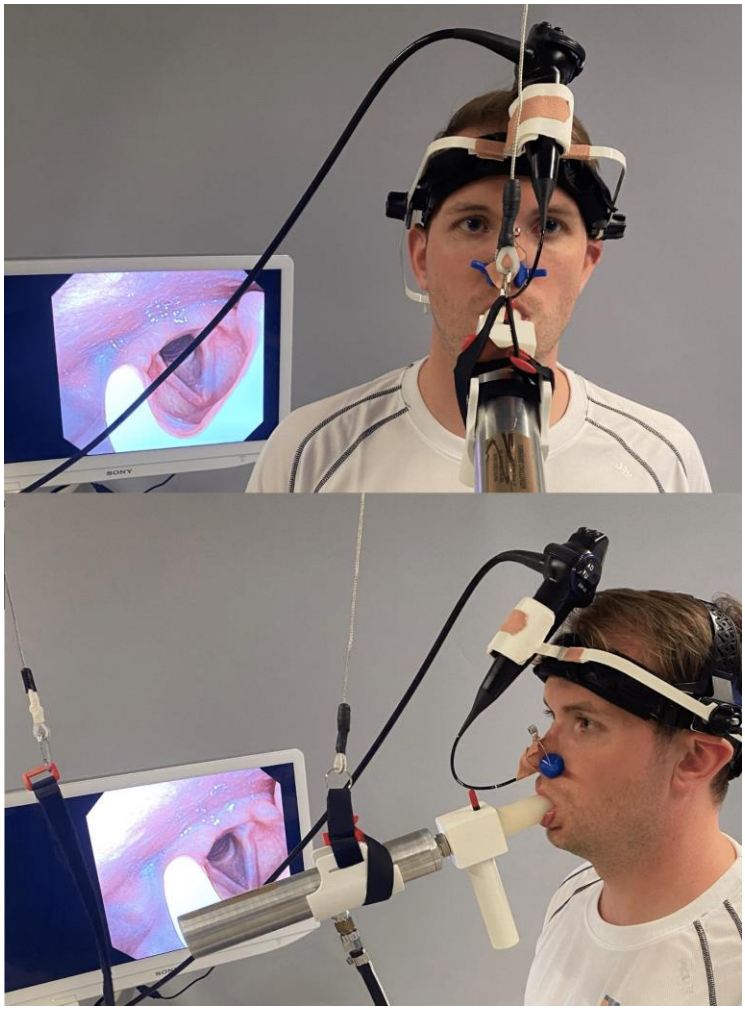

Figure 11. CLE-test with setup for breathing cold air. A setup for a continuous laryngoscopy exercise test performed on a treadmill while breathing cold air. The patients were breathing through a TurboAire Challenger™ biting over a mouthpiece. A wire connected to the ceiling was used to adjusts the height of the TurboAire Challenger™. A hose connected to a gas-output on the wall with pressurized air of approximately 7 bars, gave the air a temperature around minus 15°C. (Picture: private).

3.6.6 The CLE score

The CLE score was given according to the CLE scoring system described by Maat et al.[24] The scoring system contains four sub-groups and a sum score (illustrated in table 4). Each subgroup was graded from zero to three at glottic and supraglottic level at moderate and maximal effort. A patient was to be diagnosed with EILO if having a CLE score ≥ two on at least one of the subgroups.

Table 4. Table showing the different subgroups in a CLE score.

	Glottic	**Supraglottic**
Moderate effort (starts to run)	Subgroup A	Subgroup B
Maximal effort (aborts the test)	Subgroup C	Subgroup D
CLE sum score	E	

The CLE scores were set by an experienced and independent scorer evaluating the tests after the CLE test. When in doubt, another independent scorer was consulted to decide the score. Figure 12 shows the complete scoring system.

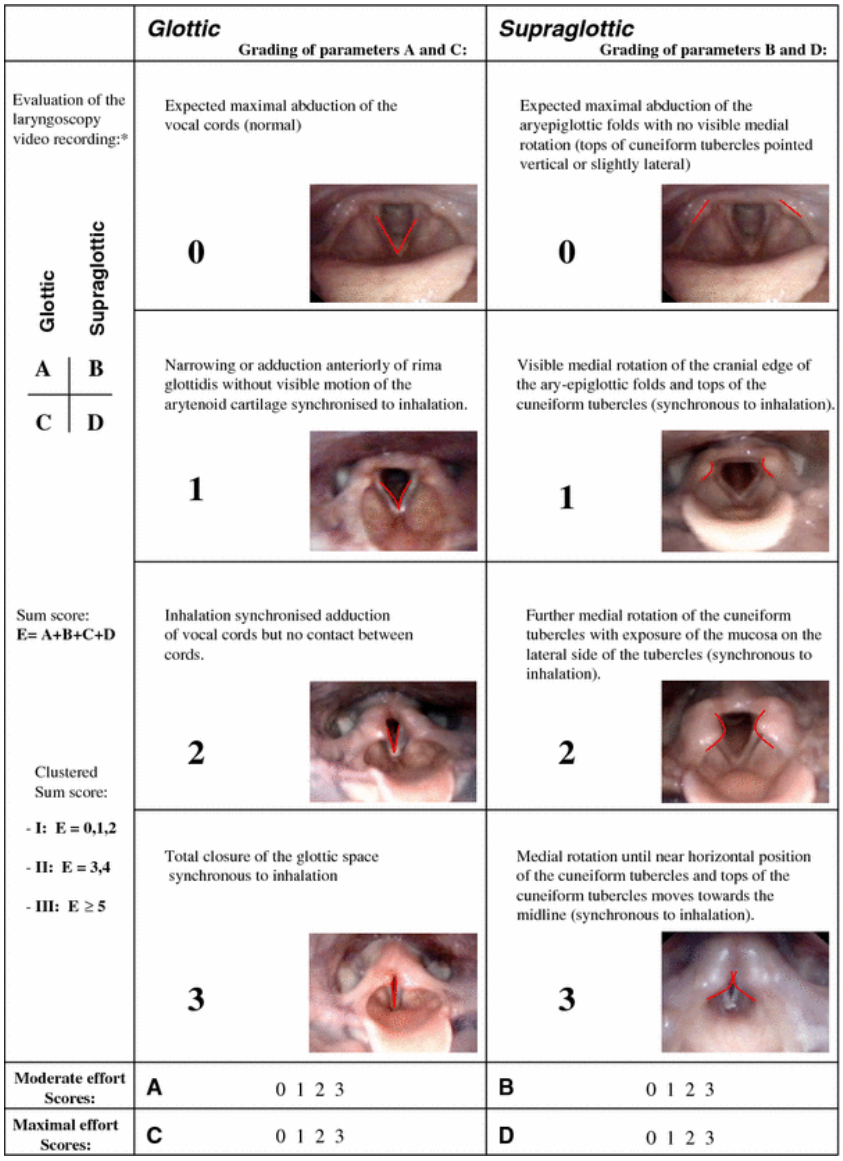

Figure 12 The CLE-scoring system. Obtained from Maat et al.,[24] approved for use by the authors. CLE score going from zero (maximal opening) to three (full closure) on each subgroup. The CLE-score is graded at moderate (A, B) and maximal (C, D) effort, at glottic (A,C) and supraglottic (B, D) level. The score A-D is summed up to a sum score (E).

3.7 Outcome variables

The primary outcome in this project was the CLE score, and the secondary outcomes were test duration, lung function, heart rate, and Borg Scale.

CLE score and heart rate was obtained during the CLE tests and therefore evaluated at moderate and maximal effort. However, if the patients reached moderate and maximal effort at a different time during the two CLE tests, results from the tests

were also compared at an identical workload. The identical workload was defined as the points in time a patient first reached moderate and maximal effort when looking at both the CLE tests. The point in time a patient first reached moderate and maximal effort when looking at both the CLE tests were thereby used to collect and analyze CLE score and heart rate at the same two points in time for both tests.

3.8 Analysis

All statistical analyses in this thesis were done in Stata/SE (College Staio, TX: StataCorp LLC. StataCorp. 2019) Version 17.0 for Windows. Paired T-tests, and Wilcoxon matched pairs signed rank test was used. A two-sided hypothesis test was performed, and a P-value below 0.05 was considered statistically significant. The dependent variables, the effect variables [51], were CLE score, test duration, lung function, heart rate, and Borg scale (CR10). The independent variables, the causal variable,[51] were breathing room air and cold air.

Data were reported as mean and range for background data, and mean and 95% confidence interval (CI) for the main results. The CLE score and Borg Scale was calculated and reported as mean, being more informative than medians and interquartile ranges.

3.9 Data collection

All testing was performed at the heart-and lung-test laboratory at the Vitality Center for Children and Youth, Department of Pediatric and Adolescent Medicine, Haukeland University Hospital, Bergen, Norway. The laboratory has a controlled environment with a temperature stabile around 20 to 22°C, and all equipment follows the guidelines for calibration and maintenance in collaboration with relevant partners. Patients were to be tested on the same time and day one week after the first visit.

3.10 Ethics

Regional Committees for Medical and Health Research Ethics (REK) were approved prior to this project, with project number REK 109946, and informed written consent was obtained from the patients. The project complied with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice guidelines, and applicable regulatory requirements. The patients could withdraw their consent at any time. All data gathered digitally was saved on the hospital's servers and questionnaires

and other relevant data written down was safely stored in a folder kept in a looked cabinet in the hospital, as describes by the REK approval.

4. Results

The main results and results not highlighted in the article are described in this section.

4.1 Demographic data

Nine patients were included, whereas eight were females. Two patients had three weeks between test one and two and one patient had three days between, the rest follow the protocol of same time and day one week between tests. Five started with breathing cold air, and four with breathing room air. They are all physically active on a recreational level. Examples of mentioned recreational activities were strength training, running, soccer, and hiking. All expressed being physically active at the level they sweated at least once a week. Seven of the nine did so either 2-3 or 4-6 times a week. Three patients have reduced their activity level due to their exercise-induced dyspnea. Two would have been more physically active if not having these issues, while three are uncertain. Background data are described in table 5.

Table 5 Background data gathered from the included patients

Variable	**Mean (Range)**
Age (years)	29.8 (21-38)
Height (cm)	171 (162-182
BMI	27 (21.1-35.2)
Gender (female/men)	8/1
VO_2 (mL/min/kg)	39.5 (27.7-48.3)
VO_2 predicted (%)	99.7 (73-127)
Type of activities, n	
Strength training	7
Indoor bandy	1
Running/jogging	4
Swimming	1
Yoga	1
Biking	1
Hiking	2
Soccer	2

BMI = body mass index.

4.2 Results from the CLE test

No difference in CLE score was found between breathing cold and room air. A significantly shorter test duration while breathing cold air compared to room air was found, with a mean difference of 15 seconds (00:28-00:02 (95%CI). Seven of the nine patients ran longer while breathing room air. However, two patients ran five and three seconds longer when breathing cold air. Two patients had incomplete heart rate data, having to exclude them from the respected variables in the analysis. Results from the CLE tests are shown in table 6.

