Norwegian University of Life Sciences
Faculty of Veterinary Medicine
Department of Companion Animal Clinical Sciences

Philosophiae Doctor (PhD)
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From horses to humans: a comparative study developing the first protocol for measuring upper airway pressures in exercising humans

Fra hest til mennesker: en komparativ studie om utvikling av første protokoll for å måle luftveistrykk hos mennesker under anstrengelse

Zoë Louise Fretheim-Kelly

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The most beautiful thing we can experience is the mysterious.
It is the source of all true art and science-
Albert Einstein

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Table of Contents

1 Abbreviations and definitions

AEF	Aryepiglottic Folds
AF	Airflow
BMI	Body Mass Index
Bpm	Beats per minute
CFD	Computational Fluid Dynamics
CI	Confidence Interval
CLE test	Continuous Laryngoscopy Exercise test
COPD	Chronic Obstructive Pulmonary Disease
CPET	Cardiopulmonary Exercise Test
CR	Coefficient of Repeatability
DLC	Dynamic Laryngeal Collapse
EIB	Exercise Induced Bronchoconstriction
EILO	Exercise Induced Laryngeal Obstruction
HR	Heart Rate
ICC	Intraclass Correlation Coefficient
Km	Kilometres
l	Litres
LoA	Limits of Agreement
LRC	Locomotor Respiratory Coupling
LRT	Likelihood Ration Tests
MDAF	Medial Deviation of the Aryepiglottic Folds
mm	millimetres
NMBU	Norwegian University of Life Sciences
NSCT	Norwegian Swedish Coldblooded Trotter
PE	Maximal inspiratory pharyngeal pressure reading
PT	Maximal inspiratory tracheal pressure reading
RER	Respiratory Exchange Ratio
RL	Laryngeal Resistance

RR	Respiratory Rate
SD	Standard Deviation
STB	Standardbred Trotter
S_w	Within Subject Standard Deviation
URT	Upper Respiratory Tract
VCD	Vocal Cord Dysfunction
VMAD	Ventromedial Arytenoid Displacement
V_T	Tidal Volume

2 List of papers

I. **Exercise Induced Laryngeal Obstruction in Humans and Equines. A Comparative Review.**
Fretheim-Kelly ZL, Halvorsen T, Clemm H, Roksund O, Heimdal JH, Vollsæter M, Fintl C, Strand E.
Front Physiol. 2019 Oct 30;10:1333. doi: 10.3389/fphys.2019.01333
PMID: 31736771; PMCID: PMC6831747.

II. **Feasibility and tolerability of measuring trans-laryngeal pressure during exercise.**
Fretheim-Kelly Z, Halvorsen T, Heimdal JH, Strand E, Vollsæter M, Clemm H, Roksund O.
Laryngoscope. 2019 Dec;129(12):2748-2753. doi: 10.1002/lary.27846
Epub 2019 Jan 30. PMID: 30698834; PMCID: PMC6900056.

III. **Reliability of trans-laryngeal resistance measurements during maximal exercise.**
Fretheim-Kelly Z, Engan M, Clemm H, Andersen T, Heimdal JH, Strand E, Halvorsen T, Røksund O, Vollsæter, M.
Manuscript in review *Laryngoscope*

IV. **A bitless bridle does not limit or prevent dynamic laryngeal collapse.**
Fretheim-Kelly Z, Fjordbakk CT, Fintl C, Krontveit R, Strand E.
Equine Vet J. 2021 Jan;53(1):44-50. doi: 10.1111/evj.13287
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3 Abstract

Dynamic obstructions of the larynx are a set of disorders that occur during exercise and are a major cause of morbidity in equines and humans. There are a number of similarities in presentation, diagnosis, pathophysiology and treatment. Both equines and humans present with exercise intolerance secondary to dyspnoea. The similarities of anatomy and certain types of dynamic collapse would suggest that the equine larynx provides a good model for human exercise induced laryngeal obstruction (EILO) (Paper I). Thus, close collaboration between veterinarians and medical doctors may lead to advancements in diagnostics and treatment in both species.

A working group at the European (human) Respiratory Society in 2015 identified the strong need for an objective outcome measure for the Continuous Laryngoscopy Exercise (CLE)-test. Therefore, the aim of Paper II of this Thesis was to develop an objective outcome measure for the CLE-test based on airway pressure measurement techniques already established in equine medicine. Seven adult volunteers ran a CLE-test with airway pressure measurement reporting tolerability for the three main interventions in the procedure. Alterations to the protocol were made and four subjects reran the test, all reported improved tolerability with the alterations. Consistent pressure data were obtained from all subjects in all tests, demonstrating that it is feasible and tolerable to measure trans-laryngeal pressure during a CLE-test.

The aims of Paper III in this Thesis were to determine the reliability of trans-laryngeal resistance measurements during a CLE-test, and to establish indicatory normal values for laryngeal resistance in humans. Thirty-one subjects were recruited to undergo two CLE-tests with airway pressure measurements. The repeatability of trans-laryngeal resistance measurement was shown to be excellent; coefficient of repeatability $0.62 cmH_2O/l/s$,

establishing trans-laryngeal resistance as a reliable outcome measure. Data from this small group indicates that mean trans-laryngeal resistance in females without endoscopic evidence of EILO (2.88cmH_2O/l/s) is greater than in males (2.18 cmH_2O/l/s). A greater degree of visual laryngeal obstruction was associated with higher trans-laryngeal resistance values in females.

Tracheal pressure measurements were performed in 130 harness racehorses presenting for high-speed treadmill videoendoscopy. As horses are obligate nasal breathers these patients often also develop airway obstruction at the level of the nares or nasopharynx, in addition to the larynx. Obstructions of the upper respiratory tract (URT) are common and can considerably increase resistance to air flow, resulting in poor performance. Measuring inspiratory and expiratory pressures provided an easy, objective outcome measure in clinical cases to supplement the subjective endoscopic grading systems currently utilized. These studies are still ongoing; however, a small cohort of these cases were recruited to perform the first objective study on the role of the snaffle bit on an equine obstructive URT disorder (dynamic laryngeal collapse associated with poll flexion, DLC) using inspiratory airway pressure readings as an outcome measure (Paper IV). Bits have often been incriminated as causing airway obstruction in horses, creating a welfare concern without any concrete evidence to support or refute this claim.

Nine Norwegian Swedish Coldblooded Trotters (NSCTs) with a previous diagnosis of DLC underwent a standardized high-speed treadmill videoendoscopy protocol, with simultaneous tracheal pressure measurements, on two separate days outfitted with either a snaffle bit or Dr. Cook Bitless bridle. The study was unable to demonstrate that a bit caused or influenced the severity of DLC in NSCTs as the mean airway pressures in the flexion phase were not significantly different between the two bridles. An interesting finding was that the presence of a snaffle bit in the mouth of the horses caused a mild but significant inspiratory obstruction in the free head carriage phase relative to when wearing the Dr Cook Bitless bridle.

In conclusion, this Thesis has demonstrated that trans-laryngeal pressure measurements during a CLE-test are feasible and tolerable in humans, providing repeatable objective data. This provides a new objective measurement tool for use in humans with airway obstruction at the level of the larynx.

Tracheal pressure measurements can be used routinely in the clinical evaluation of harness racehorses during high-speed treadmill videoendoscopy without complications. By applying this technology, we performed the first objective study investigating the role of the bit in inducing equine URT obstruction at the level of the larynx. It is hoped that our contributions will allow similar interventional studies to be performed in human patients suffering from dyspnoea with a laryngeal component. If the larynx is viewed as the entrance valve to the lower airways, future access to airway pressure data can become as important to respiratory medicine as transvalvular pressure gradients are in today's cardiology.

4 Norsk sammendrag

Dynamiske obstruksjoner av strupehodet er en gruppe lidelser som oppstår under trening, og er en viktig årsak til pustevansker hos både hester og mennesker. Det finnes en rekke likheter i presentasjon, diagnose, patofysiologi og behandling mellom disse lidelsene. Både hester og mennesker presenterer med treningsintoleranse sekundært til pustevansker. Likhetene i anatomi og visse typer dynamisk kollaps tyder på at hestens strupehode er en god modell for treningsindusert laryngeal kollaps hos mennesker (EILO) (Artikkel I). Dermed kan et tett samarbeid mellom veterinærer og leger fremme både diagnostikk og behandling hos begge arter.

En arbeidsgruppe ved det European (humane) Respiratory Society i 2015 identifiserte et sterkt behov for å formulere et objektivt utfallsmål ved kontinuerlig laryngoskopi under belastning, en såkalt CLE-test. Målet med artikkel II i denne avhandlingen var derfor å utvikle et objektivt utfallsmål for denne testen basert på trykkmålingsteknikker allerede etablert ved luftveisutredning hos hester. Syv frivillige voksne personer utførte en CLE-test, og rapporterte deretter tolerabilitet for de tre viktigste intervensjonene i prosedyren. Basert på dette ble endringer i protokollen gjort og fire personer løp testen på nytt. Samtlige rapporterte forbedret tolerabilitet med disse endringene. Konsistente resultater fra alle forsøkspersoner, og i samtlige tester viste at det er teknisk mulig og overkommelig å kunne måle luftmotstand over strupen under en CLE-test.

Målet med artikkel III i denne avhandlingen var å avgjøre hvor pålitelig en luftmotstandsmåling over strupen under en CLE-test er, og samtidig etablere normale verdier for luftmotstand over strupehodet hos mennesker. Trettien personer ble rekruttert til å gjennomføre to CLE-tester med luftveistrykkmålinger. Repeterbarheten av luftmotstandsmålinger over struphodet viste seg å være utmerket; koeffisient av repeterbarhet var 0.62

cmH2O/l/s, og bekreftet dermed trans-laryngual luftmotstand som et pålitelig resultatmål. Data fra denne gruppen indikerer at gjennomsnittlig trans-laryngual luftmotstand hos kvinner uten endoskopiske tegn på EILO (2.88cmH2O/l/s) er større enn hos menn (2.18 cmH2O/l/s). En større grad av visuell laryngeal obstruksjon var forbundet med høyere verdier av luftmotstand over strupen hos kvinner.

Trakeale trykkmålinger ble utført på 130 travhester under høyhastighets tredemølle test med videoendoskopi. Fordi hester normalt kun puster gjennom nesen, er obstruksjoner i de øvre luftveien et vanlig problem, og kan resultere i en betydelig økt motstand mot luftstrømmen. Dette vil igjen resultere i nedsatt prestasjon. Måling av inspiratoriske og ekspiratoriske trykk ga et enkelt, objektivt resultatmål i kliniske kasus som supplementerte de subjektive endoskopiske graderingssystemene man rutinemessig benytter. Disse studiene pågår fortsatt, men en liten kohort ble rekruttert til å utføre den første objektive studien om trinsebitt og øvre luftveier. Studien omhandler hvorvidt et trinsebitt er årsak til, og eventuelt i hvilken grad det påvirker en obstruktiv øvre luftveislidelse hos hest (dynamisk laryngeal kollaps forbundet med fleksjon av nakken, DLC) (artikkel IV). Bitt har ofte blitt mistenkliggort som en underliggende årsak til øvre luftveisobstruksjon hos hester. Dette har skapt en diskusjon rundt bitt og hestevelferd til tross for at man ikke har konkrete bevis for å støtte denne påstanden.

Ni kaldblodstravere som ved en tidligere undersøkelse hadde blitt diagnostisert med DLC, gjennomgikk en standardisert tredemølle protokoll, inkludert trakeale trykkmålinger på to påfølgene dager utstyrt med enten et trinsebitt, eller Dr Cook Bitless hodelag. Studien kunne ikke påvise at trinsebittet forårsaket, eller påvirket alvorlighetsgraden av DLC i hestene da det gjennomsnittlige luftveistrykket i fleksjonsfasen ikke var signifikant forskjellig mellom de to hodelagene. Et interessant funn var at det å ha et trinsebitt i munnen forårsaket en mild, men likevel betydelig forskjellig luftveisobstruksjon i den frie hodefasen i forhold til Dr Cook Bitless hodelag.

I konklusjon har denne avhandlingen vist at måling av trans-laryngeal luftmotstand ved en CLE-test er gjennomførbart og en tolerabel prosedyre hos mennesker, og gir repeterbare objektive data. Dette gir oss dermed et nytt objektivt måleverktøy for bruk hos mennesker med laryngeale luftveisobstruksjoner.

Trakeale trykkmålinger kan brukes rutinemessig i den kliniske evalueringen av travhester ved tredemølleutredninger uten komplikasjoner. Ved å bruke denne teknologien utførte vi den første objektive studien som undersøkte om bittet har en rolle i å indusere en øvre luftveisobstruksjon. Vi håper at våre bidrag vil muliggjøre lignende intervensjonsstudier hos humane pasienter som lider av dyspné med en strupehodekomponent. Hvis strupen blir sett på som inngangsventilen til de nedre luftveiene, kan fremtidig tilgang til luftveistrykkdata bli like viktig for luftveismedisin som transvalvulære trykkgradienter er i dagens kardiologi.

5 Synopsis

5.1 Introduction

5.1.1 Born to run?

Approximately 621 million humans worldwide are reported to run recreationally, with around 10 million competing in amateur events from five kilometer runs to marathons [1]. Competitive human racing dates back as far as 1829 BC [2]. Horseracing is one of the most ancient sports, having been described in literature from ancient Greece, Rome and Egypt [3]. As with human athletics the aim is to be the first over the finish line. This requires a musculoskeletal and respiratory system that perform optimally. Humans are unique in the running world as bipeds. Cursorial mammals such as the horse and greyhound are quadrupeds, they are specialist at moving at high speeds; 15-20m/s having a musculoskeletal system developed for running [4]. Humans are relatively poor sprint runners, being non-cursorial and thus have a high metabolic cost of running [4]. However, humans are exceptional endurance runners, most likely a result of evolution as persistence hunters [5]. Whether sprint or endurance running there are increased ventilatory demands that must be met, at maximal exertion these are over 20 times greater than at rest. This greater airflow puts strenuous demands on the upper respiratory tract (URT).