Table 6. Results from the CLE-test

Variable	Effort	Room air Mean (SD)	Cold air Mean	Mean difference (95% CI)	Paired t-test	Signed rank test
Test dur. (min) (n=9)	Moderate	08:07 (00:54)	07:54 (00:52)	-00:13 (-00:06-00:32)	0.155	0.317
Test dur. (min) (n=9)	Maximal	10:19 (01:27)	09:56 (01:23)	-00:15 (00:28-00:02)	0.030*	0.038*
Heart rate (bpm) (n=7)	Moderate	157.3 (19.2)	154.4 (7.5)	-2.9 (-9.6-3.8)	0.337	0.500
Heart rate (bpm) (n=8)	Maximal	180,4 (9.1)	179,6 (6.9)	-0.8 (-3.4-1.9)	0.528	0.480
Borg Scale (n=9)	Maximal	8,1 (1.7)	8 (1.4)	-0.1 (-1.1-0.8)	0.763	0.827
Glottic	Moderate	0	0	0	1.000	1.000
Supraglottic	Moderate	0.9 (0.8)	1.1 (0.6)	0.2 (-0.1-0.6)	0.170	0.157
Glottic	Maximal	1.3 (1.0)	1.1 (1.1)	-0.2 (-0.6 –0.1)	0.170	0.157
Supraglottic	Maximal	1.7 (0.5)	1.7 (0.5)	0 (-0.4-0.4)	1.000	1.000
CLE sum score (n=9)		4 (1.1)	3.9 (1.1)	-0.1 (-0.7–0.5)	0.681	0.655

The scoring system contains four sub-groups, each graded from zero to three at glottic and supraglottic level, graded at moderate and maximal effort. A higher score indicated more laryngeal closure. CLE score = Continuous laryngoscopy exercise score. bpm = beats per minute. SD = Standard deviation. * = p-value <0.05 was considered statistically significant. SD = Standard deviation. CI = Confidence interval. Dur. = duration.

The mean difference in test duration was 13 seconds at moderate effort, and 15 seconds at maximal effort, in favor of breathing room air (Illustrated in figure 13). Below the figure are results from the CLE tests when compared at identical workload.

*Figure 13. Illustration to schematically illustrate when the patients reached moderate and maximal effort while breathing room and cold air. * Indicates a statistical significance with a p-value <0.05.*

When comparing breathing cold air versus room air at identical workload at moderate effort, patients had significantly more laryngeal closure on supraglottic level, 0.6 (0.2-1) (mean difference (95%CI)). No significant difference in the CLE sum score was found (see table 7).

Table 7. The CLE-test when performed in room and cold air, and the differences between the scores and heart rate of the two tests compared at an identical workload.

Variable	Exercise intensity with identical workload	Room air Mean (SD)	Cold air Mean (SD)	Mean difference (95% CI)	Paired t-test	Signed rank test
Heart rate (bpm) (n=7)	Moderate	153.7 (6.9)	154.4 (7.5)	0.7 (-3.7-2.2)	0.576	0.641
Heart rate (bpm) (n=8)	Maximal	178.4 (9.1)	179,3 (6.9)	1.3 (-4.6-2.1)	0.413	0.656
Glottic	Moderate	0	0	0	1.000	1.000
Supraglottic	Moderate	0.6 (0.5)	1.1 (0.6)	0.6 (0.2-1)	0.013*	0.025*
Glottic	Maximal	1.1 (1.1)	1.1 (0.8)	0 (-0.7-0.7)	1.000	1.000
Supraglottic	Maximal	1.7 (0.5)	1.7 (0.5)	0 (-0.4-0.4)	1.000	1.000
CLE sum score(n=9)		3.6 (1.1)	3.9 (1.1)	0.3 (-0.6-1.3)	0.438	0.465

The scoring system contains four sub-groups, each graded from zero to three at glottic and supraglottic level, graded at identical workload at moderate and maximal intensity for both CLE tests. A higher score indicated more laryngeal closure. CLE score = Continuous laryngoscopy exercise score. bpm = beats per minute. SD = Standard deviation. * = p-value <0.05 was considered statistically significant. SD = Standard deviation. CI = Confidence interval.

4.2.1 Mean difference between those started breathing cold air versus room air

When comparing the mean difference between those who started breathing cold air with those who started breathing room air, a difference of 2 seconds in test duration was found and 0.1 in CLE sum score. These results are shown in table 8.

Table 8. Mean difference between started breathing room versus cold air.

Variables (n=9)	Room air first (n=4)			Cold air first (n=5)			Mean difference
	Room air Mean (SD)	Cold air Mean (SD)	Mean diff	Room air Mean (SD)	Cold air Mean (SD)	Mean diff.	
Test duration (min)	10:44 (1:35)	10:10 (1:22)	00:16	10:00 (1:29)	09:46 (1:22)	00:14	00:02
CLE sum score	0.25 (0.5)	0.75 (0.5)	0.5	0.8 (0.45)	1.4 (0.55)	0.6	0.1

Table describing the mean difference in test duration and CLE sum score breathing for the four who started breathing room air and the five who started breathing cold air. CLE-score = Continuous laryngoscopy exercise-score. SD=standard deviation. Diff.=difference

4.3 Spirometry

No difference in pre and post spirometry were found on either group (described in table 9) or at individual level. No differences in regard to statistical significance between paired t-test and Wilcoxon signed rank test were found.

Table 9. Spirometry test results before and after the CLE test performed in room air and in cold air.

	Room air				Cold air			
Variables	**Pre CLE test Mean (SD)**	**Post CLE test Mean (SD)**	**Mean difference (95% CI)**	**P-value**	**Pre CLE test Mean (SD)**	**Post CLE test Mean (SD)**	**Mean difference (95% CI)**	**P-value**
FVC (L)	4.63 (0.51)	4.64 (0.55)	0.01 (-0.94-0.11)	0.864	4.63 (0.51)	4.59 (0.49)	-0.04 (-0.15-0.06)	0.388
FEV_1 (L)	3.78 (0.35)	3.85 (0.44)	0.06 (-0.06-0.19)	0.271	3.77 (0.40)	3.76 (0.37)	-0.02 (-0.13-0.89)	0.656

FVC=Forced vital capacity. FEV_1 = Forced expiratory volume the first second. CLE test = Continuous laryngeal exercise test. Cold air = CLE test while breathing cold air. Room air = CLE test while breathing room air.

5. Discussion

In this section, the results will first be discussed. Thereafter, the method discussion will discuss weaknesses, strengths, theoretical grounding, and my role as a researcher.

5.1 Result discussion

The result discussion in this thesis focuses on relevant background data from the sample and results from the CLE-test, not highlighted in the article.

5.1.1 Background data

Research regarding EILO is lacking, and most studies done are performed on children, adolescences, and athletes.[2, 17, 18] This is a different population than those included in this project, being young adults suspected of EILO. Therefore, background data will be useful when trying to understand the sample investigated up against its population, and helpful when comparing the sample to previous research on EILO.

Previously done research indicates that EILO are more common among young physical active adults.[25] This corresponds to the findings in this study, where the majority were physically active to the extent that they sweated for several hours a week, with the most reported activity being strength training. Interestingly, strength training is not typically described as an activity associated with EILO among athletes.[21] However, considering that seven out of nine where diagnosed with EILO in this project, strength training might be a common arena for patients with EILO, among the young adult population. Anyway, despite type of activity, physical activity seems to be a common denominator for patients with EILO, as well does gender proportion.

The female proportion in this project is 89 percent, and even though epidemiological knowledge about EILO is lacking, a female predominance is found.[17, 18, 52] No studies have investigated the exact gender proportion, but a recent study on EILO-treatment among athletes had a similar female proportion, with 85 percent.[21] However, when investigating a possible connection between breathing cold air and EILO, an equal gender proportion should be considered for future studies. This is because an equal gender proportion can investigate a potential different response between genders when exposed to cold air. Having said that, both previous qualitative and quantitative

studies investigating both females and males exposed for cold air has not highlighted any obvious gender differences.[9, 53] Since previous studies has failed to find any obvious difference between genders when exposed to cold air, a different response between genders when breathing cold air and EILO are unlikely. The indication of no difference in genders suggests a predominant female proportion to be suitable. Still, it is noteworthy to mention that the understanding of the young adult population suspected of EILO is lacking.

The background data from the sample in this project have similarities to previous research. Despite this, when reviewing research mostly done on athletes, adolescents, and children, it is difficult to know if the included sample is representative for young adults suspected of EILO in general.[51] Therefore, to better understand the population, more research regarding young adults suspected of EILO is needed, and the results from the CLE test must be considered with caution.

5.1.2 The CLE test

The main result from the CLE test was that no significant difference in CLE sum score between breathing room and cold air was found. No difference in CLE sum score indicates that young adults suspected of EILO do not have a different CLE score when breathing cold air compared to room air. However, the 95% CI, suggests these results to be inaccurate in regard to the population as a whole, due to a wide spread in both directions. The 95% CI intends to estimate with 95% confidence what the expected mean difference will be for the population.[45] An imprecise result when it comes to CLE sum score is shown with the 95%CI being wide, at least 0.5 in favor of either room or cold air. Considering that a difference in CLE score of 0.5 is clinically relevant,[54] research with a larger sample is needed to get a more precise result. Despite the uncertain result regarding CLE sum score, a significant difference was found on one subgroup.

A significantly higher supraglottic score was found on moderate effort while breathing cold air compared to breathing room air at an identical workload. This might indicate an earlier EILO debut while breathing cold air. However, the CLE sum score showed no significant difference at identical workload. Regardless of this, the patients still had a difference in test duration.

Test duration when breathing cold air was found to be significantly shorter at maximal effort. At the same time, no significant difference in heart rate nor Borg scale was found. This implies that the patients are experiencing similar perception of breathlessness and physical demand during the tests. However, similar to the findings regarding CLE score, a wide 95%CI indicates the need for more research. Interestingly, heart rate at maximal effort had a smaller CI with a small mean difference, revealing less possibilities to find any clinically relevant difference in heart rate at maximal effort, even with a larger sample. Having said that, discussing weaknesses and strengths regarding the methodology is imperative to appropriately consider results and findings in this project.

5.2 Method discussion

The method discussion will highlight and discuss the design and possible aspects of the methodology affecting the findings, both regarding weaknesses and strengths.

5.2.1 Research design

In this project, a randomized crossover design was used. The design was deemed the best because it eliminated "between-patient variability".[50] "Between-patient variability" is the natural variation between two patients when tested, and results in a higher standard deviation (SD), compared to when this is eliminated.[55] The higher SD is a result of different patients responding differently to an independent variable, like for this project, breathing either cold or room air during the CLE tests. Hence, with a "between-patient variability" a heterogeneity in the sample would have occurred, due to the natural difference between patients, reducing the statistically power and thereby increasing the sample size needed.[50] A crossover design, however, eliminated the effect a "between-patients variability" could have had on both the result and sample size, as the design allowed each patient to be its own control, as they performed one CLE test while breathing room air and one while breathing cold air, in a randomized order. As a result, the crossover design required a relatively small sample size, which was suitable for an explorative study, lacking both knowledge regarding effect size and distribution. Randomizing the order reduced additional possible factors effecting the results.

Randomizing the order for whether the patients were to start breathing cold or room air reduced the possibilities for random errors, biases, and confounding variables, alternatively explaining the findings in this project.[56] The randomization reduces in other words these factors possibly explaining the findings, and therefore strengthen the internal validity. The internal validity is defined in this project as in what extend the results are caused by a potential difference between breathing cold air and room air.[57] Hence, randomizing the order in a crossover design is appropriate, and overall the most suitable design.

An alternative to the randomized crossover design, is a randomized control trial (RCT). An RCT is historically considered to be the gold standard regarding clinical trials.[58] However, regarding the aims of this project, an RCT would have required far more patients than with the crossover design, due to it being affected by a "between-patient variability".[50] Therefore, an RCT would have been far more resource- and time-consuming, not suitable for this project. However, even though the randomized crossover design is favorable, some common effects can weaken the internal validity of the results.

The carry-over effect is considered a common confounding variable to account for in a crossover design.[50] A carry-over is present when the exposure a patient experiences during the first test affects the second .[45] In this study, a possible exposure that had a risk of carry-over were patients having fatigue from the first test lasting until the next. To avoid this, the time span between the two visits was one week. However, one patient had only three days between the tests, increasing the risk for a carry-over effect. Still, after consulting the patient and co-researchers, the patient was included. This was because the patient was considered fit, with small chances of getting fatigue lasting for three days, especially when having in mind the treadmill protocol lasting for around ten minutes and most of the test being walking at a comfortable speed. In the light of this, it was considered unnecessary to exclude the patient from this explorative study with an already small sample. Anyhow, to control for a potential carry-over effect, mean difference for test duration and CLE sum score among those who started breathing cold air was compared with the mean difference for those who started breathing room air (Table 8) [50]. A very small difference was observed, arguing that test duration and CLE sum score had not been exposed to any relevant carry-over effect.

Having said that, by randomizing the order a potential carry-over effect was expected to be evenly distributed and therefore not affecting the results.

In contrast to the carry-over effect, the design has no obvious way to account for a potential dropout and loss of data. Most designs are vulnerable to dropouts and loss of data, but designs with small samples and a crossover, like for this project, are particularly vulnerable.[50] The results from studies using the crossover design depends on each patient to complete both tests. If dropout or loss of data occurs, the data gathered from the first or second visit will be difficult to use, due to each patient working as its own control and it is incorrect to ignore this.[45] No dropout occurred, but data regarding heart rate for two patients during one of their tests were incomplete. This resulted in their respected heart rate data to be exclude in the analysis. Considering that heart rate was essential to interpretate the patient's objective effort during the CLE tests, important data were lost. There is no obvious way to make up for the lost heart rate data to get an objective measure for their effort. That being said, the patients' perceived breathlessness and test duration did indicate, for both patients, that they performed to maximal effort during both tests. This underpinned that their CLE score, test duration and Borg Scale was acceptable to include in the analysis. Still, considering the sample being small, and data obtained from each patient is of high value. The external validity is negatively affected by the loss of data and the small sample size in general.[51]

5.2.2 The study subjects and sample size

External validity is the sample's representativity towards the population aimed for, being young adults suspected of EILO.[51] Poor external validity will therefore result in a selection bias, meaning the sample is not representing its aimed population.[59] Due to lack of knowledge regarding the population, the external validity is challenging to investigate. A strength in this project is that the background data from the sample are similar to previous research regarding gender proportion and physical activity. Also, working together with experienced physicians in this project has likely reduced the possibilities for a type of selection bias termed referral bias. A referral bias would have occurred if the patients included are different from the aimed population.[59] The physicians evaluating the included patients in this project have expertise in differential diagnostic regarding pulmonary diseases, particularly exercise-induced dyspnea, including EILO and EIB. This makes them suitable and appropriate to use in confirming

that the included patients are considered suspected of EILO. Performing spirometry pre and post the CLE tests, finding no difference in lung function associated with EIB for either of the patients, underpins that the included patients were thoroughly screened before inclusion and are likely to represent the aimed population. Still, more knowledge regarding the young adult population suspected of EILO is needed to proper evaluate the representativity, especially when considering the small sample size.

The sample size is considered to be one of the most important contributors to secure a representative sample.[51] Small samples, as found in this project, increases the possibility for a selection bias being present. With only nine patients included, this master project has failed to include the 20 patients aimed for. Thus, underpinning the need for a larger sample size in future research. That being said, since this is the first project investigating a possible connection between EILO and breathing cold air with a CLE test setup, the 20 aimed included patients are not based on a power analyze. These nine patients, on the other hand, will be useful towards finding an appropriate effect size and distribution to perform a power analyze for future research investigating EILO and breathing cold air. However, for the findings in this project, the sample size being small has some challenges.

An insufficient sample size increases the possibilities for alternative explanations, like random errors, biases and confounding variables.[45] In addition, a small sample size increases the risk of a type II error. A type II error is described as not detecting a connection between the independent and dependent variables even if it actually exists.[45] Therefore, it is important to consider the findings in this thesis with caution, no matter the finding. However, there are some strengths regarding a small sample size.

A small sample size is more doable to conduct considering reviewing patient record, performing analyses and guiding patients based on individualized findings.[60] Projects with a small sample can also be performed in a shorter period of time and are appropriate regarding explorative studies, and a master project.[60] Furthermore, a false positive result, termed type I error, is rarely found in small samples.[45] A type I error occurs when the null hypothesis is true but are still being discarded. However, a type

I error can still occur if the patients perform differently from one test to another, due to an alternative explanation.

5.2.5 Treadmill protocol

A difference in test duration between breathing cold air and room air was found, and an alternative explanation, caused by a random error, could be a difference in motivation between two tests. A patients' level of motivation is essential during the test to reach maximal effort.[61] However, to minimize random errors, like difference in motivation and day-to-day variation, researchers with good experience in the procedure and tests were used. This was thought to keep the patients motivated and comfortable to perform as equally as possible during both tests. Still, if a patient aborted the test earlier during one of the tests' due to less motivation, a random error occurs, and the internal validity is affected. To better understand the patients' motivation throughout the tests, their effort was evaluated. Borg Scale and maximum heart rate were necessary and valid tools to assess this,[49, 62] and particularly essential considering lack of previous experience with the CLE test setup while breathing cold air.

5.2.7 The CLE test setup

Even though there is no previous knowledge regarding breathing cold air during a CLE test, a strength with the setup used is that it is identical both times, except for the Turbo Aire Challenger™. Still, the newly developed test for investigating EILO while breathing cold air had some challenges that might have affected the patients, and thereby the findings. Some of the challenges with breathing cold air and the CLE setup are highlighted in the article, but elements not mentioned in the article are described below.

5.2.7.1 Oxygen consumption

Measuring oxygen consumption is the gold standard for estimating physical form.[39] This is typically performed without the CLE test setup, but a recent published study found no difference between running with and without the CLE test setup when it comes to the oxygen consumption.[63] This is a strength in the matter of getting a valid result of the measured physical form among the included patients. Having said that, if oxygen consumption is affected among the included patients by the CLE test setup while breathing cold air is unknown.

5.2.7.2 Breathing cold air

The method used in this project relied on high pressured air to produce cold air. This air was dry and not humid, like typical room air. Study comparing lung function after exposed to dry and humid air, found that half the subjects having EIB in dry air did not have EIB in humid air.[64] These findings underpin the need to investigate patients suspected of EILO in even more controlled environments, like a climate chamber. A climate chamber will allow for stable humidity, while changing the temperature, eliminating dry air as a possible confounding variable when investigating a connection between breathing cold air and EILO.

5.2.6 The CLE-score

When investigating a connection between breathing cold air and EILO, the CLE scoring system provides verifiable outcome based on live footage of laryngeal responses during exercise.[24] However, the reliability has been debated, meaning in what regard the CLE scoring system produces similar results under consistent conditions.[51] One study have found the CLE scoring system to have a low inter-rater reliability.[65] A low inter-rater reliability describes that it is a poor agreement between two independent scorers.[51] This might be due to the numbers in the scoring system being integer, even though the degree of closure is continuous. This can make it challenging for the scorer to differentiate between different degree of EILO, even though there are defined criteria (figure X). In this study, however, a strength in this regard is that all scoring was performed by the same scorer, with experience both from clinical and research work, minimizing the bias a potential misinterpretation of the CLE scoring system can have. The system having the clinicians' approval when it comes to the different scores,[24] supports the CLE score based on this system to be used in this project.

5.2.7 Equipment

The use of Vyntus Pneumo for measuring lung function, and Vyntus CPX for measuring ventilatory data during the CLE test while breathing room air, are both reliable and valid tools.[66, 67] Using valid and calibrated equipment according to guidelines from the supplier helps to secure valid and reliable measurement throughout the test period, and thereby minimized the risk of a bias affecting the analysis. [57]

5.2.8 Analysis

The primary outcome to be analyzed was the difference in CLE scores between the CLE test while breathing cold air and the CLE test while breathing room air. To investigate a potential difference in CLE score between the tests, both paired t-test and Wilcoxon matched pairs signed rank test were performed. An advantage of using a paired T-test is that a 95%CI and mean gives more describing findings, compared to using only the signed rank test, giving a P-value. No difference in the outcome regarding statistically significance were found between paired t-test and sign rank test. This supports that paired t-test was appropriate to use despite a small sample size.[45] Still, some challenges regarding the sample size and the scoring system used is important to note when it comes to the analysis.

Analyzing data with a randomized crossover design can be challenging.[50] A small sample size made it not doable to evaluate an estimate of the effect using a linear mixed model.[68] Furthermore, several of the CLE score results gave the P-value of 1.0, meaning zero difference. This might be correct, still, it might be due to the sample size being small, and the CLE scoring system only consisting of two subgroups on each level or the score only going from zero to three. This might be an issue in the sense that the analysis fails to detect small differences. However, a difference in CLE score below 0.5 on group level is not a clinically relevant finding.[54] Having said that, a patient having a CLE score of two, close to three on one subgroup at maximal effort during one test, can have a two close to one during the other. This can cause an uncertainty regarding the analysis. On that note, to better understand a connection between breathing cold air and EILO, the value of different theoretical groundings and approaches should be discussed.

5.3 Theoretical grounding

This project bases its findings in measurable variables in a quantitative design, a typical characteristic in the biomedical perspective.[40] The biomedical perspective is the dominating perspective used in research regarding EILO. However, some authors have discussed the importance of the patients own experiences and opinions after meetings with patients that express more discomfort during exercise when breathing cold air, compared to room.[2] To date, no qualitative studies have investigated the

patients' own experiences, and this represents a knowledge gap in the EILO literature. Qualitative research in a phenomenological perspective can give thorough knowledge about the patients experiences and thoughts regarding breathing cold air and their exercise-induced dyspnea.[69] In an anthropological perspective, how patients suspected with or diagnosed with EILO live their daily life, and how they are met by healthcare professionals can be explored. Including these perspectives in future research would provide necessary knowledge to better understand all aspects of EILO in the light of the biopsychosocial model. To better highlight importance of including both biological, psychological, and social factors, a relevant case is described below.

> When at the clinic performing CLE tests there is a wide specter of different patients, and none are alike. However, some similarities are seen in respect to how exercise-induced dyspnea influences daily living, and these are described through the case called Nora, 18 years. Nora is worried about the CLE test to come. However, she expresses relief about finally being here for the test. She has already used non-efficient medication for EIB for several months, even though there are no diagnostic findings associated with EIB. The last year has been particularly challenging when she exercises. Just minutes into vigorous activity she gets the sensation of being choked and starts hyperventilating. She aborts the activity, even though she feels better more or less immediately after aborting. To prevent any symptoms, her activity-level has declined the last months. She thinks about quitting recreational activities. Her motivation for keeping exercising is to be with her friends. At the clinic, she is diagnosed with EILO after the CLE test. She is thankful for being shown what happens when she experiences the sensation of being choked and starts to hyperventilate. After being told that this is a common finding for patients with EILO and it is likely to be better with guidance from the clinicians and with practice, she seems happier. She expresses that she is glad someone finally believed her, after a longer period of feeling alone and with little hope to get well.

The case described is an anecdote, based on experiences from the laboratory. However, these experiences need to be systematized.[70] This will help to better understand how Nora's exercise-induced dyspnea really affects her daily life. Increased knowledge about psychological and social factors is essential considering EILO affecting biological, psychological, and social factors. In other words, healthcare-

professionals need to better understand EILO in this regard. Still, as a first step in researching a connection between EILO and breathing cold air, investigating biological factors in a biomedical perspective is not wrong. This gives essential knowledge towards a connection. However, for future studies an isolated qualitative study or a qualitative study nested with a quantitative study should be considered. This will possibly also help health-care professionals to better understand their position and role when meeting patients with or suspected of EILO.