In modern competition equines and humans are the predominant species that compete at maximal exertion and additionally have documented laryngeal abnormalities associated with exercising at high intensity [6, 7]. As such, their URT are subject to similar strong aerodynamic stresses, potentially providing a common platform for research and understanding. Dynamic URT obstructions and their treatment are far better described in equines than in humans, suggesting a potential for added benefit for humans.

5.1.2 Dynamic obstructions of the upper respiratory tract in humans and equines

Human medicine recently agreed upon the phrase exercise induced laryngeal obstruction (EILO) [8, 9] to describe the phenomenon that in equines is usually referred to as dynamic obstruction of the URT [7]. These conditions represent comparable sets of disorders characterized by the larynx appearing normal at rest, with abnormalities seemingly induced by the increased ventilatory demands during ongoing exercise, and thereafter quickly resolving with cessation of exercise.

5.1.3 Comparative medicine

Between animal and human and medicine there is no dividing line – nor should there be. The object is different, but the experience obtained constitutes the basis of all medicine, Rudolf Virchow (1821- 1902). Comparative medicine is the study of comparable diseases in different species; the similarities give the model its relevance, but the differences often provide the most informative insights. By studying pathologies that co-exist in humans and animals, we may uncover common denominators of disease and identify new therapies, while at the same time reducing animal welfare concerns. Disease is not inflicted on these animals; instead they may in fact benefit from early access to new diagnostics, treatments and preventive techniques.

5.1.4 Comparative anatomy and physiology of the larynx

All mammalian larynxes have the same basic anatomy, a cricoid ring, a thyroid cartilage, an epiglottic cartilage, two arytenoid cartilages, with varying development of cuneiform cartilages [10]. Adaptions to the individual species' needs has led to variations on this basic structure [10]. The equine has a more funnel-shaped larynx so when the arytenoids are fully abducted the laryngeal opening has a greater diameter than the proximal trachea, allowing for large increases in airflow when fleeing a predator [10, 11]. In humans however, adaption has been made in favour of phonation, the laryngeal aperture is the narrowest part of the URT even when the arytenoids are fully abducted [10] (Figure 1). This does not however exclude the equine from being a good model for the human larynx. The basic anatomy is the same, the same muscles are innervated by the same nerves, producing the same movements. For example, both the equine and human larynx have only one arytenoid abductor; the cricoarytenoid dorsalis (equine) muscle or posterior cricoarytenoid (human) muscle [10, 12]. The difference in nomenclature occurs only due to the upright posture of the human. Damage to the recurrent laryngeal nerve in both species results in the same pathology; laryngeal hemiplegia, with failure of the cricoarytenoid dorsalis / posterior abducting the arytenoid cartilage [12, 13].

The larynx is the single greatest point of airway resistance in the human URT during mouth breathing [14]. In equines the larynx is the point of greatest turbulence in the URT, suggesting that significant resistance to airflow occurs here, although the rostral nasopharynx is subject to the most negative inspiratory pressures [11]. The larynx is therefore exposed to great aerodynamic stresses in both species.

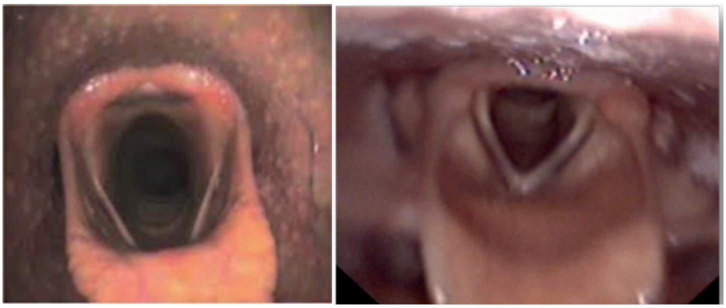

Figure 1. Normal larynx during exercise. Left: Equine. Right: Human.

During maximal exercise the respiratory system must meet the increased demands for oxygen and removal of increased carbon dioxide. This is achieved by increases in breathing frequency and tidal volume (V_T) [15]. The equine increases V_T 2-fold, from 5-8 litres (l) to 10-15 l and respiratory rate (RR) by a factor of 12-fold, from 10-15 breaths a minute to

120-140 breaths a minute [15, 16]. This results in a minute ventilation in equines at maximal exercise of 1800 l/min but it can be as high as 2400 l/min, a 15 to 20-fold increase over minute ventilation of 75-100 l/min at rest [15, 16]. In humans minute ventilation increases to 100-120 l/min in healthy adults (300 l/min in elite athletes). This represents a 15-20-fold increase over resting ventilation, the V_T increasing from 0.5 l to 2.5 l and breathing frequency from 12-15 breaths a minute to 50-70 [17]. Although the absolute values are much greater in equines the ventilatory response to exercise is similar in both species, with the minute ventilation at maximum exercise being approximately 15-20 times greater than at rest.

The equine is an interesting model for study of the respiratory system, as despite the high respiratory capacity compared to body size [18] the URT is the limiting factor to high intensity exercise in equines [19, 20]. In humans it is the cardiovascular system [17]. This makes the study of the respiratory system in equines interesting as derangements become all the clearer when it is the performance limiting system.

5.1.5 Aerodynamic principles

When attempting to understand airflow through the URT, the trachea can be simplified to a tube with a narrowing at the laryngeal aperture allowing resistance to airflow to be described by the laws of flow dynamics through a tube. Resistance to laminar flow of a non-compressible fluid in a tube can be derived by the Hagen-Poiseuille resistance formula; the resistance to flow is inversely proportional to the radius of the tube to the fourth power (Figure 2). Thus, when the radius of the tube is halved the resistance increases 16-fold. This explains why even a small loss of airway radius causes significant resistance to flow and thus dyspnoea, and why individuals with a narrower larynx will have a greater resistance to breathing giving a greater sensation of dyspnoea. However, in equines at exercise the Reynolds-number is greater than 2100 indicating that flow is turbulent not laminar [11]. One would assume this also be the case in humans. When flow is turbulent reduction in lumen radius does not have as pronounced effect on airway resistance as when flow is laminar [11]. The simplified model assuming laminar flow is considerably easier to calculate and understand and gives an approximation of the changes expected, it must just be remembered that the change will not be as pronounced as the Hagen-Poiseuille resistance formula would predict.

$$\text{Resistance} = \frac{8 \times \text{length} \times \text{viscosity}}{\pi \times (\text{radius})^4}$$

Figure 2. Hagen-Poiseuille resistance formula.

When considering the effect of failure of full abduction of the structures of the larynx, or in the case of humans the normal larynx which is the narrowest portion of the upper airway, the Bernoulli principle is an important aerodynamic principle [21]. Derived from the laws of conservation of energy it describes how a reduction in diameter of the airway will lead to increased flow velocity and a decrease in intramural pressure through the narrow section. The total energy within a closed system; the upper airway must remain constant. The energy within the system is in three forms: kinetic (flow velocity), (intramural) pressure and potential energy. The potential energy is considered constant as there is no change or minimal change in height. Therefore, if flow velocity increases, the intramural pressure must decrease in order to keep the total energy constant. This faster moving, lower pressure air jet is known as the Venturi effect [21]. This may explain why once an obstruction occurs in the upper airway there is a tendency for it to worsen over time and for other neighbouring structures to collapse into the airway lumen, as there is less pressure exerted on structures and thus greater muscular effort is required to hold them abducted [22, 23]. Hence, when considering resistance to airflow through the upper airway in both equines and humans the effect of airway radius on resistance to airflow can be approximated by the Hagen-Poiseuille resistance formula. Individuals with a narrow airway will experience greater resistance to flow and thus feel greater dyspnoea. When there is a natural or pathological narrowing of the airway, the Bernoulli principle describes the reduction in intramural pressure making it increasingly difficult to keep structures abducted through neuromuscular action.

5.1.6 Clinical presentation

In both equines and humans, the common presenting complaint is reduced exercise tolerance occurring secondary to dyspnoea, which is typically characterized by inspiratory stridor that worsens as exercise intensity increases and resolves within a few minutes of cessation of exercise [7, 24-26]. In humans, panic reactions occasionally occur as a response to breathlessness [27]. Anecdotally, corresponding "stress and avoidance behaviours" have been reported in equines, associated with occasions of dyspnoea at racetracks.

In humans, first time presentation of EILO is most frequent in active youngsters, with those partaking in competitive sport being overrepresented [27, 28]. Symptoms might initially be vaguely described; however, on closer questioning some very typical features will be revealed. The inspiratory phase of the breathing cycle being more affected than the expiratory phase, and symptoms are at their worst when ventilation requirements are at their most intense, with symptoms typically abating as ventilation decreases – unless panic occurs. This pattern clearly contrasts the expiratory dyspnoea of Exercise Induced Bronchoconstriction (EIB) that peaks after cessation of exercise (Figure 3). Nevertheless, these conditions are too frequently confused, often with unfortunate consequences [29, 30]. However, care must be taken when interpreting self-reported symptoms, as many patients find it hard to attribute symptoms to a particular phase of respiration, and a number of studies suggest that self-reported symptoms are a poor prediction of EIB and EILO [28, 31].

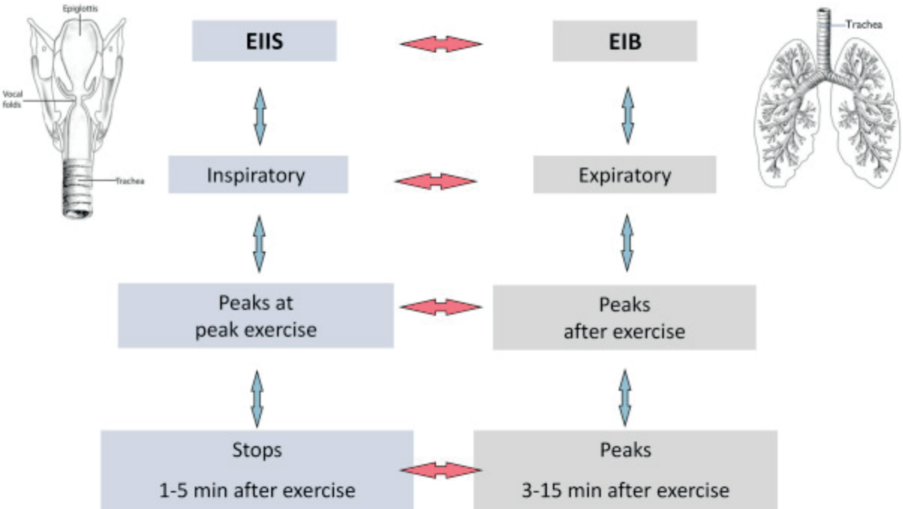

Figure 3. The characteristics of exercise-induced inspiratory symptoms (for example EILO) versus exercise induced bronchoconstriction (EIB).

Similarly, symptoms of dynamic obstruction of the URT in equines often present around 2-3 years of age, as performance expectations increase [7]. Trainers report the horse not exerting itself as fully as before, abnormal respiratory noise at higher exercise intensities, and poorer race performance in terms of placings and earnings [24]. Again, upon closer questioning, trainers and jockeys can often time the respiratory noise to inspiration [7]. Abnormal breathing patterns may occur, with uncoupling of the typical locomotor respiratory coupling mechanism (LRC), which is systematically observed to be 1:1 in galloping horses but sometimes also occurs in trotting horses [32, 33]. Horses with respiratory tract disease have been shown to adopt a 2:1 pattern when galloping, taking 1 breath over 2 strides [32]. This may reflect "breathlessness" and an effort to regulate pathological dyspnoea [34]. By increasing the time over which a breath is inhaled, flow rate and pressures within the airway can be reduced, thereby exerting less stress on the upper airway structures [32]. No such adaptations have been reported in humans with EILO, although a change in timing of the respiratory cycle is an area of research interest in this patient group.

5.1.7 Diagnosis

Dynamic obstructions by definition require the upper airways to appear normal at rest, and that abnormal function is revealed during ongoing exercise. Thus, visualization of the larynx during exercise is required for a diagnosis in both species, allowing clinicians to determine which structures are implicated, as well as when and in what sequence they become involved [7, 29, 35].

In equines, treadmill endoscopy protocols have been in use for more than three decades [35]. All apply the same principles of running the horse at the trot or gallop, until symptoms are seen or to fatigue, with an appropriately positioned endoscope in place during the entire test [7, 23, 36]. Most protocols include a method to determine the level of exertion, such as a heart rate monitor, ECG or gas flow analysis (Figure 4). Some centres include periods of free head carriage and periods of poll flexion to mimic real race or riding conditions, as recent articles report that head position can induce or aggravate many disorders [37, 38]. During the last decade overground endoscopes with GPS tracking have been developed, which allows the videoendoscopic test to be performed in the field [39]. Although overground endoscopy cannot be standardized as a treadmill test can, it does allow testing in the same environment in which symptoms occur [40]. This allows evaluation of cases that are not suitable or for those who do not have access to high-speed treadmill facilities.

Figure 4. A horse undergoing high-speed treadmill videoendoscopy with tracheal pressure readings at the equine hospital, NMBU.

Pressure readings from the trachea and pharynx have become important tools at equine university clinics to allow objective, precise measurement of the degree of inspiratory and/ or expiratory airflow obstruction (Figure 4) [41, 42]. This has allowed the development of computational fluid dynamics (CFD) models, and consequently a better understanding of pathological processes and treatment planning [43].

The major differentials and co-morbidities for exertional dyspnoea in the equine are lower airway problems, cardiac disease and poor fitness. Bronchoalveolar lavage is routinely performed as part of a respiratory performance workup to determine lower airway inflammatory disease status. ECG recordings during treadmill testing allow evaluation of any arrhythmias that may be present. It should be noted however that arrhythmias are commonly reported in racehorses and their clinical significance varies [44-46].

In humans the continuous laryngoscopy exercise (CLE)-test was similarly developed to study the visual presentation of the larynx during ongoing exercise in patients complaining of symptoms that on close questioning appeared to originate in the upper airways. The CLE-test as described by Heimdal et al. in 2006 [47], is a complete incremental cardiopulmonary exercise test; including tidal flow volume loops, breath-by-breath gas analyses, ECG, video of the upper torso and head, and sound recordings, combined with an appropriately positioned endoscope in place for the entire test (Figure 5). The data is synced into one single file to allow storage and subsequent analyses [47]. A number of modes of exercise can be used, but the CLE-test is most often performed on a treadmill or a cycle ergometer controlled via software to produce a standardized exercise test. As with equine patients, replicating real life conditions where symptoms occur is useful and as such, laryngoscopy during rowing, stairclimbing and even swimming have been described [48, 49]. The benefit of this is the recreation of the conditions in which symptoms occur; the disadvantage is that these modes of exercise are harder to standardize, which poses a problem when evaluating effects from interventions or when conducting research.

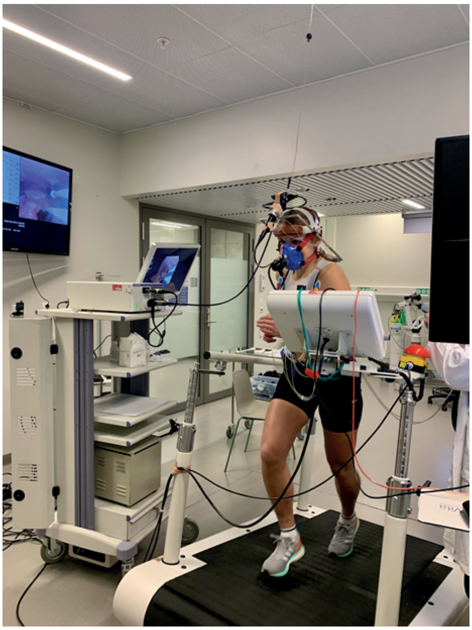

Figure 5. The CLE-test set-up. An incremental cardiopulmonary exercise test (respiratory parameters and ECG) is combined with laryngoscopy.

As with equine patients, it is fundamental to view the larynx throughout the complete exercise test and recovery. This allows identification of the structures that are implicated, when and in what sequence they become involved and as such, a full visual representation on how the situation evolves; information that collectively is critical for appropriate treatment [29]. For example, failing to observe excessive supraglottic tissue collapse prior to a glottic obstruction may result in patients receiving conservative treatment that may not be efficient. Excessive supraglottic tissue obstruction is usually better treated by surgery (although the glottic component also needs to be addressed). Likewise, in equines, failure to diagnose aryepiglottic fold collapse being secondary to failure of full arytenoid abduction and thus only treating the aryepiglottic fold collapse, will not result in full clinical improvement and return to performance. Thus, for both equines and humans, video laryngoscopy performed throughout an exercise test is by definition required to make a definitive diagnosis, and also imperative to institute appropriate treatment, although so far only in equines at larger veterinary clinics are these images supplemented by objective airway pressure measurements.

5.1.8 Grading of severity and degree of obstruction

Grading systems for the degree of obstruction caused by luminal collapse or medialization of the various structures in the upper airway (mainly the larynx), have been developed independently for both humans [50] and equines [37], and are surprisingly similar. These systems are based on subjective visual assessments (Figure 5). The subjective nature of visual assessment makes it a poor outcome measure for interventions and for research.

In equines, tracheal pressure measurements, first described by Nielan et al, 1992, have provided an objective measure for research for nearly three decades [41]. The larynx functions as the entrance valve to the lower airway. Thus, techniques for tracheal and pharyngeal pressure measurements throughout a full CLE-test in order to determine the normal and excessive pressure drops during high intensity exercise over this so vitally important valve have been proposed to meet the request for an objective outcome measure in a recent literature review [8]. Encouraged by the successes obtained by veterinarians in objectively quantifying obstructions in equines [42, 51, 52], it is hoped that a better understanding of these pressure relationships will facilitate our understanding of laryngeal function in humans. Thus, application of equine veterinary research and practice has great potential to aid the understanding of the pathophysiology and treatment of EILO, and thereby contribute to the provision of quantifiable data that can be used to guide therapy. This is the major goal underpinning this Thesis.

5.1.9 Pathophysiology underlying dynamic upper airway collapse

The inciting cause of dynamic upper airway collapse varies and has not been determined for humans. In equines, a number of forms of dynamic collapse seem to be due to anatomic / functional phenotypes for which certain breeds seem predisposed [37, 53]. However, all result in an initial local narrowing of the airway, which with further exercise often worsens [54]. These pressure/flow/size relations are described mathematically by the Bernoulli principle and Hagen-Poiseuille formula, as described in 5.1.5.

5.1.10 Specific pathologies

In humans, laryngeal obstruction is divided into supraglottic (structures rostral to the vocal folds) and glottic (at the level of the vocal folds) [9]. There is no such division in the equine. In the following paragraphs, each specific structure that is subject to collapse and thus potentially can obstruct the airflow will be described. It is hoped that by measuring airway pressure that our understanding of these obstructions will be improved, currently they are only subjectively described. Although each structure is described separately, most upper airway collapse involves multiple structures, this is termed complex airway collapse [7, 22, 55].

5.1.10.1 Supraglottic collapse (human) versus medial (axial) deviation of the aryepiglottic folds (MDAF) (equine)

The aryepiglottic folds (AEF) are found both in humans and equines, and are membranous tissues that run from the epiglottis to the arytenoid cartilages, serving a protective function during swallowing [10]. In both equines and humans they are supported by the corniculate cartilages and in humans and some equines by the cuneiform cartilages [10, 56].

In equines a number of mechanisms have been suggested to lead to MDAF; e.g. failure of full abduction of the arytenoids, lifting of the epiglottis, a structural weakness of the tissue, an excess of fold tissue or stretching of the fold tissue secondary to other upper airway obstructions, resulting in laxity [22, 23]. The result is displacement of the folds axially (medially) during inspiration (Figure 6), as the lax tissue cannot withstand the increasingly negative pressure created by the increasing airflow induced by the increased ventilation of exercise [56]. This is supported by research using computer modelling, that shows that the AEF contralateral to the unilateral laryngeal collapse is subject to more negative airway pressures compared with horses free from pathology [43]. Histological examination has shown that collapsing AEFs have focal inflammation and oedema similar to that seen in cases of laryngomalacia in humans [57]. It has not been established if these histological changes are causative or a result of trauma induced by the airflow during collapse [57].

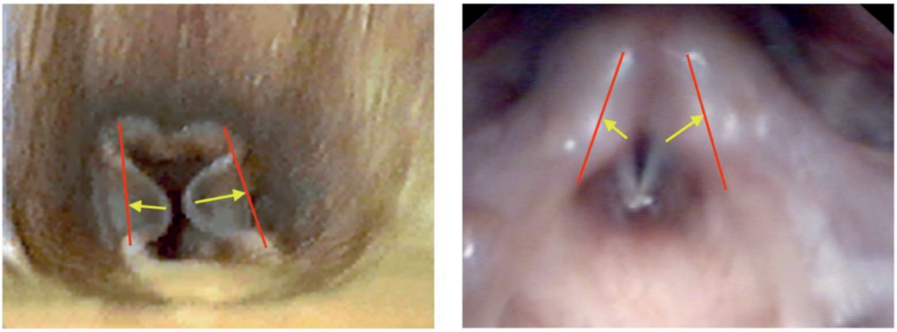

Figure 6. Endoscopic photographic images during exercise of: left; marked medial deviation of the aryepiglottic folds in an equine. Right; grade 3 supraglottic EILO in a human. Red lines denote expected line of aryepiglottic fold in a normal subject. Yellow arrows denote degree of collapse to normal

In humans with EILO, there is virtually no knowledge on basic pathophysiology; however, the aerodynamic principles described in horses are one of the main causal theories currently under investigation [8]. The larynx is protected from inspiratory collapse by a rigid cartilage skeleton, particularly the cricoid cartilage, and by muscular actions that provide support for the glottis and supraglottic structures. Reidenbach (1998), suggested that a possible reason for the inward collapse of the aryepiglottic folds may be insufficient anchorage to the cartilage skeleton of the larynx [58]. A flaccid, oedematous, swollen and/or superfluous mucosa of the arytenoids and of the aryepiglottic folds may contribute by disturbing the airflow, thereby inducing change from laminar to turbulent flow at an earlier stage. A retroflexed or omega-shaped epiglottis may contribute in a similar way or represent an obstruction by itself [59]. Airway pressure readings in humans with EILO may provide an objective answer to this, as they have done in horses. Other theories include laryngeal hypersensitivity, neurological reflex arc alterations, and psychological stresses; however, currently all theories lack supporting evidence. Given the complex functions of the larynx, it is likely that the pathophysiology of EILO is multifactorial, and that the aetiology varies between individuals, as seems to be the case in horses [55]. It seems likely that an anatomical or physiological anomaly predisposes the AEFs to collapse at the increasing negative intraluminal pressures induced by the greater airflow of exercise (Figure 6). This theory finds some support by findings in equines of significant associations between severity of deviation of the AEF and increasing number of upper airway abnormalities detected [22, 55].

5.1.10.2 Left laryngeal neuropathy

In equines and humans failure of full abduction of the arytenoid cartilage may occur unilaterally or bilaterally; however, only unilateral failure will be reviewed here, as bilateral failure is uncommon in the horse and results in dyspnoea at rest and is as such not a dynamic condition which is the focus of this Thesis [7].

The most common equine cause of left sided failure of arytenoid abduction is the condition 'recurrent laryngeal neuropathy', caused by distal neuronal axonopathy of the recurrent laryngeal nerve [13, 60]. In more advanced cases, this failure of abduction may be seen during resting laryngoscopy, which makes it a non-dynamic condition. It is important to view the larynx during exercise in "early" cases, as the grade of collapse can improve as well as worsen during exercise, due to variations in the recruitment of muscle fibres with corresponding variations of the clinical course [60, 61].

In humans, damage to the left recurrent laryngeal nerve during surgery for patent ductus arteriosus, mediastinal and head and neck surgery is a recognized surgical complication and was a leading cause of left sided laryngeal hemiplegia. Recent increased attention to this has reduced incidence resulting in idiopathic cases being more common [62-65]. This condition corresponds with equine 'recurrent laryngeal neuropathy' grade 4 (end stage), as this implies complete disruption of motor innervation to the posterior cricoarytenoid muscle (Figure 7).

Figure 7. Endoscopic photographic images during exercise of: left; Left-sided recurrent laryngeal neuropathy in an equine. Right; left laryngeal hemiplegia in a human. Red lines denote expected position of arytenoid cartilage in a normal subject. Yellow arrows denote degree of collapse to normal.

Other types of malpositioning of the arytenoids that result in airway obstruction include medial luxation of one arytenoid apex under the other [66]. In equines this is termed ventromedial arytenoid displacement (VMAD) [66]. This is hypothesised to be due to the transverse arytenoid muscle not being able to support the axial aspect of the arytenoid [67]. One study of VMAD noted post-mortem that the transverse arytenoid ligament was excessively wide. It is clear that further research is needed to determine the pathophysiology of this disorder [67].

In humans, scissoring of the corniculate cartilages has been anecdotally described, seemingly with a similar visual appearance to VMAD. Ventromedial arytenoid displacement results in obstruction of airflow through the most posterior part of the glottic opening. Computational flow dynamics models have shown that the main course of airflow is through the posterior portion of the glottis, above the vocal process [43, 68]. So, although malpositioning of the arytenoids does not visually appear to represent a significant obstruction, it may indeed prove to be, as it obstructs the main channel of airflow. Further research is needed to confirm or refute this in clinical cases. Airway pressure measurements are currently being utilized to evaluate all types and degrees of URT obstruction in equines, including

VMAD; hopefully providing information that will aid our understanding of the significance of dynamically malpositioned arytenoids also in humans.

5.1.10.3 Vocal cord dysfunction in humans and equines - Combined supraglottic and glottic collapse in humans versus DLC in equines

Vocal cord dysfunction (VCD) is a controversial diagnosis in humans, there is debate surrounding its cause in terms of physical triggers or if it is entirely a psychological response [69, 70]. For the purpose of this discussion we will consider it only in response to exercise as a trigger. In equines, vocal fold collapse in the absence of collapse of other structures is uncommon but has been described and experimental studies have shown that cricothyroid muscle dysfunction due to superior laryngeal nerve damage is the underlying mechanism [71]. This results in a lack of tension on the vocal folds, which results in a passive drawing into adduction when the ventilatory volume increases. In humans, VCD may occur as the only or the primary obstruction but more commonly occurs secondary to supraglottic (i.e. aryepiglottic fold) collapse [27, 29], giving a visual impression similar to what is labelled DLC in equines (Figure 8).

Dynamic laryngeal collapse occurs during poll flexion (flexion of the head relative to the neck) and results in passive collapse of the vocal folds, arytenoid cartilages, and in many cases the AEF [54]. The underlying mechanism seems related to phenotype since innervation to the larynx and associated muscles is normal [72]. The clinical phenotype is an equine with a rostral positioned larynx relative to the mandible accompanied by a narrow intermandibular space [73]. This results in the larynx being compressed by the hyoid apparatus during head /neck flexion, preventing full abduction of the arytenoid cartilages during exercise [73].

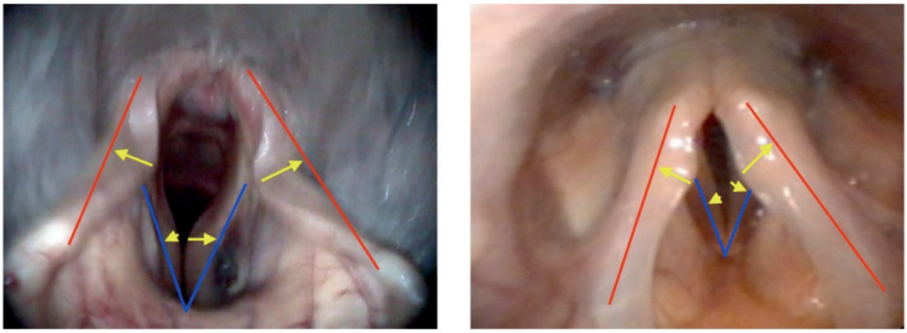

Figure 8. Endoscopic photographic images during exercise of: left; moderate dynamic laryngeal collapse in an equine. Right; grade 3 combined supraglottic and glottic EILO in a human. Red lines denote expected line of aryepiglottic fold in a normal subject. Blue lines denote expected line of vocal fold in normal subject. Yellow arrows denote degree of collapse to normal.