5.4 My role as a researcher

I have been participating in research projects for the last few years, involved in planning, organizing, data collection and analyzing. Several of these projects have been focusing on EILO and this has given me an insight into EILO and what might be necessary and interesting to investigate. The environment with co-researchers and different projects had guided me into a research-field I find challenging and meaningful. At the same time, I must be aware of potential factors that might affect me in performing a reliable and valid master project.

My role as a researcher in this project is likely to be affected by my previous experiences.[71] Firstly, my involvement in a large research group with competent, experienced, motivated, and resourceful co-researchers are relevant to highlight. Being a part of this kind of environment sometimes makes it challenging to ask questions regarding choices and the methodology in different projects. That being said, my involvement in this research environment has at the same time given me experience, knowledge, and confidence to ask relevant questions. This has helped me to better understand what I am investigating, as well as given me the confidence needed to ask questions to help secure reliable and valid research.

Secondly, as a master student and researcher, I want to be a part in evolving the research field regarding EILO with this project. If the study can give an insight into something new about EILO, it will help the research field. However, being a part of a large research group might increase the pressure on myself. This could be both positive and negative. Positive in the way that I am genuinely interested in performing a valid and reliable study, to further strengthen the research-field. On the other hand, I might get blinded by potential biases due to the influence from co-researchers and

previous projects. This realization has resulted in more pressure on myself, than expected prior to this master project, to write an adequate master thesis and article.

Thirdly, due to previous experiences and discussions with co-researchers and literature regarding EILO and breathing cold air, I had expectations to the outcome of this project. This is known as preconception in qualitative studies [72] and is describing that my known or assumed assumptions possibly could affect this master project. The hypotheses in quantitative studies, like for this project, are a result of an assumption of something being of interest. This supports that my preconception based upon, among other things, my previous involvement in other research-projects has affected my focus in this project. I also assume that this project was possible for me to do due to my previous experience and job. Possibly making me more receptive to becoming personally involved in the outcome, and underpins the need for me to use my position to openly describe this procedures, testing and analysis done through this project. In combination with new knowledge and guidance, I am aware that my research environment and I might influence each other and our approach to investigating a potential connection between breathing cold air and EILO. That being said, I hope that highlighting multiple factors possibly affecting my role in this project helps to consider the findings reliable, valid, and done in an appropriate way.

6.0 Conclusion

No significant difference in CLE sum score was found between breathing cold air and room air. The patients had a shorter test duration while breathing cold air. When scoring the tests on an identical workload, a significant higher supraglottic score at moderate intensity while breathing cold air was found. However, due to a small sample size, these findings must be considered with caution. Research with more patients is necessary to determine a possible difference between breathing cold air and room air among patients suspected of EILO in the young adult population, preferably done in a climate chamber. Qualitative and nested studies should also be considered for future studies, to give a broader picture of how EILO while breathing cold air influences their daily living.

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The Article,

Exercise-Induced Laryngeal Obstruction (EILO) while breathing cold air.

The article is aimed for publication in British Medical Journal (BMJ) Open Sport and Exercise Medicine.

Criteria for an ordinary research article in BMJ Open Sport and Exercise Medicine:

Numbers of tables/figures allowed: 6

Original Research should not exceed 3000 words, excluding references and tables.

For all original research we ask you to provide in 3-4 bullet points subheadings "What is already known", and "What are the new findings", highlighting the clinical relevance of your work.

For the main body of the paper, we encourage short introductions when the rationale of the study is obvious, i.e. it may be as short as 3 paragraphs if that addresses "Why we did it".

We encourage the use of subheadings in the methods, results and discussion. We find it hard to imagine a discussion that has fewer than two subheadings.

References must be numbered consecutively in the order in which they are mentioned in the text.
Journals from BMJ use a slightly modified version of Vancouver referencing style.

For further details see Attachment 3. British Medical Journal, formatting your paper.

Regarding this article:

Word count: 2857

Table/figures: 6

WHAT IS ALREADY KNOWN?

- Exercise induced laryngeal obstruction (EILO) is an important cause of exertional breathing problems.
- The prevalence of EILO among winter athletes is high.
- A connection between breathing cold air and EILO has been discussed.

WHAT ARE THE NEW FINDINGS?

- Patients suspected of EILO had a shorter test duration during the CLE test while breathing cold air, compared to when breathing room air.
- When breathing cold air, a significant higher supraglottic score at moderate effort was found on an identical workload.
- The indicated laryngeal responses with cold air support the need for more research.

INTRODUCTION

Exercise induced laryngeal obstruction (EILO) describes laryngeal airflow obstruction during increased exercise,[1] by some termed Vocal Cord Disorder when the obstruction is due to the vocal cords. Common symptoms are dyspnea, stridor (abnormal respiratory sounds during inspiration), wheezing, throat tightness and chest tightness.[2] The Continuous Laryngoscopy Exercise test (CLE-test) is the gold standard for diagnosing EILO.[3, 4] The EILO prevalence is reported to be between five and seven percent in the general young population.[5, 6] EILO is an important differential diagnosis for a variety of respiratory diseases, exercise-induced bronchoconstriction (EIB) being the most common.[7-9]

Breathing cold air may trigger airway obstruction in patients with EIB,[10-12] and it increases the likelihood of correctly diagnosing EIB during a standardized EIB test-protocol.[12, 13] Despite EIB being the most common differential diagnosis, it remains to be investigated systematically among patients with EILO.

One study have found the EILO prevalence to be 27 percent among cross-country skiers,[14] which led to Al-Alwan & Kaminsky[15] proposing that cooler air entering the airways might induce laryngeal closure. They underpin an increased risk of closure during increased intensity with mouth-breathing, to cope with the need for a higher minute ventilation. Cooler air entering the airways may impair the mucosa and the vocal cords and lessen the laryngeal abduction. Laryngeal closure is the normal reaction when exposed to irritants, and a recurrent stimulus, like cold air, might lead to an abnormal laryngeal adduction during exercise in the cold.[15]

A study report that respiratory complaints often are worsened in cold climate and that patients' ability to perform during exercise is reduced.[2] Another study also found that environmental factors might induce EILO, and that inspiratory stridor was reported in a higher fraction of athletes doing outdoor sports (8.3%) than indoor sports (2.5%).[16] No studies have systematically investigated if breathing cold air affects patience suspected of or with EILO. Therefore, we aimed to evaluate potential differences in laryngeal obstruction among young adults (18 to 40 years) suspected of EILO when breathing cold air compared to room air.

METHOD

Research design

In this explorative study, a randomized crossover design was used to investigate nine patients with respiratory symptoms indicating EILO. The patients completed two CLE-tests each, one while breathing room air (temperature 20 to 22°C) and one while breathing cold air (temperature minus 15°C). The order of the tests was randomized. The CLE-tests were conducted no closer than three days in between and no more than three weeks apart. The crossover design is illustrated in figure 1.

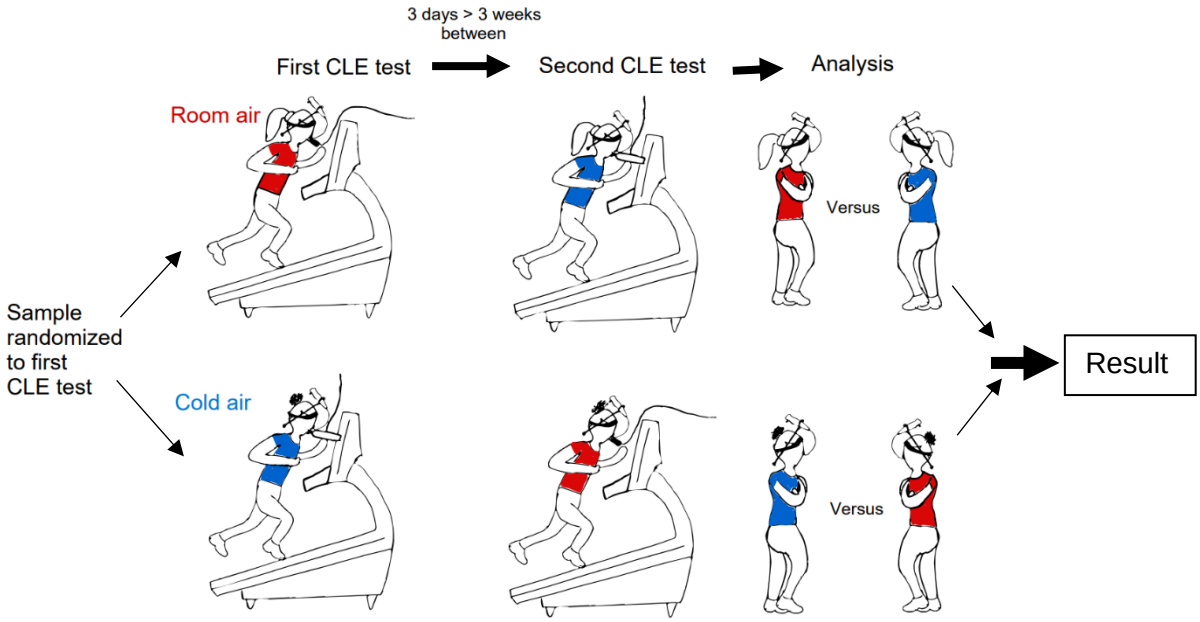

Figure 1. The figure shows the crossover design used. CLE = Continuous Laryngeal Exercise.

All patients were recruited from a specialist clinic for pulmonary diseases. They were excluded if having known lung diseases and infections or a history of smoking.

The regional ethics committee granted ethical approval (REK 109946), and informed written consent was obtained from the patients.

Spirometry

Spirometry was performed pre and post CLE-tests, measuring maximal expiratory flow volume curves.[17]

The test protocol

The test protocol was an incremental preset step running test on a treadmill (Woodway PPS 55 Med, Weil am Rheid, Germany). The protocol is a modified Bruce protocol,[18] starting at low intensity and gradually increasing either speed, incline or both every minute until maximal effort for each patient.

The continuous laryngeal exercise test (CLE test)

The CLE test was performed according to the setup described by Heimdal et al.[3] A solution of 0.5 ml Lidocaine Hydrochloride (40 mg/mL) was applied in one of the nostrils. A flexible fiberoptic laryngoscope (Olympus ENF-V2, Tokyo, Japan), diameter 3.4 mm, was inserted via the nostril into the pharyngeal space, to a position giving an optimal view of the larynx. The laryngoscope was attached to a custom-made headgear and fixed through a plug in the nasal opening, taped externally to the wings of the nose. A nose clip closed the nasal opening and helped stabilizing the laryngoscope. The laryngoscope allowed for continuous video and audio recording of the laryngeal inlet during the test. The patients used a special mouthpiece connected to a device to breathe in the cold air, thus preventing oxygen consumption (VO_2) to be measured when breathing cold air. However, VO_2 was measured when breathing room air.

Breathing cold air

The Turbo Aire Challenger™ (Equilibrated Bio Systems, Ind., Melvillle, NY) makes it possible to test ventilatory responses when breathing cold air. The device is connected to a tube connected to the wall allowing for air to flow through. A pressure around 7 bar provides an airflow of 240 L/min, creating a temperature of minus 15°C. The Turboaire Challenger™ unit hung from the ceiling and was adjusted to the patient's height. See Figure 1 for the setup prior to a CLE test while breathing cold air.