In both humans and equines, VCD and DLC cause inspiratory obstruction at the glottic level, frequently causing near complete obstruction of the glottis, characterized by a loud stridor [55]. Glottic obstruction appears to cause human patients the most distress, and cases associated with panic attacks often have a glottic component [74]. A noteworthy possible difference between glottic collapse in equines versus humans, is that the vocal folds are drawn in passively in equines, due to lack of maintenance of arytenoid abduction [71, 75]. Contrary, in humans, active adduction of the vocal folds is the most common cause of glottic obstruction [74].

5.1.10.4 Epiglottic retroflexion (retroversion)

Epiglottal retroflexion arises when the apex of the epiglottis retroflexes and thereby covers the rima glottis, causing an obstruction of the entrance to the larynx [29, 76]. Collapse of the margins of the epiglottis can also occur in equines[37]. Epiglottic retroflexion is rare in humans and uncommon in equines [55, 76]. In humans, it is often associated with an epiglottis that is omega shaped or with a high resting position and can be seen as a form of supraglottic collapse. The range of retroflexion varies from an epiglottis that fails the anterior rotation normally seen as exercise intensity increases, thereby disrupting airflow, to the epiglottis completely retroflexing into the rima glottis (Figure 9). In equines, the condition has been associated with neuromuscular dysfunction of the hyoid musculature and with damage to the hypoglossal nerve, and consequently hyoepiglotticus muscle paralysis or dysfunction [76]. In a clinical commentary by Ahern (2013), the most common cause of epiglottic retroflexion in equines was trauma to the hypoglossal nerve as a complication of previous URT surgery, other causes included idiopathic and URT infection[76].

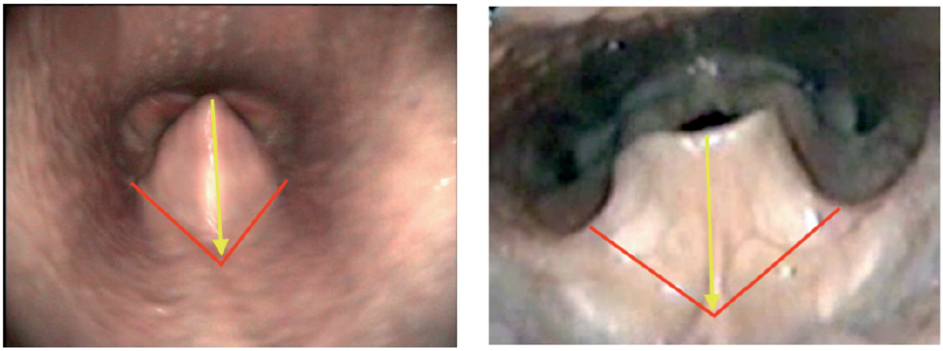

Figure 9. Endoscopic photographic images during exercise of: left; epiglottic retroversion in an equine. Right; epiglottic retroversion in a human. Red lines denote expected margins of epiglottis in a normal subject. Yellow arrows denote degree of collapse to normal.

Currently these dynamic abnormalities are usually evaluated subjectively in the clinical setting with endoscopy performed during exercise. It is hoped that in the future airway pressure measurements will allow objective evaluation of their degree of severity, and a fuller description of airway derangements associated with these forms of airway collapse. It is hoped that this Thesis will establish protocols for measuring airway pressures in the URT in both equines and humans allowing eventually for their routine clinical use. This will allow for numerical grading of the degree of airway obstruction, which will aid planning and post-operative evaluation of treatment.

5.2. The study

5.2.1 Aims of the PhD Thesis

The overall aim of this Thesis was to develop an objective outcome measure for upper airway pressure measurements in humans during a CLE-test using a comparative medicine approach. The studies in this Thesis had the following specific aims in order to achieve this:

- To review the relevant published literature in order to establish the scientific basis for a comparative medicine approach in equines and humans. Participation in clinical investigations at the Equine hospital, Faculty of Veterinary Medicine, NMBU and Haukeland University Hospital to enable comparisons between dynamic upper airway obstructions in equines and humans to be made (Paper I).

- To develop and refine the technique of performing upper airway pressure measurements for clinical use in harness racehorses during high-speed treadmill exercise videoendoscopy.

- To use this experience and established techniques to develop the first ever protocol to objectively assess upper airway function during a maximal exercise test in humans (Papers II and III).

- To demonstrate the use of upper airway pressure measurements as an objective outcome in a clinical setting in a cohort of horses; specifically to use tracheal pressure measurements to determine the role of the action of the bit on the tongue in inducing DLC in horses during periods of poll flexion (Paper IV).

5.2 Materials and Methods

A brief summary of the materials and methods is provided in this section. For complete methodology see Papers II-IV.

5.2.1 Subjects

Human subjects were recruited from the staff, students and associates of Haukeland University Hospital, University of Bergen and Western Norway University of Applied Sciences, Bergen. The subjects were 24-60 years of age and included 18 females and 15 males. Five subjects (3 males and 2 females) were enrolled in both study II and III.

In the period from January 2017 to December 2020, 199 Standardbred trotters (STB) and NSCTs presented to the equine hospital, NMBU for high-speed treadmill videoendoscopy. These horses presented due to abnormal respiratory noise and/ or poor performance, or racing fitness evaluation. From this subject pool 9 NSCTs diagnosed with moderate to marked DLC were recruited for a prospective study, exploring the role of the action of the bit on the tongue in inducing DLC associated with poll flexion (Paper lV).

5.2.2 The Continuous Laryngoscopy Exercise (CLE)-test (Papers II and III)

The CLE-test was developed and first described by Heimdal et al, 2006 [47]. Briefly, subjects were attached to a 12-lead portable ECG, and the right nostril and nasal cavity were anesthetized with 4% lidocaine. An endoscopic video camera system (Olympus Visera, CLV-S40, Tokyo, Japan) was connected to a fibreoptic laryngoscope (Olympus ENF-V3, Tokyo, Japan) advanced through a modified facemask (Hans Rudolph, Inc., Kansas City, MO, USA) through the nasal cavity to the oropharynx. A video camera was placed in front of the subject and a microphone attached to the headset to document external images and sounds. Finally, the ergo-spirometry unit was attached to the facemask. Subjects ran on a treadmill, Ergo ELG70

(Woodway, Weil am Rhein, Germany), to individual experience of exhaustion using a modified Bruce protocol with 60 second incremental intensity steps. Gas exchange variables were recorded using a Jaeger CPX unit (Vyntus, Hochberg, Germany).

5.2.3 The standardized equine treadmill protocol (Paper IV)

Horses were trained to run on the treadmill before testing, either the day before or just before testing. Horses wore standardized racing equipment (a light harness, bridle, checkrein and long reins) and were driven by an experienced technician. Heart rate (HR) was monitored continuously during the treadmill test using a 2-channel telemetry ECG with three leads. Self-adhesive electrodes were positioned under the harness against the left chest wall at 20 cm (right leg-black) and 30 cm (right arm-red) below the topline, slightly left of ventral midline (left leg-green) and along the right chest wall 20 cm below the topline (left arm-yellow). The ECG device was secured to the harness, HR response was monitored in real time and reviewed after the test. Warm-up on the treadmill consisted of 3 minutes at 5m/s and then 2-3 minutes at 8-10m/s. The treadmill was stopped and a videoendoscope passed through the right nostril and positioned near the tip of the epiglottis, giving a clear view of the arytenoid cartilages and vocal folds. The endoscope was secured to the bridle with Velcro straps and tape. The NSCTs trotted at 8.5 m/s on a 1.5-degree incline while the STBs trotted at 10m/s on a 1.5-degree incline. The 4-minute treadmill protocol consisted of 4 x 1minute phases. Phases alternated between free head carriage, phases 1, 3 and poll flexion achieved by applying tension to the reins and driving the horse onto the bit, phases 2 and 4. The degree of poll flexion was within the limits normally achieved in training and racing. Horses ran for 4 minutes or until they were unable to maintain position on the treadmill despite humane encouragement. Horses were monitored to ensure a HR of >200 Beats/min (Bpm) was reached within the first minute of the protocol. Endoscopy videos were recorded continuously throughout the test. The change in phase was marked on videoendoscopic recordings by briefly visualizing the region of the pharyngeal recess for 1-2 seconds before returning to visualise the larynx. On completion of the test, horses were jogged and walked until their HR and RR normalized.

5.2.4 Pressure measurements during the CLE-test (Papers II and III)

The methodology described above for the CLE-test was followed (5.2.2). Once the laryngoscope was *in situ* in the nasopharynx, lidocaine (4%) was used to anesthetize the vocal folds and proximal trachea. This was administered via an Olympus Spray tip catheter (PW-6C-1) producing a mist of lidocaine. One ml was sprayed as the test-subjects expressed a long “e” (vocal folds closed) and one ml with the vocal folds abducted. Further doses were given as required, judged by the test-subject eliciting a glottic closing reflex when the catheter tip contacted the laryngeal inlet. Two pressure sensors (Mikro-Cath 825-0101, Milar, Houston, USA) were introduced through the work channel, the first positioned approximately at the fifth tracheal ring and the second at the tip of the epiglottis (Figure 10). The laryngoscope was fixed to the headset as were the sensors which were connected to a data acquisition box (Powerbox 8/35, ADIInstruments, Oxford, UK). The data were collected and stored on a MacBook Pro, Apple laptop using LabChart 8.0 software. Data acquisition was set at 40Hz. Maximum inspiratory pressure and inspiratory flow rate from the last 10 breaths of the CLE-test (when the CLE grade is subjectively evaluated in the standard CLE-test) were used to calculate trans-laryngeal resistance at this time point.

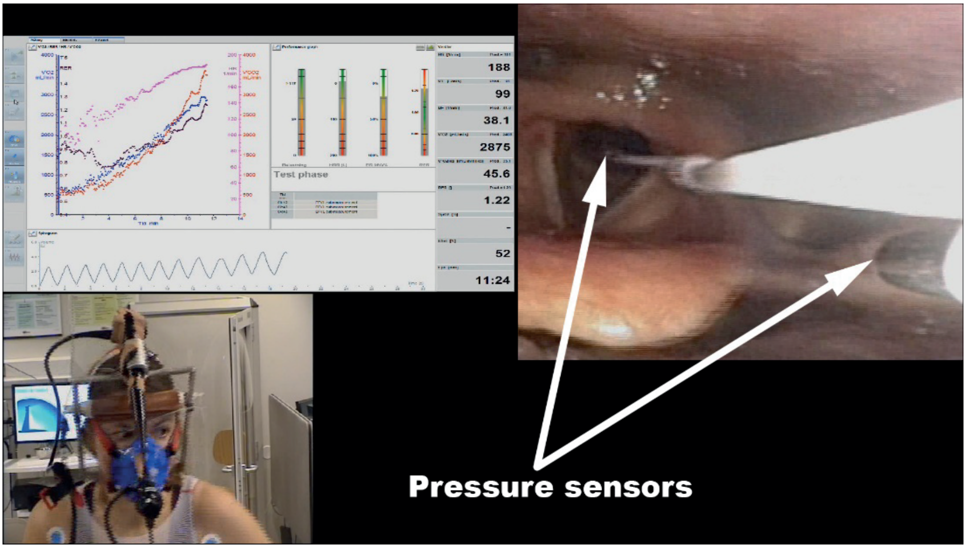

Figure 10. Screenshot from the monitor demonstrating the combined airflow data, endoscopy image (pressure sensors labelled) and video and sound recording. A program is being developed that will also include the pressure data stream.

5.2.5 Pressure measurements during the equine treadmill test

The above described protocol for the treadmill test (5.2.3) was followed to the point of placing the endoscope. The pressure sensor catheter was then passed through the right nostril ventral to the endoscope, to lie approximately 30cm into the cervical trachea and then secured to the external nares with a single suture. The catheter was housed within a protective 150cm polyethylene tube with 6 side holes starting 8 catheter diameters from the sealed tip and connected to a data acquisition box (Powerbox 8/35, AdiInstruments, Oxford, United Kingdom). Data were collected and stored on a MacBook Pro, Apple laptop (Apple Inc. Cupertino, Ca, USA) using LabChart 8.0 software. Data acquisition was set at 40Hz. The pressure transducer was calibrated using the inbuilt calibration system before each test. Tracheal pressures were recorded continuously throughout the test. Time was noted on the tracheal pressure measurements at the start of each phase. Maximum inspiratory and expiratory tracheal pressures were taken from 10 consecutive breaths during the last 15 seconds of each phase. The number of breaths per 10 seconds was noted from this time point.

5.2.6 The grading system for endoscopic imaging- human (Paper III)

Endoscopic video recordings were evaluated by 3 experienced clinicians with extensive experience of CLE testing and evaluation. The recordings were graded at the end of the CLE-test at the time interval where pressure data was collected. The Maat score [50] was used, grading supraglottic (aryepiglottic folds) and glottic (vocal folds) levels separately, grade 0 being no adduction, grade 1 mild adduction, grade 2 moderate and grade 3 severe adduction. The expression 0/2 denoted grade 0 glottic adduction and grade 2 supraglottic adduction. Grade 0/0 and 0/1 were considered normal (Figure 11).

5.2.7 The grading system for endoscopic imaging- equine (Paper IV)

Endoscopic video recordings were evaluated by two blinded diplomates of the European College of Veterinary Surgeons, with extensive experience of evaluating upper airway endoscopies. Recordings were viewed once all horses had been tested. Dynamic laryngeal collapse was graded numerically (0-3) by published grading scales [75], see Figure 11. Arytenoid cartilage collapse and vocal fold collapse were graded separately by consensus with 0 being normal position of the structure and 3 being marked adduction of the structures. The grade was determined by the most severe grade persistent for 10 seconds. The presence or absence of other URT abnormalities were also noted.

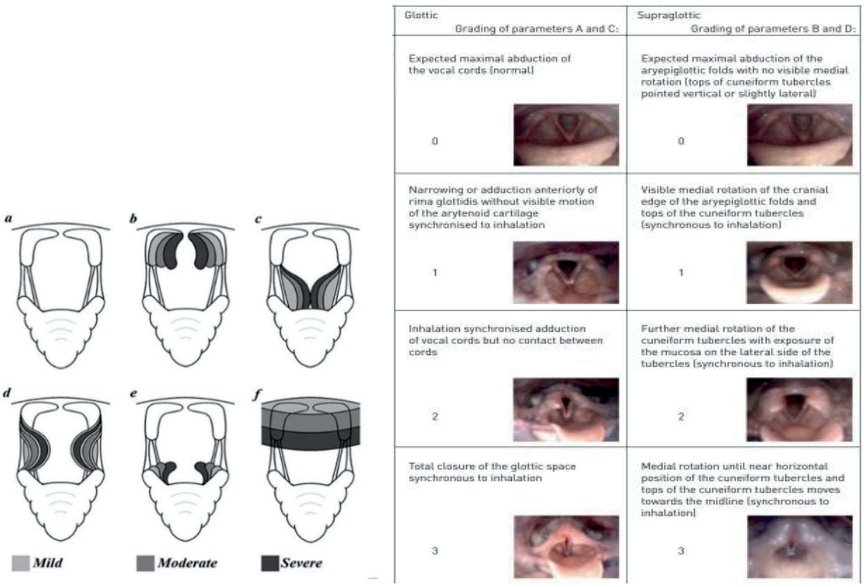

Figure 11. Subjective grading systems for equine and human laryngeal collapse. Left; the equine grading system. Schematic illustration of a) normal larynx, b) arytenoid cartilage collapse, c) vocal fold collapse d) medial deviation of aryepiglottic folds, e) collapse of margins of epiglottis, f) pharyngeal roof collapse. Light grey: mild, grey: moderate, dark grey: marked (75). Right; the human grading system illustrated by endoscopic photographic images during exercise from the larynx showing the different grades of laryngeal obstruction at the glottic and supraglottic levels (50).

5.2.8 Calculation of trans-laryngeal resistance (Papers II and III)

Trans-laryngeal resistance gives a direct objective measure of the resistance to breathing at the level of the larynx. Due to variation in respiratory frequency and V_T measuring trans-laryngeal pressure without flow data would not take into consideration these variations which directly affect pressure within the airway. Breath by breath variation in V_T necessitates that an average of 10 breaths is used in order to give a representative value for the individual. 10 consecutive representative (no swallow, speaking, cough that would affect the pressure value) breaths at the end of the CLE-test are identified. The time point at the start of the 10 breaths is identified and noted. In the CPET flow data time offsets are identified from the transcript at the start of the test. The time offset, if present is used to find the time corresponding to the pressure data time. The

10 consecutive inspiratory flow rates are then matched to the corresponding pressure value. Trans-laryngeal resistance is calculated by:

RL = (PT – PE) / AF

RL Laryngeal Resistance (cmH2O/l/s)
PT Maximum inspiratory tracheal pressure reading (cmH2O)
PE Maximum inspiratory pharyngeal pressure reading (cmH2O)
AF is airflow during inspiration in l/s as determined by breath by breath data from Jaeger CPX.

5.2.9 Tolerability scale human (Paper II)

Subject-reported Likert score of 1, no discomfort, to 5, intolerable (Figure 12), was used to determine tolerability of three aspects of the procedure: insertion of the scope, application of lidocaine to the laryngeal aperture and running the CLE-test with pressure sensors *in situ*. This gave a range of tolerability score from 3 (no discomfort in any aspect of the test) to a maximum score of 15 (all aspects intolerable).

Grade	1	2	3	4	5
Description of discomfort level	No discomfort	Mild discomfort	Moderate discomfort	Great discomfort	Intolerable discomfort

Figure 12. Likert score chart

5.2.10 Head position equine (Paper IV)

Head position was evaluated as similar poll flexion angle and head height were required in order for correct comparisons and data inclusion to be made between the snaffle bridle and Dr Cook Bitless bridle. Horses were filmed with a digital video camera placed at a fixed point to the left of the treadmill. White adhesive markers were placed on the left side by the bit, on the bridle by the ear base and on the withers. The angle between these markers and ratio of head to withers height over the treadmill bar were used to calculate poll flexion angle and head height (Figure 13). Three still images at 20, 30 and 40 seconds into each flexion phase of the test were obtained. The still frames were then used to determine an average poll flexion angle and head height ratio for that phase. Equivalent phases in the snaffle and Dr Cook Bitless bridle in each horse were then compared to determine that a similar poll flexion angle and head height were achieved in each bridle.

Figure 13. Placement of the markers and measurements made. Green lines show ratio of poll to withers height, calculated by dividing length of poll to treadmill bar height in mm by length of withers to treadmill bar height in mm. Red lines show poll flexion angle; the angle between the two red lines gives the angle of poll flexion in degrees.

5.2.11 Rein tension equine (Paper IV)

Rein tension was measured via inline "Super Samson" spring balances (Salters, FKA Brands Ltd), to ensure that approximately the same amount of rein tension was applied in both the Dr Cook Bitless and snaffle bit bridle. The amount of force (weight) applied to the right and left rein was measured at 20, 30 and 40 seconds into the flexion phase. The average of these three measurements determined the tension for that phase. No tension was applied to the reins during the free phases.

5.2.12 Statistical analysis of airway pressure readings

Repeatability of trans-laryngeal resistance (Paper III)

Data were reported as means with standard deviations (SD) and differences with 95 % confidence intervals (CI). The coefficient of repeatability (CR) for trans-laryngeal resistance defines the value below which the absolute difference between two replicate measurements is expected to be found with 95% probability [77, 78]. Briefly, the CR is calculated as follows: The variance of the two observations from each subject is calculated by determining the difference between the two measures, squaring the value, and dividing by two. The square root of this value gives the within subject standard deviation (S_w). The CR can then be calculated: CR= 2.77 x S_w and accounts for both random and systematic errors. Both CR and CR % (CR as a percentage of the pairwise mean) were reported. The coefficient of repeatability is directly related to 95% limits of agreement (LoA) [77]. A plot of the SDs of the mean differences between repeated tests were made to visualize the relationship between the repeated tests, where the 95% LoA between the two tests were expressed as +/- 1.96 SD of the differences [79]. One-sample t-test versus zero was used to examine for systematic bias between the two tests. The differences between tests were regressed on the average to test for proportional bias, i.e., whether the differences were influenced by the numerical magnitude of the measurement [80].

A preliminary normal range for trans-laryngeal resistance was calculated based on data from participants with normal laryngeal findings (defined here by CLE-scores 0/0-1) reported as the mean value with SD and 95 % CI. Stratified by gender, the Kendall's Tau-b correlations coefficient (r) was used to measure the association between the trans-laryngeal resistances across the CLE categories. Statistical significance was set at $p < 0.05$. Statistical calculations were performed using the statistical software SPSS version 25 (IBM SPPS Statistics, Armonk, NY, USA) and MedCalc version 19.5.3 (MedCalc Software Ltd, Osted, Belgium).

Use of airway pressure measurements as an objective outcome measure in equine clinical cases affected with DLC (Paper IV)

Summary statistics of tracheal inspiratory pressure (mean, SD, minimum and maximum values) were calculated for each horse, separated by bridle type and time period. Linear mixed model analysis with each horse as a random effect and a compound symmetry (exchangeable) correlation structure were fitted to assess the impact of bridle on tracheal inspiratory pressure. Two models were fitted; with bridle as categorical variable Snaffle free phase (S1), Snaffle flexion phase (S2), bitless free phase (B1) and bitless flexion phase (B2) and with the difference between free and flexion phase for snaffle and bitless as dichotomous variable (S1-S2 vs B1-B2). Intraclass correlation coefficients were calculated based on the variance estimates from the models to give an estimate of the level of clustering in the data. Models with and without the horse random effect were compared with likelihood ration tests (LRT). Assumptions for linear mixed models were evaluated.

Ethical approval

For horses all procedures were approved by the Faculty of Veterinary Science, NMBU in accordance with national legislation for ethical animal research. For humans all studies were approved by the Regional Committee on Medical Research Ethics of the Western Norway Health Region Authority (2017/636/REK vest**).**

5.3 Results

A summary of the main results is provided below. The full results can be found in Papers II-IV.

5.3.1 Treadmill tests with airway pressure measurements in humans and equines

All humans and horses completed all treadmill tests without adverse effects or injury. Collection of pressure data from 130/199 of equine treadmill cases that presented to the equine hospital, NMBU from February 2017 to December 2020 showed that including tracheal pressure measurements required minimal extra time and equipment (Table 1). Mild erythema and bleeding from the tracheal wall were commonly observed in those horses where bronchoalveolar lavage was performed 1-2 hours after the treadmill test. This did not appear to be of any consequence for any of the horses. The main reasons for failure of pressure data collection in horses were: the catheter tube being blocked by mucus (1 case); the horse breaking the catheter (4 cases); catheter irritation causing excessive coughing resulting in poor data (1 case); and unavailability of the author to carry out pressure measurement (63 cases). The pressure data collected from these client-owned clinical cases will be used in future studies when sufficient data has been collected for analysis.

	Total treadmill cases	Pressure data obtained	Pressure data not obtained	No data due to catheter blocked/ irritation	Catheter broke	Unavailability of staff to measure pressure
2017	66	21	45	2	4	39
2018	47	23	24	0	0	24
2019	46	46	0	0	0	0
2020	40	40	0	0	0	0
Total	199	130	69	2	4	63

Table 1. Summary data of all treadmill cases presented to the equine hospital, NMBU.

Paper IV, included nine NSCT, aged between four and seven years, including four mares, four stallions and one gelding. One horse failed to reach a HR of >200Bpm during the exercise test so was not eligible for inclusion in the study. Another horse failed to achieve similar degrees of poll flexion in the snaffle and bitless bridle thereby excluding it from the study. Only two horses completed all four phases during the two consecutive days of exercise testing. The remaining horses fatigued either during phase 3 (free head carriage) or 4 (poll flexion). Therefore, data analysis was performed on phase 1 (free head carriage) and phase 2 (poll flexion) for the remaining seven horses.

Pressure data was successfully recorded in all human subjects (Papers II and III). Flow data was successfully recorded in all human subjects allowing calculation of trans-laryngeal resistance. Five human subjects reported a sore throat and some increased secretions post testing, which resolved without treatment within a couple of days. All human subjects reached maximal exertion during the CLE-test as demonstrated by plateau of VO2 and HR and an RER of 1.2 or above. Five subjects failed to complete a second test within two weeks due to unrelated illness or time constraints. This prevented their inclusion in the repeatability study; however, their data could be included in the indicatory values part of the study. Of the 31 human subjects recruited for study III, 15 had no evidence of EILO. Sixteen subjects had various grades of supraglottic and combined glottic and supraglottic EILO like collapse witnessed endoscopically during testing.

	Female n = 18		Male n= 13	
Characteristics	mean	SD	mean	SD
Age, years	32.4	7.4	39.2	11.5
Weight, Kg	64.6	7.6	85.2	7.5
Height, cm	166.9	6.4	183.5	5.3
BMI, kg/m^2	23.2	2.5	25.3	2.0

Table 2. Descriptive characteristics of the 31 subjects participating in the repeatability study on trans-laryngeal resistance measurements during maximal treadmill exercise. Abbreviations: BMI: body mass index; SD: standard deviation

5.3.2 Tolerability and feasibility of measuring trans-laryngeal pressure in humans (Paper II)

Continuous measurement of trans-laryngeal pressure and airflow rate during a CLE-test is possible, feasible and tolerable, as determined by collection of data and subject reported Likert Score (Paper II). Pressure and flow data were collected throughout all tests in all individuals and was consistent and followed physiological trends (Figure 17). The major factors affecting tolerability were insertion of the laryngoscope through the nares and application of topical lidocaine to the laryngeal aperture. Both were improved with adjustments in technique, improving the average Likert score from 3 to 2.

5.3.3 Repeatability coefficient for trans-laryngeal resistance during maximal exercise in humans (Paper III)

The repeatability coefficient for trans-laryngeal resistance during strenuous treadmill exercise in this select population of adult humans (Paper III) was 0.62cmH2O/l/s. Assuming normal distribution it is expected that 95% of repeat measures will lie within +/- 0.62cmH2O/l/s of the preliminary value.

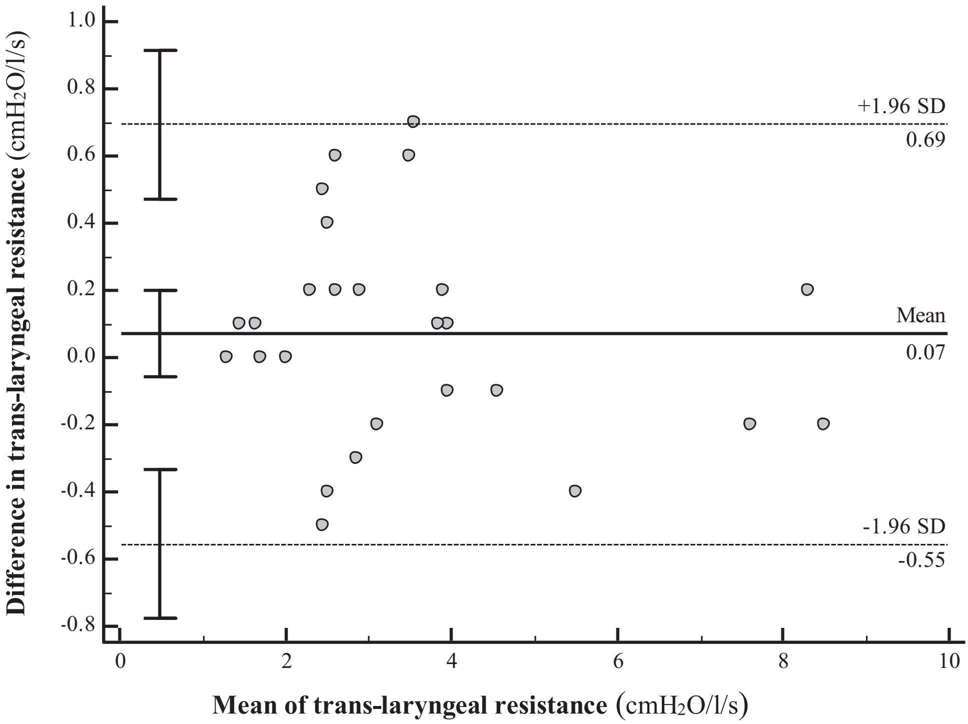

Figure 14. Agreement between laryngeal resistance obtained from 26 subjects examined twice while performing a maximal treadmill exercise test. The horizontal lines depict the mean difference between the laryngeal resistance obtained in test 1 and test 2, whereas +/- 1.96 standard deviations of this difference represent the 95% limits of agreement between the two tests. The 95 % confidence intervals for the mean, the upper limit of agreement and the lower limit of agreement are indicated by vertical lines.
The main difference was 0.07 cmH_2O/l/s, the upper limit of agreement was 0.69 cmH_2O/l/s and the lower limit of agreement was -0.55 cmH_2O/l/s.