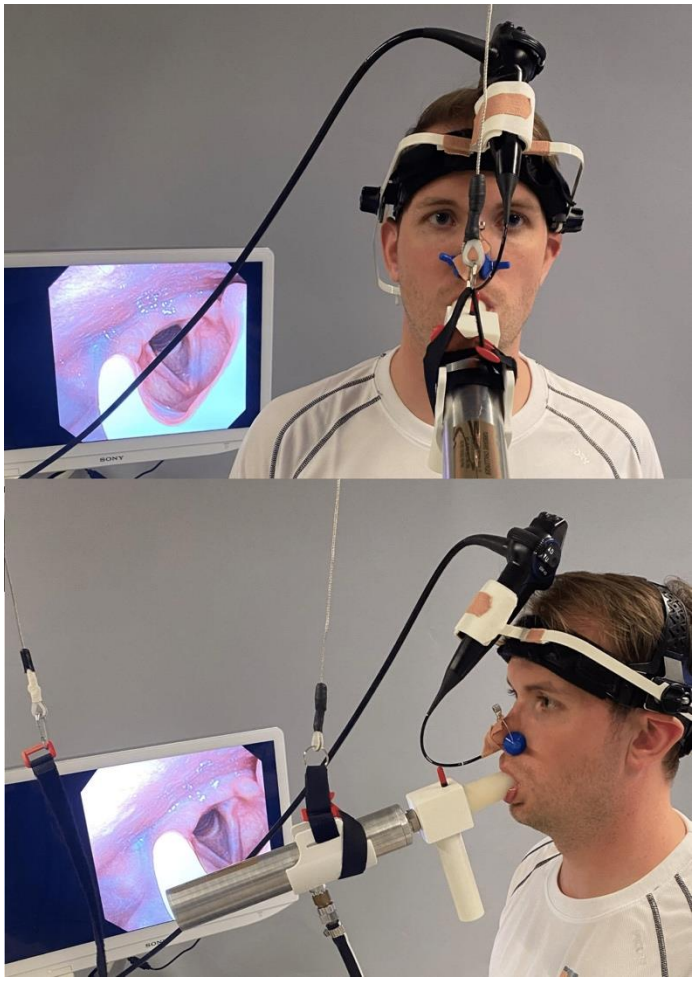

Figure 1. CLE test with setup for breathing cold air. A setup for a continuous laryngoscopy exercise test performed on a treadmill while breathing cold air. The patients were breathing through a TurboAire Challenger™ biting over a mouthpiece. A wire connected to the ceiling was used to adjusts the height of the TurboAire Challenger™. A hose connected to a gas-output on the wall with pressurized medical air of approximately 7 bars, gave the air a temperature around minus 15°C. (Picture: private).

CLE score

CLE score was graded according to Maat et al.[19] The degree of laryngeal closure was evaluated at moderate and maximal effort at the supraglottic and glottic level. Moderate effort at supraglottic level is defined as one subgroup, summing up to a total of four subgroups. The CLE-score ranges from zero to three on each subgroup. A score of three represents full laryngeal obstruction. Moderate effort is defined as the transition when a patient chooses to change from walking to running. Maximal effort is when the patient ceases to run, due to fatigue, respiratory distress, or a combination. EILO is defined as having a CLE score of at least two in one of the four subgroups.

If a difference in test duration between the two tests were found, the results from the CLE test between breathing room and cold air for each patient were also compared at an identical workload. The identical workload was defined as the points in time a patient first reached moderate and maximal effort when looking at both the CLE tests. The points in time a patient first reached moderate and maximal effort when looking at both the CLE tests were thereby used to collect and analyze CLE score and heart rate at the two defined two points in time.

Laryngeal movements were evaluated during the CLE test. The CLE scores were set by an experienced and independent scorer evaluating the tests later. It was not possible to blind the scorer regarding whether the patient was breathing cold or room air. This is due to a difference in sound and video recordings between the tests. The order of tests for scoring was random, making it more challenging for the scorer to remember the patients' previous CLE scores. When in doubt, another independent scorer was consulted to decide the score.

Heartrate

To monitor the heart rate, four electrodes connected to CUSTOMED 100/110 BT EKG (Munich, Germany) were placed on the patient's chest and further to the CUSTOMED software (Vyarie, Germany) by Bluetooth.

Borg scale (CR10)

Borg CR10 Scale estimated breathlessness immediately after exercise, further denoted Borg Scale (Borg, 1998). The scale goes from 0 (no breathlessness) to 10 (extreme breathlessness).

Statistical Analysis

Descriptive analyses were used to assess means, range and standard deviations (SD) of demographic and clinical characteristics. Paired t-test with 95% Confidence interval (CI) and Wilcoxon matched-pairs signed rank test were performed to assess differences between variables collected during the two testing occasions. The criterion for statistical significance was p-value ≤ 0.05. All analyses were conducted using Stata/SE (College Staio, TX: StataCorp LLC. StataCorp. 2019) Version 17.0 for Windows.

RESULTS

Nine patients (eight females) were included and completed both tests no more than three weeks apart. Seven patients had a CLE score associated with EILO on both tests, while the remaining two did not have EILO. Background data and laryngeal findings are shown in table 1.

Table 1. Background data for the patients.

Variable	**N=9**
Category	**Statistic**
Age in years, mean (SD)	29.8 (21-38)
Height in cm, mean (SD)	171 (162-182
BMI, mean (SD)	27.1 (21.1-35.2)
VO_2-uptake (mL/min/kg), mean (SD)	39.5 (27.7-48.3)
VO_2 predicted (%), mean (SD)	99.7 (73-127)
Glottic or supraglottic subgroup score ≥ 2	7/9
Laryngeal obstruction at maximal effort in room air	
Supraglottic subscore ≥2	7/9
Supraglottic subscore <2	2/9
Glottic subscore ≥2	4/9
Glottic subscore <2	5/9
Laryngeal obstruction at maximal effort in cold air	
Supraglottic subscore ≥2	7/9
Supraglottic subscore <2	2/9
Glottic subscore ≥2	3/9
Glottic subscore <2	6/9

Characteristics of the nine included patients with suspicion of Exercise Induced Laryngeal Obstruction (EILO). BMI=body mass index. VO_2 = oxygen consumption. SD = Standard Deviation

CLE test

The CLE sum score was not different between the two tests (-0.1 (-0.7-0.5) (mean difference (95%CI), and there were no differences in either the glottic nor the supraglottic score. The patients had a significant shorter test duration when

breathing cold air, -00:15 (00:28-00:02) (mean difference (95%CI)), but no difference in heart rate, -0.8 (-3.4-1.9) (mean (95%CI)) or Borg scale, -0.1(-1.1-0.8) (mean (95%CI)). The results from the CLE tests are shown in table 2. Both paired t-tests and Wilcoxon Signed rank test gave same results in matter of statistical significance.

Table 2. The results from the CLE test when performed in room air and in cold air.

Variable	**Exercise intensity/ effort**	**Room air Mean (SD)**	**Cold air Mean**	**Mean difference (95% CI)**	**P-value**
Test dur. (min) (n=9)	Moderate	08:07 (00:54)	07:54 (00:52)	-00:13 (-00:06-00:32)	0.155
Test dur. (min) (n=9)	Maximal	10:19 (01:27)	09:56 (01:23)	-00:15 (00:28-00:02)	0.030*
Heart rate (bpm) (n=7)	Moderate	157.3 (19.2)	154.4 (7.5)	-2.9 (-9.6-3.8)	0.337
Heart rate (bpm) (n=8)	Maximal	180,4 (9.1)	179,6 (6.9)	-0.8 (-3.4-1.9)	0.528
Borg Scale CR10 (n=9)	Maximal	8,1 (1.7)	8 (1.4)	-0.1 (-1.1-0.8)	0.763
Glottic	Moderate	0	0	0	1.000
Supraglottic	Moderate	0.9 (0.8)	1.1 (0.6)	0.2 (-0.1-0.6)	0.170
Glottic	Maximal	1.3 (1.0)	1.1 (1.1)	-0.2 (-0.6 –0.1)	0.170
Supraglottic	Maximal	1.7 (0.5)	1.7 (0.5)	0 (-0.4-0.4)	1.000
CLE sum score (n=9)		4 (1.1)	3.9 (1.1)	-0.1 (-0.7–0.5)	0.681

The scoring system contains four sub-groups, each graded from zero to three at glottic and supraglottic level, graded at moderate and maximal effort. A higher score indicated more laryngeal closure. CLE score = Continuous Laryngoscopy Exercise score. bpm = beats per minute. SD = Standard deviation. * = p-value <0.05 was considered statistically significant. SD = Standard deviation. CI = Confidence interval. Dur. = duration.

The patients reached moderate and maximal effort at a different time during the two CLE tests, and results from the CLE test between breathing room and cold air for each patient were also compared at identical workload. When comparing CLE score at an identical workload, a higher supraglottic score at moderate effort when breathing cold air was found (0.6 (0.2-1) (mean difference (95%CI)). There was no difference in CLE sum score, nor heart rate and Borg Scale (Table 3).

Table 10. The CLE-test when performed in room and cold air, and the differences between the scores and heart rate of the two tests compared at an identical workload.

Variable	Exercise intensity with identical workload	Room air Mean (SD)	Cold air Mean (SD)	Mean difference (95% CI)	P-value
Heart rate (bpm) (n=7)	Moderate	153.7 (6.9)	154.4 (7.5)	0.7 (-3.7-2.2)	0.576
Heart rate (bpm) (n=8)	Maximal	178.4 (9.1)	179,3 (6.9)	1.3 (-4.6-2.1)	0.413
Glottic	Moderate	0	0	0	1.000
Supraglottic	Moderate	0.6 (0.5)	1.1 (0.6)	0.6 (0.2-1)	0.013*
Glottic	Maximal	1.1 (1.1)	1.1 (0.8)	0 (-0.7-0.7)	1.000
Supraglottic	Maximal	1.7 (0.5)	1.7 (0.5)	0 (-0.4-0.4)	1.000
CLE sum score(n=9)		3.6 (1.1)	3.9 (1.1)	0.3 (-0.6-1.3)	0.438

The scoring system contains four sub-groups, each graded from zero to three at glottic and supraglottic level, graded at identical workload at moderate and maximal intensity for both CLE tests. A higher score indicated more laryngeal closure. CLE score = Continuous laryngoscopy exercise score. bpm = beats per minute. SD = Standard deviation. * = p-value <0.05 was considered statistically significant. SD = Standard deviation. CI = Confidence interval.

Spirometry

No difference in lung function was found pre and post any of the CLE tests when breathing cold air or room air (See table 4).

Table 4. Spirometry test results before and after the CLE test performed in room air and in cold air.

	Room air				Cold air			
Variables	**Pre CLE test Mean (SD)**	**Post CLE test Mean (SD)**	**Mean difference (95% CI)**	**P-value**	**Pre CLE test Mean (SD)**	**Post CLE test Mean (SD)**	**Mean difference (95% CI)**	**P-value**
FVC (L)	4.63 (0.51)	4.64 (0.55)	0.01 (-0.94-0.11)	0.864	4.63 (0.51)	4.59 (0.49)	-0.04 (-0.15-0.06)	0.388

FEV_1 (L)	3.78 (0.35)	3.85 (0.44)	0.06 (-0.06-0.19)	0.271	3.77 (0.40)	3.76 (0.37)	-0.02 (-0.13-0.89)	0.656

FVC=Forced vital capacity. FEV_1 = Forced expiratory volume the first second. CLE test = Continuous laryngeal exercise test. Cold air = CLE test while breathing cold air. Room air = CLE test while breathing room air.

DISCUSSION

In young adults suspected of EILO, we found no difference in the degree of laryngeal obstruction when they were tested with the CLE test breathing cold air compared to room air. When comparing room and cold air at an identical workload, the supraglottic CLE score at moderate effort was higher when breathing cold air. The patients had a shorter test duration when breathing cold air compared to room air. There were no differences in heart rate or perceived breathlessness.

The CLE score and cold air

A plateau during maximal effort is recognized when the ventilatory work does not increase concomitantly with increased work.[20] A plateau can typically be concluded on ventilatory data when analyzing VO_2.[21] In our study, VO_2 could not be measured when breathing cold air, as the Turboaire Challenger^{TM} is connected to the patient with a mouthpiece. Thus, heartrate and Borg scale were noted at peak performance, to evaluate effort and perceived breathlessness. Our study indicated no differences in either heartrate or Borg scale when breathing cold air compared to breathing room air. There was also no difference in CLE sum score, indicating that the degree of EILO might not be affected by breathing cold air. However, the supraglottic CLE score was higher at moderate effort when breathing cold air at an identical workload compared to the CLE test breathing room air. A difference in supraglottic score might indicate a possibly increased ventilation with cold air inducing laryngeal closure earlier. However, there was no difference in heart rate, thus we cannot conclude that there was a difference.

Cold air and supraglottic obstruction

Cold air requires the mucosa to moisten and heat the air, which may result in a net transport of water out of the cells of the airways.[22] No studies have investigated how this might affect laryngeal obstruction. However, if cold air can result in supraglottic structures becoming more flaccid, this could explain a higher supraglottic score earlier. The moistening and heating of the inspired air requires energy, and more energy when the inspired air is colder.[22] In our study, the patients ran shorter when breathing cold air, and they had higher supraglottic CLE scores at moderate effort at an identical workload. Whether this is due to higher energy expenditure for heating and moistening the cold air, or whether it is due to changes in the laryngeal mucosa, remains unknown. An explanation may also be that the test subjects perceived breathing cold air as more uncomfortable, causing more laryngeal obstruction earlier. However, the Borg Scale, being a reliable tool for scoring the perceived breathlessness,[23] was not different, and the same applied to the maximal heart rate obtained during the two tests. Taken together, this indicates that breathing cold air was not perceived very different than breathing room air by the patients. The pathophysiological mechanisms for the development of EILO remains improperly understood.

Another explanation for the difference in supraglottic score at moderate effort, might be the high pressure airflow through the Turbo Aire Challenger™. The flow from the device travels into the mouth and upper airways through a mouthpiece. A high flow might induce protective laryngeal reflexes as it hits the laryngeal mucosa. The Bernoulli's principle states that as the flow increases, the pressure lowers, and a lower pressure inside a flexible, or even flaccid tube, like the larynx, may cause the walls of this tube to adduct or close.[24] Therefore, the observed earlier supraglottic obstruction in cold air could be caused by the increased airflow delivered by the Turbo Aire Challenger™.

Test duration

The patients ran for a shorter time when breathing cold air compared to room air, despite no differences in CLE sum score. That being said, reduced performance is found among athletes without lung disease when working out in the cold.[25] The included patients running shorter when breathing cold air is therefore not surprising.

However, the Turbo Aire Challenger™ might affect the patients' performance. The device might mechanically affect the patients' effort and breathing pattern by causing noise and being more unstable at rapid speeds compared to the lighter mouthpiece used when breathing room air.

Considerations

A previous study reported that being surrounded by cold air might impact the lung capacity.[26] However, the Turbo Aire challenger™, is attached to the patients with a mouthpiece, thus, the cold air travels directly into the airways. Therefore, whether patients with suspicion of EILO is affected when being surrounded by cold air cannot be investigated in this study.

The Turbo Aire Challenger™ produced cold, but also dry air. Dry air might cause bronchoconstriction among patients with EIB.[27] Similar mechanisms may also come into play in patients with EILO. More research is needed to explore environmental factors and their influence on the development of EILO.

The CLE test setup while breathing cold air

As an explorative study using a newly developed test to investigate a potential connection between breathing cold air and EILO, some challenges regarding the CLE test setup while breathing cold air are further described.

Breathing sounds

During a standard CLE test, respiratory sounds are normally registered. Noise from the TurboAire Challenger™ made it difficult to register respiratory sounds properly during the test, and it was periodically challenging to differentiate between inspiration and expiration. The patients also had difficulty communicating verbally while breathing cold air, and hand-signs were necessary to agree on before starting the test.

Video recording

It was more challenging to get satisfactory video recordings of the larynx while the patients were breathing cold air, compared to a standard CLE-test. In combination with nose clips and tape, the laryngoscope was steadier and gave better video

recordings throughout the test, though possibly still not as steady as fixing the laryngoscope through a mask as usually done.

A strength with the setup is the experienced professionals that had performed multiple tests beforehand. The tests have mostly consisted of the ordinary CLE setup with breathing room air, but the CLE setup while breathing cold air is very similar to the other setup. Hence, observant and well-trained testers reduce possible weaknesses with little extra resources to secure sufficient testing.

Breathing cold air

The measured temperature in the TurboAire Challenger™ was at least minus 15°C, both pre- and posttest. However, it is challenging to predict the exact temperature of the air that passed through the larynx, as the inspired cold air gets heated up as it enters the mouth. However, this is no different from breathing cold air during an EIB test while exercising outside in the cold. In the lab, tape and a nose clip helped prevent air from entering through the nose and heating up the cold air.

Strengths and weaknesses

The clinic recruiting the test subjects has years of clinically experience regarding patients suspected of EILO. When investigating a potential connection between breathing cold air and EILO, the randomized crossover design was found suitable. A strength with the design is its ability to eliminate the “between-patient variability” as each patient is its own control,[28] allowing for a relatively small sample size. That being said, the main weakness of the study is still the small number of patients included, indicating that findings should be cautiously interpreted. The patients had a VO_2 which was normal and a mean BMI close to the mean for Norwegian adults.[29] Though this might indicate the patients included should be representative, we still lack sufficient knowledge of the young adult EILO population.

Cold air breathing may elicit bronchoconstriction in patients with airway hyperreactivity.[30] Spirometry pre and post the CLE tests were performed to ensure that lung function differences did not affect the test results.

CONCLUSION

In patients suspected of Exercise-Induced Laryngeal Obstruction (EILO), we found no difference in the degree of laryngeal obstruction when they were tested with the Continuous Laryngeal Exercise (CLE) test breathing cold air compared to room air. When compared at an identical workload, the supraglottic CLE score at moderate effort was higher when breathing cold air, and the test duration was shorter when breathing cold air compared to room air. More research is necessary to explore environmental factors and their influence on EILO.

COMPETING INTEREST

The authors have no conflicts of interest relevant to this article to disclose.

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Attachement 1. “Spørreskjema før første CLE-test”

Spørreskjema

SPØRRESKJEMA FOR FØRSTE CLE-TEST

1.1 Hadde du unormal lyd i pusten som spedbarn?

□ Ja □ Nei

1.2 Har du blitt operert/vært i narkose?

□ Ja □ Nei

Hvis Ja, hva slags kirurgi? _____

1.3 Hvor mange øvre luftveisinfeksjoner (forkjølelser) har du hatt siste 12 mnd?

□ Ofte (>10 ganger) □ Av og til (4-10 ganger) □ Sjeldent (0-3 ganger) □ Aldri hatt

1.4 Hvor ofte har du vært hes de siste 12 mnd?

□ Ofte (>10 ganger) □ Av og til (4-10 ganger) □ Sjeldent (0-3 ganger) □ Aldri vært

1.5 Er du plaget med halsbrann/sure oppstøt (gastroøsofagal refluks)?

□ JA □ NEI

Hvis JA: 1.5.1 Har du fått påvist såkalt gastroøsofagal refluks? □ JA □ NEI

2. ASTMA

2.1 Har du diagnosen astma (dvs. astmasymptomer utenom anstrengelse)?

□ JA □ NEI □ USIKKERT

2.2 Har du brukt astmamedisiner?

□ JA □ NEI

2.2.1 Hvilke astmamedisiner har du brukt?_____

_____

__ 2.2.2 Bruker du astmamedisiner nå (ca. de siste 2-3 månedene)?

□ JA □ NEI

2.2.4 Hvilke astmamedisiner bruker du nå?_____

_____

__ 2.2.5 Bruker du astmamedisin ved trening?

□ JA □ NEI

2.2.6 Bruker du astmamedisin utenom trening?

□ JA □ NEI

2.2.7 I hvor stor grad synes du astmamedisinene som du står på nå hjelper deg?

□ LITE/INGEN BEDRING □ NOE BEDRING □ STOR BEDRING

2.3 Hvor ofte har du hatt astmaanfall siste 12 mnd?

□ INGEN □ 1-3 GANGER □ 4-12 GANGER □ > 12 GANGER

HAR DU ANDRE SYKDOMMER/DIAGNOSER?

_____

_____

_____

_____

BRUKER DU ANDRE MEDISINER?

_____

_____

_____

_____

5. PUSTEBESVÆR

5.1 I hvilke situasjoner opplever du pusteproblemer?

□ I hvile □ Ved lett anstrengelse (gange)

□ Ved moderat anstrengelse/trening (eks. jogging)

□ Ved kraftig anstrengelse (hard trening / konkurranse)

5.2 Er det noe som gjør at du lettere får slike pusteproblemer?

□ Anstrengelse □ Sigarettrøyk □ Røykos □ Sterke lukter/parfyme □ Kulde □ Fuktig/rått vær (tåke) □ Psykisk belastning/stress □ Støv □ Varme (Syden) □ Trestøv/kjemikalier □ Reflux □ Søvn

□ Nesetetthet

□Annet:_____

6. ANSTRENGELSESUTLØST PUSTEBESVÆR

6.1 Hvor gammel var du da pusteproblemer ved anstrengelse startet?_____år

6.2 Hvordan vil du beskrive problemet ditt under anstrengelse? Hvor riktige er disse utsagnene for deg?

	Aldri	**Noen ganger**	**Nesten Ofte**	**Hver alltid**	**gang**
6.2.1. Jeg har problemer med innpust	1	2	3	4	5
6.2.2. Jeg har problemer med utpust	1	2	3	4	5
6.2.3. Jeg føler tetthet/smerte i hals	1	2	3	4	5
6.2.4 Jeg føler tetthet/smerte i bryst	1	2	3	4	5
6.2.5. Jeg har følelse av å bli kvalt	1	2	3	4	5
6.2.6. Jeg har følelsen av at et «lokk» stenger i halsen	1	2	3	4	5
6.2.7. Jeg får følelse av panikk	1	2	3	4	5
6.2.8. Jeg hører unormal lyd/piping i pusten	1	2	3	4	5
6.2.9. Jeg blir redd når pustevanskene opptrer	1	2	3	4	5
6.2.10 Jeg kan kontrollere pustevanskene når de kommer	1	2	3	4	5

6.3 Hvordan påvirker problemet deg i hverdagen?

	Aldri	**Noen ganger**	**Nesten Ofte**	**Hver alltid**	**gang**
6.3.1 Jeg lar være å presse meg fysisk pga pustevanskene	1	2	3	4	5
6.3.2 Pusteproblemene hindrer meg i å trene	1	2	3	4	5

6.3.3 Jeg har problemer med å løpe opp en trapp mellom to etasjer	1	2	3	4	5
6.3.4 Jeg har problemer med mosjon/gymnastikk	1	2	3	4	5
6.3.5 Jeg har problemer under lett trening	1	2	3	4	5
6.3.6 Jeg har problemer under hard trening eller idrettskonkurranser	1	2	3	4	5
6.3.7 Problemene er verre under konkurranser enn ved tilsvarende hard trening	1	2	3	4	5

6.4 Dersom symptomene oppstår når du er fysisk aktiv, hvor lang tid tar det før pustevanskene forsvinner etter at du har stoppet aktiviteten.