5.3.4.1 Normal trans-laryngeal resistance values for adult humans at maximal exercise (Paper III)

The mean (SD) trans-laryngeal resistance in 6 females without videoendoscopic evidence of medialization of laryngeal structures during strenuous exercise was 2.88 (0.50) cmH2O/l/s. In nine males without videoendoscopic evidence of medialization of laryngeal structures during strenuous exercise this value was 2.18 (0.50) cmH2O/l/s (Figure 15).

5.3.4.2 Trans-laryngeal resistance values for adult females with visual evidence of EILO during maximal exercise (Paper III)

Five adult female subjects with combined glottic and supraglottic laryngeal obstruction during exercise had a mean trans-laryngeal resistance of 6.54 (1.79) cmH2O/l/s. Seven females with isolated supraglottic laryngeal obstruction during exercise had a mean trans-laryngeal resistance of 4.31 (1.44) cmH2O/l/s (Figure 15).

5.3.4.3 Trans-laryngeal resistance values for males with visual evidence of EILO during maximal exercise (Paper III)

Four male subjects with videoendoscopic evidence of combined glottic and supraglottic laryngeal obstruction during strenuous exercise had a mean trans-laryngeal resistance of 2.2 (0.61) cmH2O/l/s. (Figure 15)

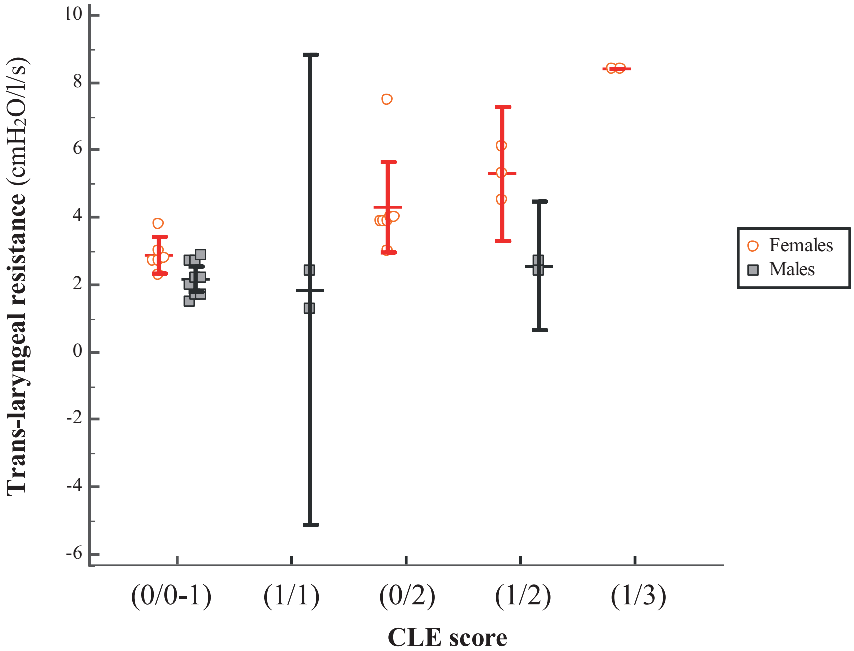

Figure 15. Trans-laryngeal resistance plotted against CLE score by sex. In females there is a correlation between increasing CLE score and increasing trans-laryngeal resistance.

5.3.4.4 Tracheal pressure readings in horses wearing a Dr Cook bitless bridle versus a snaffle bit bridle during strenuous exercise (Paper IV)

The change in mean inspiratory pressure between the free and flexion phases was significantly greater (P<0.001) in the bitless bridle (-15.2 +/- 12.3 cmH2O) than the snaffle bridle (-9.8 +/- 7.9cmH2O). Mean inspiratory pressure in the snaffle bridle (-32.3 +/- 6.3 cmH2O) in the free phase was significantly (P<0.001) more negative than in the bitless bridle (-28.5 +/-6.9 cmH2O). However, in the flexion phase there was no significant (P = 0.2) difference between the two bridles; snaffle (-42.1 +/- 10.8 cmH2O) and bitless bridle (-43.7 +/- 15.6 cmH2O). The change in inspiratory pressure between the free and flexion phases was greater in the bitless bridle due to the snaffle bit bridle having a more negative inspiratory pressure in the free phase, whilst both bridles had very similar inspiratory pressures in the flexion phase (Table 3).

Horse nr.		1	2	3	4	5	6	7	All horses
inspiratory pressure free phase	Snaffle bit bridle	-35.5 ±6.0	-35.8 ±3.6	-29.9 ±2.6	-41.7 ±3.6	-26.1 ±2.5	-25.7 ±2.2	-31.6 ±2.2	**-32.3±6.3**
	Bitless bridle	-31.6 ±3.6	-32.2 ±3.6	-29.0 ±2.8	-38.4 ±2.8	-28.3 ±3.1	-19.1 ±3.1	-21.0 ±2.2	**-28.5±6.9**
inspiratory pressure flexion phase	Snaffle bit bridle	-53.8 ±3.7	-45.7 ±2.3	-43.3 ±4.5	-50.9 ±10.1	-30.0 ±2.9	-25.8 ±2.0	-45.3 ±4.0	-42.1±10.8
	Bitless bridle	-47.4 ±2.6	-44.0 ±4.6	-43.9 ± 4.3	-73.8 ±9.8	-26.0 ±3.7	-26.9 ±2.7	-43.8 ±2.3	-43.7±15.6
Difference between inspiratory pressure in free and flexion for each type of bridle	Snaffle bit bridle	-18.3	-9.9	-13.4	-9.2	-3.9	-0.1	-13.7	**-9.8±7.9**
	Bitless bridle	-15.8	-11.8	-14.9	-35.4	+2.3	-7.8	-22.9	**-15.2±12.3**

Table 3. Summary of mean (± SD) inspiratory tracheal pressure in cmH_2O recorded in seven horses exercising on a treadmill with head position free and flexed and with two different bridle designs (snaffle bit and bitless). Bold text denotes values that are significantly different.

5.4 Discussion and future prospects

5.4.1 Materials and methods

Our experiences testing 130 equine clinical cases and using airway pressures as an objective outcome measure (Paper IV)

Recruiting horses during the study period was limited by repeated outbreaks of salmonella at the equine hospital during 2018 and 2019 resulting in hospital closure. The subsequent Covid-19 pandemic resulted in further challenges limiting the caseload which resulted in having to modify the aims for the equine part of the Thesis. These included establishing normal pressure ranges for active NSCT and STB racehorses, as well as classifying obstruction severity by pressure derangements. We were unable to obtain enough horses for each category. Data obtained from the 130 clinical cases will therefore be used in these ongoing projects. The study has however demonstrated that routine use of tracheal pressure measurements as a supplement to high-speed treadmill videoendoscopy provides objective data for diagnosis, treatment, and response to treatment. It has been suggested that in order to evaluate respiratory limitation on performance definitively it is necessary to measure URT mechanics: flow and or pressure [81]. This numerical data allows us to classify different URT obstructions viewed endoscopically as mild, moderate or severe; and as inspiratory, expiratory or combined in a definitive objective manner. The observations and experiences gained evaluating the equine cases were also essential to the development of the human pressure measurement protocol. The same type of sensors and recording equipment were utilized during a similar standardized treadmill test performed to fatigue on an incline.

Nine NSCTs were diagnosed with DLC during the study period, whose owners consented to participation in the study. This represented 4.5% of all cases presented for treadmill endoscopy during this period. A further 8 NSCTs were diagnosed with DLC during this time but the owners declined participation in the study, or the horse was not eligible for inclusion. To date research horses are most often used for studying upper airway mechanics during exercise [18, 42, 82, 83]. Paper IV used client owned active racehorses with naturally occurring URT obstruction which make the results more applicable to the population of horses seen in the clinical environment. This does however limit the invasiveness of techniques that can be employed and the availability of horses.

Tracheal pressure measurement is minimally invasive as well as giving direct objective information about the severity of the obstruction. Having flow measurements in equines as in the human studies would improve our data set and understanding of airway mechanics. However, flow masks in equines have been controversial as they may cause positive end-expiratory pressure as it is difficult to make a mask that handles the volumes of air moved in a respiratory cycle of a horse through the turbine without limiting flow [84]. Newer masks are reported to have addressed these issues [82].

Experience gained in the human studies in this Thesis would suggest that the combination of airflow and pressure to give resistance is the optimal outcome measure. A conclusion given some support in a review of equine performance testing [82]. There are a number of studies that use flow [85, 86], airway pressure [87] or endoscopy [7, 66] individually or a combination of two of these techniques [51, 88]. However, no studies up to now have combined all three in order to provide airway resistance data with visual assessment. Our human studies (Papers II and III) appear to be the first in mammals achieving this goal.

Resistance is a more reliable outcome measure because it takes account of changes in V_T and RR thereby giving a more accurate overview. When pressure is the sole measure it must be assumed that V_T and RR are constant, as pressure will change due to changes in the volume of air being moved. For example, if an obstruction occurs and an individual only breathes 70-80% of the V_T then the pressure will not be as greatly affected as less volume is being moved in the same time period. However, the individual will experience dyspnoea as adequate ventilation is not achieved and will quickly fatigue [89]. By measuring airflow and the pressure drop over the larynx an accurate measure of the effect of obstruction on ventilation can be evaluated. However, this also requires more equipment, expertise and time and is therefore thus perhaps best reserved for research use in equines or specialist exercise laboratories in human hospitals.

For routine examination in equines tracheal pressure provides a reliable outcome measure and allows for evaluation of the entire URT from the nares to trachea [82].

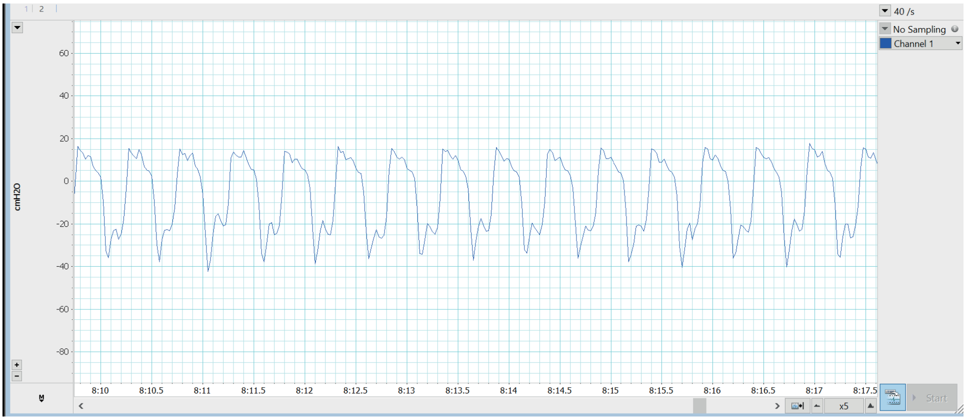

Figure 16. Example of tracheal pressure tracing from the end of the treadmill exercise test in a normal STB active racehorse in poll flexion. The maximum inspiratory and expiratory pressures from 10 consecutive breaths are used to give an average for each phase. Note the consistent breathing frequency and minimal variation in breath to breath pressures and tracing form.

As obligate nasal breathers equines are also subject to obstructions occurring within the nares and nasopharynx during strenuous exercise [89, 90]. Measurement of inspiratory and expiratory tracheal pressure in combination with high-speed treadmill endoscopy provides a precise numerical value (Figure 16) in combination with visual information as to whether airway collapse is present. It has been previously demonstrated that the presence of a 9mm endoscope does not affect airway pressure measurements in horses [42], so these can be used concurrently. We always use 10 consecutive breaths during the final 10-15 seconds of each one-minute phase to record mean inspiratory and expiratory pressure, along with RR. These values were for example precise enough to determine that mean inspiratory pressure (Paper IV) during the free phase was significantly ($p<0.001$) more negative with the snaffle bit bridle (-32.3±6.3cmH2O) versus the same horses wearing a Dr Cook Bitless bridle (-28.5±6.9cmH2O). In our experience, the RR stays fairly constant in horses during the exercise test until fatigue. It has also been previously demonstrated that elite harness racehorses do not demonstrate a significant effect of time on their inspiratory airway pressure values as the test progresses. In the same study, horses affected with DLC demonstrated a progressive worsening of inspiratory tracheal pressures over time until fatigue along with progressive laryngeal collapse [54]. This is a real-life example of the Bernoulli principle in respiratory physiology. After the induction of laryngeal collapse by poll flexion, the narrowing of the airway leads to increased local airflow velocity with resultant decrease in intraluminal pressures. This is a self-perpetuating process, which leads to rapid fatigue, especially when exercising on an incline as experienced by the horses in Paper IV.

Our experience testing humans, development of a protocol for measuring trans-laryngeal pressures in exercising humans (Papers II and III)

The study population was limited to the staff, students and associates of Haukeland University Hospital, Bergen University and the Western Norway University of Applied Sciences. This population is not representative of the population that typically presents at the EILO clinic, who are predominantly teenagers [27]. It was considered that as trans-laryngeal pressure measurements during exercise had never been performed in humans before that an adult population with a better understanding of the protocol and risks involved would be more ethically justifiable. Furthermore, obtaining medical ethics approval in Norway is easier in study populations of over 18 years of age than in younger individuals. For the initial tolerability study (Paper II), the subjects recruited were associated with the research group. This may introduce bias as all were familiar with the CLE-test and EILO and had an interest in a positive outcome of the study. This may have resulted in higher "tolerability" scoring than naive individuals may have given. However, anecdotally during the repeatability study subjects reported similar levels of tolerability.