□ 0-5 min. □ 5-15 min. □ 15-45 min. □ 45 min. eller mer

6.5 Når du er fysisk aktiv, hvor mye er du plaget av dine pustevansker?

0	1	2	3	4	5	6	7	8	9	10	
Ingen plager											Verst tenkelig

6.6 Totalt sett i livet ditt, hvor mye er du plaget av dine pustevansker?

0	1	2	3	4	5	6	7	8	9	10	
Ingen plager											Verst tenkelig

6.7 Hvordan opplever du at helsen din er nå? (sett bare et kryss)

□ Dårlig □ Ikke helt god □ God □ Svært god

7. AKTIVITETSNIVÅ

7.1 Hvor mange ganger i uken driver du med idrett, eller mosjonerer du så mye at du blir andpusten og/ eller svett?

□ Hver dag □ 4-6 ganger i uken □ 2-3 ganger i uken □ En gang i uken □ En gang i mnd □ Mindre enn en gang i mnd □ Aldri

7.2 Hvor mange timer i uken driver du med idrett, eller mosjonerer du så mye at du blir andpusten og/eller svett?

□ Ingen □ omtrent ½ time □ omtrent 1 time □ omtrent 2-3 timer □ omtrent 4-6 timer □ 7 timer eller mer

7.3 Hvilken type idrett driver du med?_____

7.3.1 På hvilket nivå?

□ Mosjon □ Lokallag □ Regionalt nivå □ Nasjonalt nivå □ Internasjonalt

7.3.2 Har du trappet ned aktivitetsnivået ditt grunnet problemer?

□ JA □ NEI

7.3.3. Hvis ja, på hvilken måte har du endret aktivitetsnivået?

□ Måtte slutte med idrett □ Måtte trappe ned på nivå □ Har skiftet type idrett

7.4 Hvis jeg ikke hadde disse pusteproblemene, ville jeg vært mer fysisk aktiv

□ JA □ NEI □ Vet ikke

Takk for at du tok deg tid til å svare!

Attachment 2. Turbo Aire Challenger™

Equilibrated Bio Systems, Inc.

900 Walt Whitman Road • Melville, NY 11747 • (516) 673-6660

TECHNICAL INFORMATION BULLETIN

FIELD ENGINEERING	APPROVED BY:	Application Note 1.01
SUBJECT: Temperature Measurements:		DATE: 12/1/86 MODEL: TAC-1 FROM:

Temperatures measured at the mouthpiece of the TAC-1 will be over-estimated (reading will be warmer than expected) unless there is a patient on the system or the mouthpiece is blocked.

When there is no patient on the system (Fig. 1), flow from the cold air generator entrains room air (at room temperature) and raises the temperature exiting the mouthpiece. With a patient on the system, the 240 l/min from the generator exceeds the flow requirements for the patient and the balance of the flow leaves the exhalation port. Without room air entrainment, mouthpiece temperatures will be as expected.

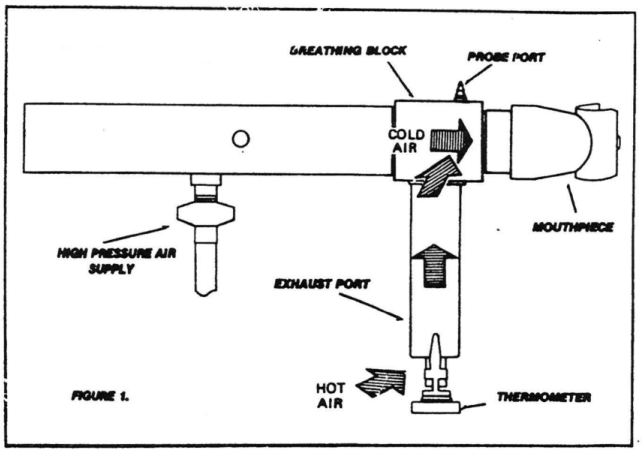

FIGURE 1.

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TECHNICAL INFORMATION BULLETIN

FIELD ENGINEERING	APPROVED BY: AB	Application Note 1.02
SUBJECT: Breathing Block Configurations:		DATE: 12/1/86 MODEL: TAC-1 FROM: AB

If patients complain about the air rushing at them from the generator, the patient breathing block can be reconfigured so that they breathe as a side stream to the cold air flow.

The TAC-1 was designed with both ports on the block having identical threads so that the mouthpiece and the exit ports could be exchanged (Fig. 1). This configuration is also useful if the TAC is used during exercise with a head mount valve support.

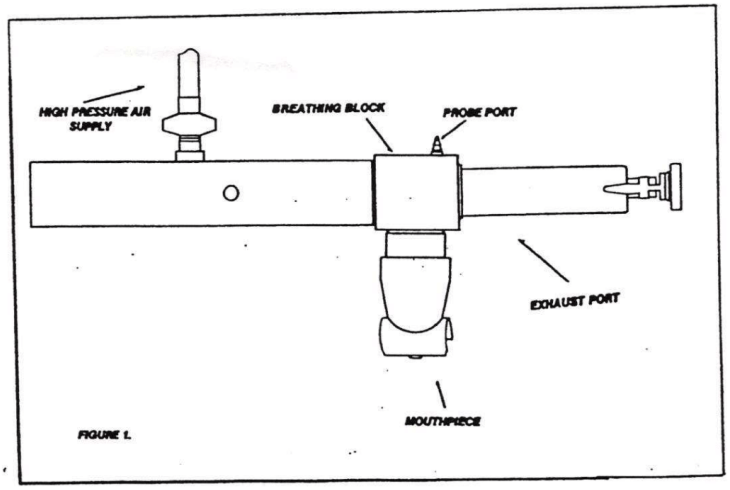


FIGURE 1.

Equilibrated Bio Systems, Inc.
TurboAire Challenger - TAC-1
Troubleshooting New Installations

Only very rarely are Equilibrated Bio Systems' products found to be defective in performance. This is especially true with regard to the **TurboAire Challenger** because of the simplicity and reliability inherent in the design of the **TAC-1** cooling generator. Occasionally, however, new installations report a failure to produce the expected cooling performance. In almost all such cases, one of four common problems is the cause of the performance difficulty.

Equilibrated Bio Systems, Inc. is pleased to assist anyone using its products with troubleshooting. However, to save you time, we suggest you check for the following four common problems before contacting EBS.

Inlet Pressure - The most common cause of poor performance is **low inlet pressure**. For optimum results, inlet pressure should be full line pressure (80-110 PSIG). Pressure gauges used to check this inlet pressure should be installed **at the TAC-1**, not at the source of the compressed air. Once proper operation has been established, the gauge may be moved to a more convenient location. **Note: The pressure must be maintained at full flow rate.**

A restriction in the inlet line will also decrease cold output. The specified compressed air supply line, the **TAC-HPH**, maintains a minimum ID of 1/4". This, coupled with the **TAC-HFR**, a 100 PSI, high flow rate regulator, are guaranteed to produce the desired cooling.

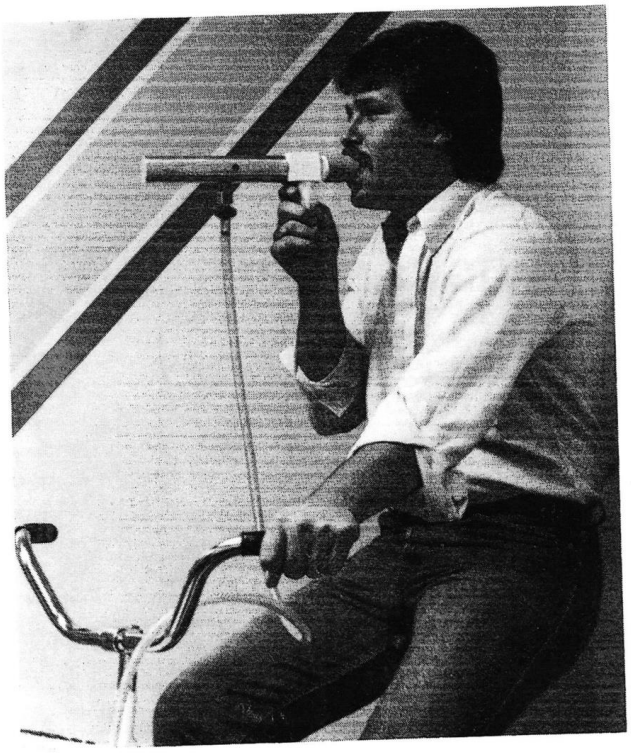
Inlet Temperature - The **TAC-1** will give best performance if **inlet air is no hotter than room temperature**. Air lines run over radiators, near heat producing devices, in direct sunlight, etc. will heat the inlet air and cause a corresponding rise in the temperature of the cold outlet of the **TAC-1** also.

Back Pressure - Under no circumstances should a back pressure be applied to the cold outlet of the **TAC-1**. **Back pressure causes serious reduction in cooling**. If you use alternative breathing block configurations for the cold air, it should not have a cross sectional area smaller than the cold outlet of the **TAC-1**. Attempts to direct cold air through small nozzles will always hurt performance.

Tighten Cold Cap - In the **TAC-1**, the generator internals are retained by an O-ring seal and a threaded cap. If the cap is disassembled, it is important that the **cold end fitting be reassembled tightly**. This is not simply to insure that the O-ring seals but additionally to seat the mechanics properly. Loose caps adversely affect performance.

Caution: Never block the hot air escape holes near the red sleeve. Restriction of hot air will not increase cold air output and may be dangerous.

Turboaire Challenger



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Turboaire Challenger

Introduction:

The Turboaire Challenger was designed to generate dry, sub-zero air for the testing of patient response to its inhalation. The Turboaire works on clean dry compressed air at 100 psig. The temperature drop achieved is dependent on the driving pressure and the inlet gas temperature. A 100 psig pressure source will produce air in the -10 to -20 degree celsius range.

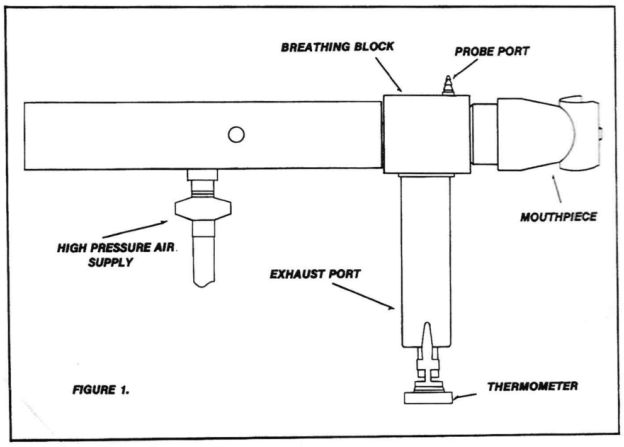

FIGURE 1.

Operating Instructions:

1. Connect the inlet of the Turboaire Challenger with a high pressure hose to a compressed air source at 100 psig.

2. Mount the acetyl breathing block on the Challenger. Hand tighten the thumb screw (Do not over-tighten) and attach a mouthpiece.

3. Clip the covered thermometer to the inside of the exhaust port. Be certain that the dial does not block the port.

4. Have the patient breathe the cold air for the duration of time in your protocol (see reference section for published protocols).

Notes:

1. An "H" cylinder (2200 PSIG) will last approximately 16 minutes. A "K" cylinder (3000 PSIG) will last approximately 24 minutes.

2. Variations in the accuracy of the regulator gauge will alter the outlet temperature. The Turboaire Challenger outlet temperature should be monitored and the pressure regulated to set the temperature to the desired level. Increasing the pressure will lower the outlet temperature (see cautions).

3. Referenced protocols have suggested having the patient breathe cold air at resting ventilation for 4 minutes. This is followed by 4 minutes breathing cold air at a ventilation level equal to 15 to 25 times their baseline FEV1 and then another 4 minutes at resting ventilation levels. Repeat function measurements are made immediately following cold air breathing and at 5 minute intervals for fifteen minutes.

References:

1. Strauss RH, McFadden ER, et. al. Influence of heat and humidity on the airway obstruction induced by exercise in asthma. J Clin Invest 61:433-440, 1978.

2. Weiss ST, et. al. Airways responsiveness in a population sample of adults and children. Am Rev Resp Dis 129:898-902, 1984.

3. McLaughlin FJ, Dozor AJ. Cold air inhalation challenge in the diagnosis of asthma. Pediatrics 72:503-509, 1983.

4. Jones NL, Campbell EJ. Clinical Exercise Testing. 1st edition, 1975 WB Sauders publisher.

Cleaning and Sterilization:

There are no moving parts or consumable materials in the Challenger and therefore it should not be disassembled for cleaning or maintenance.

1. The Turboaire Challenger should not be submerged in liquids. Sterilization should be by low temperature ethylene oxide.

2. The breathing block should be washed in warm water with a mild detergent. Sterilization may be by ethylene oxide or cold liquid disinfectants.

Cautions:

1. The metal heat discharge end of the Turboaire Challenger can become uncomfortably warm. Do not allow the patient to hold the unit by this end.

2. If the gas supply is not clean or contains water, you may get icing and obstruction of the Challenger outlet which will diminish the efficiency and raise airway temperature.

3. Do not exceed 200 PSIG at the inlet.

4. For use only by direction or prescription of a licensed physician.

5. If CO2 is added to the inspired gas, be certain to monitor expired CO2 to avoid accidental hypercapnea.

Figures 2 and 3 can be used to select power outputs requirements for desired ventilation levels (Ref 5).

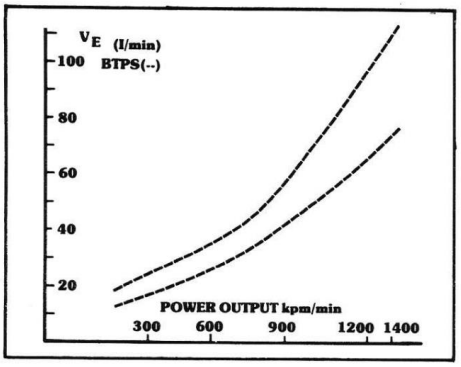

Figure 2: Expected ventilation range at selected power output for normal males.

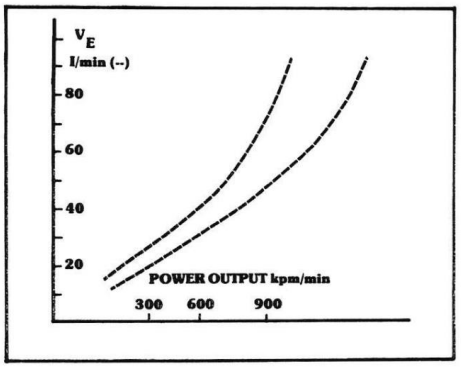

Figure 3: Expected ventilation range at selected power output for normal females.

Warranty:

Equilibrated Bio Systems, Inc. warrants the Turboaire Challenger against defects in materials and workmanship for a period of one year from date of purchase. We will repair or replace, at our option and without charge, any items that prove defective during the warranty period provided that the proper maintenance procedures described in the manual are followed.

The foregoing warranty is in lieu of all other warranties, expressed or implied, including but not limited to, the implied warranties of merchantability and fitness for a particular purpose.

Disassembly of the Challenger voids all factory warranties.


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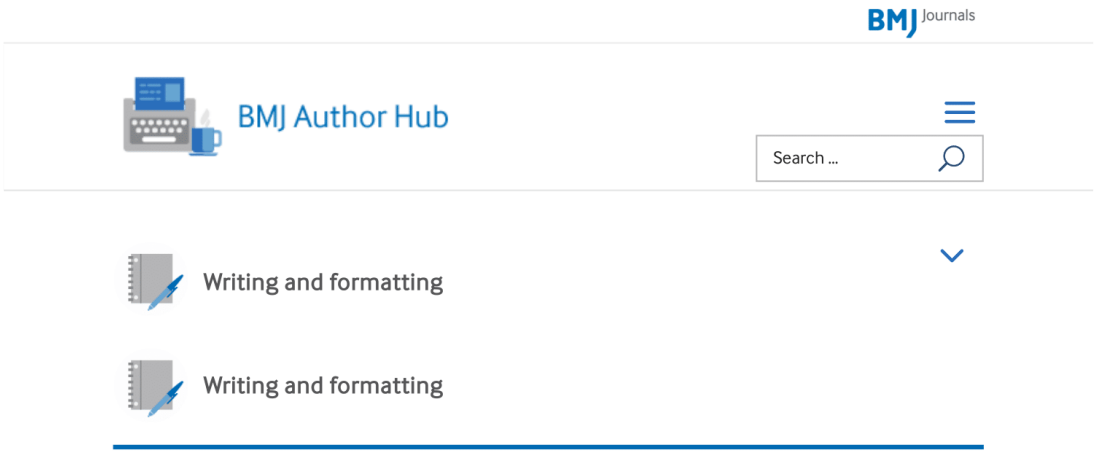
Attachment 3. British Medical Journal, formatting your paper.



In this section:

Formatting your paper

These are general formatting guidelines across BMJ, please always refer to journal-specific instructions for authors for article type specifications. You can browse the titles on our Journals website. If you are looking to submit to *The BMJ*, please visit this section.

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Title page

The title page must contain the following information:

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- Full name, postal address and e-mail of the corresponding author
- Full name, department, institution, city and country of all co-authors
- Word count, excluding title page, abstract, references, figures and tables

This is excluded for the journal *BMJ Quality and Safety* which operates triple-blind peer review.

Back to top >>

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Authors can usually opt to (or are required to) choose keywords relevant to the content of the manuscript during the submission process. This assists in the identification of the most suitable reviewers for the manuscript. The selected keywords should also be included in the abstract itself.

Back to top >>

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Back to top >>

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The manuscript must be submitted as a Word document (*BMJ Case Reports* and *Veterinary Record Case Reports* request that authors submit using a template which should also be in Word format). PDF is not accepted.

The manuscript should be presented in the following order:

- Title page
- Abstract, or a summary for case reports (Note: references should not be included in abstracts or summaries)
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- Tables should be in Word format and placed in the main text where the table is first cited. Tables should also be cited in numerical order
- Acknowledgments, Competing Interests, Funding and all other required statements
- References. All references should be cited in the main text in numerical order

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Please remove any hidden text headers or footers from your file before submission.

Back to top >>

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Acronyms and abbreviations should be used sparingly and fully explained when first used. Abbreviations and symbols must be standard. SI units should be used throughout, except for blood pressure values which should be reported in mm Hg.

Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.

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Back to top >>

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Back to top >>

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For figures consisting of multiple images/parts, please ensure these are submitted as a single composite file for processing. We are unable to accept figures that are submitted as multiple files.

During submission, ensure that the figure files are labelled with the correct File Designation of "Mono Image" for black and white figures and "Colour Image" for colour figures.

Figures are checked using automated quality control and if they are below the minimum standard you will be alerted and asked to resupply them.

Please ensure that any specific patient/hospital details are removed or blacked out (e.g. X-rays, MRI scans, etc). Figures that use a black bar to obscure a patient's identity are not accepted.

Back to top >>

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Tables should be in Word format and placed in the main text where the table is first cited. Tables must be cited in the main text in numerical order. Please note that tables embedded as Excel files within the manuscript are NOT accepted. Tables in Excel should be copied and pasted into the manuscript Word file.

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Back to top >>

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Back to top >>

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Citing in the text

References must be numbered sequentially as they appear in the text. References cited in figures or tables (or in their legends and footnotes) should appear at the end of the reference list to avoid re-numbering if tables and figures are moved around at peer review/proof stage. Reference numbers in the text should be inserted immediately after punctuation (with no word spacing)—for example,[6] not [6].

Where more than one reference is cited, these should be separated by a comma, for example,[1, 4, 39]. For sequences of consecutive numbers, give the first and last number of the sequence separated by a hyphen, for example,[22-25]. References provided in this format are translated during the production process to superscript type, and act as hyperlinks from the text to the quoted references in electronic forms of the article.

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Journals from BMJ use a slightly modified version of Vancouver referencing style (see example below) available in Endnote. Note that The BMJ uses a different style.

BMJ reference style

List the names and initials of all authors if there are 3 or fewer; otherwise list the first 3 and add 'et al.' (The exception is the Journal of Medical Genetics, which lists all authors). Use one space only between words up to the year and then no spaces. The journal title should be in italic and abbreviated according to the style of Medline. If the journal is not listed in Medline then it should be written out in full.

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Example references

- **Journal article:** 13 Koziol-Mclain J, Brand D, Morgan D, et al. Measuring injury risk factors: question reliability in a statewide sample. *Inj Prev* 2000;6:148–50.
- **Abstract/supplement:** 16 Roxburgh J, Cooke RA, Deverall P, et al. Haemodynamic function of the carbomedics bileaflet prosthesis [abstract]. *Br Heart J* 1995;73(Suppl 2):P37.
- **Preprints:** Rostami A, Sepidarkish M, Leeflang M, et al. First snap-shot meta-analysis to estimate the prevalence of serum antibodies to SARS-CoV-2 in humans. MedRxiv 20185017 [Preprint]. September 02, 2020 [cited 2020 Sep 20] https://doi.org/10.1101/2020.08.31.20185017. *More information about preprints >>*
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- **Electronic letters:** Bloggs J. Title of letter. *Journal name* Online [eLetter] Date of publication. url eg: Krishnamoorthy KM, Dash PK. Novel approach to transseptal puncture. *Heart* Online [eLetter] 18 September 2001. http://heart.bmj.com/cgi/eletters/86/5/e11#EL1
- **Book:** 15 Howland J. Preventing Automobile Injury: New Findings From Evaluative Research. Dover, MA: Auburn House Publishing Company 1988:163–96.
- **Chapter in a book:** 14 Nagin D. General deterrence: a review of the empirical evidence. In: Blumstein A, Cohen J, Nagin D, eds. Deterrence and Incapacitation: Estimating the Effects of Criminal Sanctions on Crime Rates. Washington, DC: National Academy of Sciences 1978:95–139.
- **Legal material:** Toxic substances Contro Act: Hearing on S776 Before the Subcommittee of the Environment of the Senate Comm. on Commerce, 94th Congress 1st September (1975).
- **Law references:** The two main series of law reports, Weekly Law Reports (WLR) and All England Law Reports (All ER) have three volumes a year e.g. Robertson v Post Office [1974] 1 WLR 1176

There are good historical precedents for the use of square and round brackets. Since 1891, round ones have referred to the date of the report, square ones to the date of publication of the report. Apart from not italicising the name of the case, we use the lawyers' style; be careful with punctuation, e.g. Caparo Industries plc v Dickman and others [1990] 1 All ER 568-608.

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Back to top >>

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Back to top >>

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