In study III participants were asked if they had exercise-induced dyspnoea as part of the inclusion criteria. A surprising number (16/31) of the participants in this study had visual evidence of EILO, some with considerable medialisation of the supraglottic structures and mild glottic obstruction. All included subjects had reported themselves as having no history of exercise associated dyspnoea. Although the subjects in the study were not competing athletes and/or teenagers, the groups typically reporting performance limited by EILO, they did all reach maximal exertion as determined by RER and stable HR max. None reported EILO symptoms post testing or cited such symptoms as their reason for stopping. This raises several questions around normal and abnormal laryngeal function. Can some degree of medialisation of the structures be normal and not cause performance limiting obstruction? It should be noted that all subjects with a degree of laryngeal obstruction (grade greater than 0/1) had greater respiratory resistance than the normal individuals (grade 0/0 and 0/1). This has implications for equine practice. Equines with reported poor performance are routinely endoscopically examined, and any deviation from normal fully abducted structures is often considered abnormal and the potential cause of the poor performance. Perhaps some degree of obstruction can be normal and does not affect performance? However, these horses are elite athletes and even small deviations from full abduction may have significant effects on performance when all systems are working at their maximum limits [89]. In humans dyspnoea is a psychosomatic symptom, further complicating evaluation [91]. Frequently endoscopically observed collapse does not correspond to degree of reported dyspnoea. If an individual reports dyspnoea, or in the case of equines is referred for poor performance and an URT obstruction is observed, how can we be sure this observed obstruction is the cause of the dyspnoea or poor performance? This is the value of an objective measurement tool. Airway pressure readings will be able to tell us if the obstruction is increasing airway resistance and as such is the cause of the dyspnoea and that treatment will improve symptoms.

Measuring the pressure drop over the larynx has challenges in humans that are not present in equines. Humans have a hyper-reflexive larynx and increased sensitivity in the upper airways compared with equines which necessitates local anaesthesia of the nasal cavity, nasopharynx, and larynx. This poses some challenges both in terms of how to apply the local anaesthetic and if lidocaine affects airway function and thus test outcome. It was observed in both study II and III that the mucous membranes of the nasopharynx showed mild oedema and there were increased secretions in some individuals. Baier et al reported that application of topical lidocaine did not affect total airway resistance in humans [92]. However a study in equines found lidocaine to affect upper airway resistance, but predominantly by affecting the pharyngeal region [93]. As it is the pressure drop over the larynx that is measured in isolation in Papers II and III, pharyngeal pressure changes are unlikely to affect this pressure difference. However further investigation of this is warranted, especially as the Baier study was conducted at rest.

Other sources of error in the repeatability study could come from leaks from the facemask, especially around the modified stopper the laryngoscope was inserted through. Although a good fit is made at the start of the test the mask is stressed during the test by the weight of the laryngoscope through it. Furthermore, the laryngoscope had a working channel to insert the sensors through. This was sealed as best possible, but air may have “escaped” from here. As with all biological studies the particular form of the subject on the day of testing and learning from previous experience of the test in the repeat measure could affect outcome. However, there was no trend in the test one verses test two results (Paper III) as tested by regressing the average to test for proportional bias. These issues are similar in equines (Paper IV), which is why the bridle type run with first was alternated.

5.4.2 Humans and equines are comparable to a point (Paper I)

The systematic literature review demonstrated that equines and humans have a number of similarities in laryngeal anatomy, function, pathology and response to exercise. These similarities combined with the equine larynx being hypo-reflexive compared with the human larynx make the equine larynx easier to study and a good model for humans [19, 94]. Interestingly both the CLE-test for humans and treadmill test for equines along with the visual endoscopic grading scales have been developed independently but are very similar (Figure 11) [40, 47, 50]. However, both tests had lacking objective evaluation, the CLE-test has lacked an objective outcome measure and in equines an easy to use objective measure that allows routine clinical use has not been fully developed. This makes a compelling argument for comparing equines and humans.

It should be noted that equines do not have the sex differences in lung function and laryngeal size that humans have [10]. This is likely due to both male and female equines requiring a respiratory system that allows them to flee from predators with equal ability. Humans however have had differing gender roles in terms of hunting and other physical pursuits such as warfare, that may explain the difference seen between the sexes. Data from the Paper III study suggests that female humans have a greater trans-laryngeal resistance than males. This is in line with the sex differences in laryngeal inlet size and our understanding of the significant effect aperture diameter has on resistance to flow. In equine clinical cases and in the subjects recruited for Paper IV no such difference in tracheal pressure was noted between the sexes.

The population of equines studied during this thesis were highly trained athletes competing in professional races. The human subjects included individuals who train recreationally or compete at a non-profession level. This makes any comparisons between these groups in terms of performance difficult as they are not training or competing to similar levels. In future research it will be interesting to see if there are any differences between elite human athletes and recreational athletes in terms of laryngeal resistance values. Is it possible that athletes will have lower trans-laryngeal resistance resulting in a reduced work of breathing allowing for a competitive advantage? Elite NSCTs were not subject to increasingly negative tracheal pressure measurements over time, suggesting that their URT were able to maintain better airway patency at maximal exertion [54]. This impacts work of breathing and thus performance. Will we see a similar response in elite human athletes compared with recreational athletes?

5.4.3 CLE test with trans-laryngeal pressure measurements is feasible and tolerable (Paper II)

The tolerability study was the first time trans-laryngeal pressure had been measured in humans during exercise. It demonstrated that trans-laryngeal pressure could be continuously measured during a CLE-test. The pressure measurements were reliably recorded following trends in airflow and breathing frequency (Figure 17). There were no adverse events however, the procedure is invasive. The tolerability was acceptable with an average of 2 in Likert Score- mild discomfort. It would be preferable to improve the tolerability, especially since the patients the test is developed for are young teenagers and adults with breathing difficulties. When assessing the function of the larynx, as with other internal structures there is a challenge in minimising discomfort, and it is unlikely that all discomfort can be removed. When considering tolerability, the discomfort level has to be weighed up against the information obtained from the test. The objective data obtained by measuring trans-laryngeal pressure gives direct information about the resistance to airflow and thus the level of obstruction at the larynx. The need for an objective measurement of obstruction at the larynx has been a major research goal [8]. The importance of the larynx in dyspnoea both as an independent source of airflow obstruction or as a contributing factor to intrathoracic obstructions is becoming increasingly clear [31, 95-97]. Visual examination of the larynx during exercise or at rest does not give us information as to how much the larynx is contributing to airway resistance and thus dyspnoea. Directly measuring the variable you are interested in gives the most accurate information about its effect on the system.

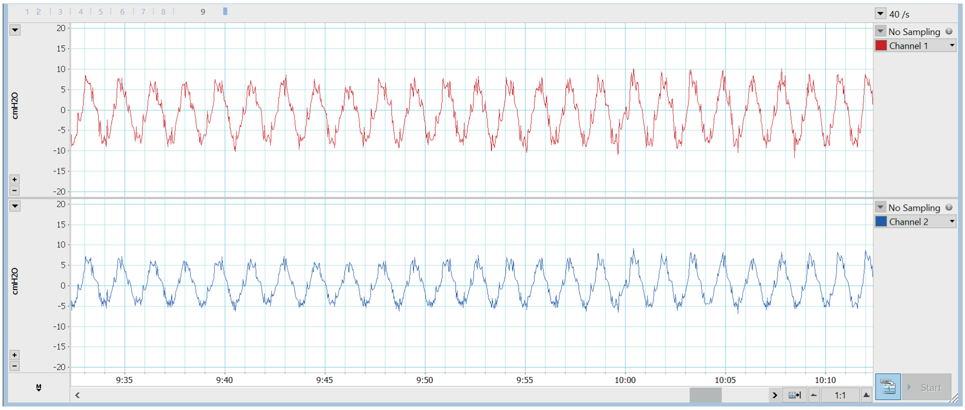

Figure 17. Example of pressure tracing from the end of a CLE test, the blue line depicting the epiglottic and the red line the tracheal pressure readings. At 40Hz maximum and minimum data are recorded, as seen by even consecutive peaks. There is minimal background interference as seen by tracings with minimal secondary displacements. The epiglottic and tracheal tracings are temporally aligned indicating that the tracings are well correlated.

The two main tolerability issues were the diameter of the laryngoscope causing nasal discomfort and application of lidocaine to the laryngeal aperture. Advancements in technology are allowing development of smaller diameter laryngoscopes. Alternatively, the use of a bronchoscope which has a smaller diameter may provide a solution to this tolerability issue, whilst still allowing insertion of the pressure sensors through the work channel. Application of topical lidocaine to the laryngeal aperture is essential to avoid laryngospasm. There are a number of described techniques for in office anaesthesia of larynx for ear, nose and throat specialists [98]. In this study we opted to spray the larynx with a fine mist via a catheter inserted through the work channel of the laryngoscope. This technique was considered to be relatively simple for the operators to use, compared with for example injection via the cricothyroid membrane. The technique allows for dose adjustment based on the observed individual response and required minimal extra equipment. However, the initial spray caused coughing and moderate discomfort, a Likert score 3 for some individuals. Future studies will test the effectiveness of nebulised lidocaine to assess if this technique can give adequate anaesthesia of the larynx, at a safe dosage, without affecting test performance.

The tolerability study revealed an interesting finding; that as exercise intensity increased individuals adopted differing breathing patterns. Some preferentially increased V_T, others breathing frequency. The ratio of inspiration to expiration time also varied more than expected between individuals. This highlighted the importance of calculating trans-laryngeal resistance where flow rate is taken into account. In Paper II minute ventilation was used to calculate flow and thus resistance. With the variations seen in inspiratory to expiratory ratio, V_T and breathing frequency it became evident that inspiratory flow data had to be matched breath for breath to pressure measurements. This method was used in the repeatability Paper (III) and will be used in future studies.

5.4.4 Indicatory normal values for trans-laryngeal resistance. Trans-laryngeal resistance is greater in subjects with visual evidence of EILO than normal subjects (Paper III)

Study III was the first study to report trans-laryngeal resistance during an incremental exercise test to maximal exertion in humans. The study produced indicatory normal ranges for healthy adult males and females, and for those with laryngeal obstruction comparable with EILO. Trans-laryngeal resistance in normal subjects was 33% greater in women (2.88+/-0.50cmH2O/l/s) than in men (2.18 +/-0.50cmH20/l/s). This finding is compatible with females having a narrower laryngeal aperture than males. Although females generally have lower minute ventilations than males, the radius of the aperture has the greatest influence on resistance through a tube. Halving of the radius of the tube increases resistance four-fold. Flow is however turbulent at maximum exercise, so the effect will not be as pronounced as this laminar flow equation would suggest, but it is still pronounced. Thus, the smaller diameter of the female larynx compared with the male likely results in the increased resistance measured in Paper III.

An important observation was that increasing grade of visual laryngeal obstruction is associated with greater laryngeal resistance. Had there been no association between trans-laryngeal resistance and degree of obstruction visualised, it would render trans-laryngeal resistance not useful as an outcome measure for the CLE-test. For females with isolated supraglottic obstruction the mean resistance was 4.31cmH2O/l/s whereas for those with a more severe combined glottic -supraglottic obstruction the mean resistance was 6.54cmH2O/l/s. These values were well outside the presumed normal range for the research subject group. This further supports the use of trans-laryngeal resistance as an outcome measure as it clearly differentiates between normal and affected individuals.

The gender difference observed in normal individuals appears to also be present in individuals with visual evidence of EILO. This could be expected as females have a narrower aperture to begin with and therefore greater resistance to airflow than male individuals. The effect of further loss of radius due to medialisation of laryngeal structure will have a greater effect on resistance. These are interesting observations. However, they are based on a small number of individuals, most notably there were only four males with a CLE grade concurrent with visual evidence of EILO. This likely explains why we could not identify a trend in males. Studies with larger cohorts are needed to confirm these findings.

5.4.5 There is good repeatability of the trans-laryngeal pressure measurements (Paper III)

In order for trans-laryngeal resistance to be a valid outcome measure it must be a reliable measure. Study III established that the repeatability co-efficient was 0.62cmH20/l/s indicating that for any given measure the repeat measure would be expected to lie within +/-0.62cmH2O/l/s assuming a normal distribution. Thus, when using trans-laryngeal resistance as an outcome measure a change greater than 0.62cmH2O/l/s is required to demonstrate a difference or effect of intervention. Trans-laryngeal resistance has a similar repeatability compared to other respiratory outcome measures such as static lung volumes and diffusion capacity [99, 100]. Having little internal variation is preferable as it allows for small changes caused by an intervention to be detected. Furthermore, the repeatability co-efficient was unrelated to the magnitude of measurement meaning that the relative reliability can be expected to increase with increasing resistance, making the test more useful in patients with EILO as their trans-laryngeal resistance is higher than normal individuals. The fact that trans-laryngeal resistance appears to be related to laryngeal aperture cross-section suggests that this value is mainly determined by laryngeal cross-sectional area and airflow dynamics. This lends support to the notion that dynamic URT collapse (EILO) in humans is determined by phenotype and airflow mechanics, as is the case in horses [54, 73]. Further studies are needed to confirm whether this is the case.

5.4.6 Using inspiratory airway pressure recordings as an objective outcome measure for an intervention in horses (Paper IV)

Bits have often been incriminated as a cause of URT obstruction in horses, and therefore may present an animal welfare issue [34, 101]. This was the first study to objectively evaluate the effect of a bit on inducing a specific equine URT disorder: DLC. This disorder is witnessed in susceptible horses when these individuals are being restrained on the bit and reins during training and racing. We were interested in determining the role of the action of the snaffle bit on the tongue and hyoid apparatus, and whether this was what caused DLC. Tongue position influences the relative position of the hyoid apparatus and larynx [102-104]. For example, tongue-ties which pull the tongue forward have long been used as a treatment for dorsal displacement of the soft palate in racehorses [105]. This study could not provide any clear evidence that the presence of a snaffle bit in the horse's mouth influences the development or severity of DLC. As with previous studies it was a change in head and neck position induced by rein tension that was the key event in provoking DLC [54, 106]. Poll flexion induced by rein tension caused an increase in tracheal inspiratory pressure and visible signs of laryngeal collapse on endoscopy whether the horse was outfitted with either the snaffle bit or Dr Cook Bitless bridle during testing. In free head carriage, without rein tension, the larynx appeared normal visually and inspiratory tracheal pressures were within normal limits.

An interesting finding in this study was that in free head carriage the snaffle bit caused a mild (-3cmH_2O), but significant obstruction to inspiration as measured by tracheal pressures. There has been longstanding controversy over the effect of the bit on upper airway mechanics and the bits role in inducing upper airway obstruction [34, 90, 101]. This finding was an interesting and unexpected result and warrants further investigation. We know that increasing negative pressures due to greatly increased airflows of exercise are responsible for inducing dynamic URT collapse in horses [107]. It could be hypothesised that although the bit may not be the inciting event in airway collapse, the increase it causes in inspiratory pressures increases the likelihood of collapse of susceptible structures. However, this needs to be further investigated in both normal horses and those with pathologies. This study had only 7 subjects, all of the same breed, all with DLC, so its findings can therefore not be generalised to other forms of dynamic airway collapse.

This study provides further support for the use of tracheal pressure measurements as a clinical diagnostic and research tool in equines and hopefully in the future for humans. Tracheal pressure was successfully measured in all the clinical cases included in Paper IV twice, as well as in the majority of clinical cases that presented to the equine hospital during the period of this Thesis. Meaningful objective numerical data that guided diagnosis and treatment were obtained. Using tracheal pressure measurement in routine clinical testing is safe, feasible, quick and provides an accurate numerical measure on the degree of inspiratory or expiratory obstruction present.

5.4.7 Future prospects

Having established a method for measuring the trans-laryngeal pressure difference over the larynx the future prospects are numerous. However as previously highlighted larger studies are needed to determine the resistance values associated with health and disease. Also, values in cohorts more relevant to EILO are needed. The larynx plays a central role in both upper and central airway pathologies. Until now it has not been possible to objectively evaluate the role the larynx plays in these pathologies. Before the test can be used on human patients some additional improvement in tolerability would be beneficial. Furthermore, understanding if topical lidocaine affects laryngeal function in humans will also be vital to being able to use this protocol in further research and as a clinical diagnostic tool as it has been in equine patients. Trans-laryngeal pressure measurements are included in work packages for the HelpILO project (https://www.helpilo.com/) that will investigate both EILO and the role the larynx plays in complex asthma and in failure/ poor tolerance of non-invasive ventilation.

An exciting prospect is the development of CFD models. Pressure readings from the airway during strenuous exercise are essential to the development of CFD models but have been lacking in human medicine. With the techniques developed in this Thesis this is now possible. Real pressure measurements and airflow data from an individual combined with three-dimensional imaging; computerised tomography (CT) are needed in order to test an as close to reality model as possible. Computational fluid dynamics models have already been produced for equines [11] and provide important clinical decision-making information [43]. Developing these CFD models further would be the first step in developing these for exercising humans and would allow doctors to test out irreversible treatments such as surgery before performing them on the patient.

For equines, establishing normal inspiratory and expiratory tracheal pressure values for the population of active harness racehorses presenting to the equine hospital, NMBU will be the next research priority. Further to this, we intend to categorise the different dynamic URT obstructive disorders using objective airway pressure measurements. Previously obstructions have been subjectively categorised in equine and human medicine by visual assessment and grading, which has resulted in controversy as to the severity of certain forms of obstruction. A numerical measure of the change in airway pressure will answer this question. The research group has recently acquired a large grant from the Norwegian-Swedish Equine Research Foundation for this follow-up project.

5.5 Implications of the study

This study has demonstrated that it is feasible and tolerable to measure the trans-laryngeal pressure drop during an incremental exercise test in humans. The test has good repeatability making it a suitable objective outcome measure for evaluating the upper airway. The understanding of upper airway flow dynamics is a priority of current respiratory research; this study presents a tool for achieving that. This is further supported by the equine part of the study where measuring tracheal pressure has provided an objective outcome measure in a clinical population of horses, helping to guide diagnostics, and answering a research question, whilst seemingly having no adverse effects on the horse.

The study also highlights the value of comparative research. An established objective outcome measure from equine veterinary research: airway pressure measurement, has been modified for use in humans. A reliable objective outcome measure for the CLE-test was the result. Data gathered from humans, where flow is easier to measure has highlighted the importance of V_T and RR changes on pressure, independent of the presence of airway obstruction, leading us to consider this problem in equines. The lack of a direct relationship between reported dyspnoea and degree of visual obstruction in humans also draws attention to not just assuming any degree of collapse observed in equines is responsible for their poor performance. Having a numerical value for the degree of airway obstruction present is of enormous value in treatment decision making and as a research tool. If the larynx is viewed as the entrance valve to the lower airways, future access to airway pressure data can become as important to respiratory medicine as transvalvular pressure gradients are in today's cardiology.

5.6 Conclusion

This Thesis has demonstrated important findings for both human and equine medicine and established airway pressure measurement as an objective tool for both human and equine research as well as exploring the possibility for its clinical use. It has been established via a systematic literature review that humans and equines have comparable upper airway structures and suffer similar dynamic obstructions of the laryngeal inlet resulting in exertional dyspnoea (Paper I). Tracheal pressure measurements have previously provided an objective outcome measure in equine research. In human research there was no objective outcome measure, this led to Paper II and III that demonstrate that trans-laryngeal pressure measurement is tolerable and feasible, yielding repeatable measurements. This lays the foundation for using airway pressure measurements as an outcome measure and to better understand upper airway dynamics in humans. The research suggests that women have higher trans-laryngeal resistance than men, and that individuals with visual evidence of EILO have higher trans-laryngeal resistance than normal individuals. The research also seems to demonstrate a direct cause and effect relationship between airflow and pressure parameters and dynamic upper respiratory collapse in humans, as in equines.

This Thesis has produced the first objective evaluation of the effect of the bit on upper airway mechanics in equines. Paper IV established that the bit had no clear effect on the development or severity of DLC. However, the bit appeared to cause a mild airway obstruction in these horses when in free head carriage (no rein tension). The presence of the bit in the horse's mouth may break the airtight seal otherwise created by the lips, which helps hold the soft palate in apposition to the dorsal aspect of the tongue. This needs to be further investigated.

6 References

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7 Papers

I Exercise Induced Laryngeal Obstruction in Humans and Equines. A comparative review

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Exercise Induced Laryngeal Obstruction in Humans and Equines. A Comparative Review

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Dynamic obstructions of the larynx are a set of disorders that occur during exercise in equines and humans. There are a number of similarities in presentation, diagnosis, pathophysiology and treatment. Both equines and humans present with exercise intolerance secondary to dyspnea. During laryngoscopy at rest, the larynx appears to function normally. Abnormalities are only revealed during laryngoscopy at exercise, seemingly triggered by increased ventilatory demands, and quickly resolve after cessation of exercise. Lower airway disease (asthma being the most prevalent condition), cardiac disease and lack of fitness are the major differentials in both species. Laryngoscopic examination during exercise should be performed from rest to peak exertion to allow for a comprehensive diagnosis, including where the airway collapse begins, and thereafter how it progresses. Dynamic disorders with most visual similarity between humans and equines are: aryepiglottic fold collapse (both species); equine dynamic laryngeal collapse (DLC) relative to some forms of human combined supraglottic/glottic collapse; and epiglottic retroversion (both species). Quantitative grading techniques, such as airway pressure measurement, that have proven effective in veterinary research are currently being piloted in human studies. Conditions that appear visually similar are treated in comparable ways. The similarities of anatomy and certain types of dynamic collapse would suggest that the equine larynx provides a good model for human upper respiratory tract obstruction during exercise. Thus, close collaboration between veterinarians and medical personal may lead to further advancements in understanding pathophysiologic processes, and enhance the development of improved diagnostic tests and treatments that will benefit both species.

Keywords: comparative medicine, Exercise Induced Laryngeal Obstruction (EILO), exercise laryngoscopy, exercise dyspnoea, larynx, equine upper airway disorders, dynamic laryngeal collapse

DYNAMIC OBSTRUCTIONS OF THE UPPER RESPIRATORY TRACT IN HUMANS AND EQUINES

Human medicine recently agreed upon the phrase Exercise Induced Laryngeal Obstruction (EILO) (Christensen et al., 2015; Halvorsen et al., 2017) to describe the phenomenon that in equines is usually referred to as dynamic obstruction of the upper respiratory tract (Lane et al., 2006a). These conditions represent comparable sets of disorders characterized by the larynx appearing normal at rest, with abnormalities seemingly induced by the increased ventilatory demands during ongoing exercise, and thereafter quickly resolving with cessation of exercise.

COMPARATIVE MEDICINE

Between animal and human and medicine there is no dividing line – nor should there be. The object is different but the experience obtained constitutes the basis of all medicine, Rudolf Virchow (1821–1902). Comparative medicine is the study of comparable diseases in different species; the similarities give the model its relevance, but the differences often provide the most informative insights. By studying pathologies that *co-exist* in humans and animals, we may uncover common denominators of disease and identify new therapies, while at the same time reducing animal welfare concerns. Disease is not inflicted on these animals; instead they may in fact benefit from early access to new diagnostics, treatments and preventive techniques.

WHY INVOLVE EQUINES IN THE STUDY OF EILO IN HUMANS?

Equines and humans are the predominant species that compete at maximal exertion and additionally have documented laryngeal abnormalities associated with exercising at high intensity. As such, their upper respiratory tracts are subject to similar strong aerodynamic stresses, potentially also providing a common platform for research and understanding. Dynamic upper respiratory tract obstructions and their treatment are far better described in equines than in humans, suggesting a potential for added benefit for humans. This article compares current knowledge on presentation, diagnostics and treatment of this phenomenon, aiming to uncover common features that might lead to better treatments in both species.

SEARCH STRATEGY

Guidelines set out by Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) where used for guidance. The Cochrane Database, Ovid Medline, PubMed, and Web of Science where searched using three search domains for equine disease and four domains for human disease. Each search domain was combined using "AND" and within each domain combined with "OR." The first domain for equine disease included, "Dynamic," "maximal exercise," "treadmill exercise," and "poor performance." Second domain, "Upper respiratory tract," "soft palate," "DDSP," "epiglottis," "laryngeal," "aryepiglottic fold," "dynamic laryngeal collapse (DLC)," "arytenoid," "upper airway," and "vocal fold." Third domain "collapse," "disease," "obstruction," "stridor," and "respiratory noise." For human, "Exercise," "exertional," "maximal exercise," "exercised-induced," "athletes," "episodic," "treadmill," "high-intensity exercise," "paradoxical," and "functional." Second domain, "induced," "inducible," "stimulated," and "provoked." Third domain, "laryngeal," "vocal cord," "vocal fold," "larynx," "glottis," "upper respiratory tract," "laryngomalacia," "soft palate," and "upper airway." Fourth domain, "obstruction," "dysfunction," "dyspnoea," "stridor," "obstruction," "inspiratory," "laryngeal dyskinesis," "asphyxia," "upper airway obstruction," and "vocal fold dysfunction." Last search date was 27/12/2018. Reference lists from included studies were checked for other studies and Ph.D. thesis on dynamic collapse from NMBU regarding equines and Bergen University/Haukeland University Hospital regarding humans were used. The search results were first scanned by title and inappropriate articles removed. Abstracts of remaining articles were read for relevance, and those articles most appropriate to poor performance and dyspnea due to laryngeal obstruction at exercise in equines and humans were read fully. The information obtained from reading these articles was then used to collate a comparative review of laryngeal induced dyspnea at exercise in equines and humans.

CLINICAL PRESENTATION

In both equines and humans, the common presenting complaint is reduced exercise tolerance occurring secondary to dyspnea, which is typically characterized by inspiratory stridor that worsens as exercise intensity increases and resolves within a few minutes of cessation of exercise (Newman et al., 1995; Beard, 1996; Rundell and Spiering, 2003; Lane et al., 2006a). In humans, panic reactions occasionally occur as a response to breathlessness (Roksund et al., 2009). Anecdotally, corresponding "stress and avoidance behaviors" have been reported in equines, associated with occasions of dyspnea at racetracks.

In humans, first time presentation of EILO is most frequent in active youngsters, with those partaking in competitive sport being overrepresented (Roksund et al., 2009; Nielsen et al., 2013). Symptoms might initially be vaguely described; however, on closer questioning some very typical features will be revealed. The inspiratory phase of the breathing cycle being more affected than the expiratory phase, and symptoms are at their worst when ventilation requirements are at their most intense, with symptoms typically abating as ventilation decreases – unless panic occurs. This pattern clearly contrasts the expiratory dyspnea of Exercise Induced Bronchoconstriction (EIB) that peaks after cessation of exercise. Nevertheless, these conditions are too frequently confused, often with unfortunate consequences (Chiang et al., 2008; Roksund et al., 2017). However, care must be taken when interpreting self-reported symptoms, as many patients find it hard to attribute symptoms to a particular phase of respiration, and a number of studies suggest that self-reported symptoms are a poor prediction of EIB and EILO (Nielsen et al., 2013; Johansson et al., 2014).

Similarly, symptoms of dynamic obstruction of the upper respiratory tract in equines often present around 2–3 years of age, as performance expectations increase (Lane et al., 2006a). Trainers report the horse not exerting itself as fully as before, abnormal respiratory noise at higher exercise intensities, and poorer race performance in terms of placings and earnings (Beard, 1996). Again, upon closer questioning, trainers and jockeys can often time the respiratory noise to inspiration (Lane et al., 2006a). Abnormal breathing patterns may occur, with uncoupling of the typical locomotor respiratory coupling mechanism (LRC), which in galloping horses is 1:1 (Fitzharris et al., 2015). Horses with respiratory tract disease have been shown to adopt a 2:1 pattern when galloping, taking 1 breath over 2 strides (Fitzharris et al., 2015). This may reflect "breathlessness" and an effort to regulate pathological dyspnea. By increasing the time over which a breath is inhaled, flow rate and pressures within the airway can be reduced, thereby exerting less stress on the upper airway structures (Fitzharris et al., 2015). No such adaptations have been reported in humans with EILO, although change in timing of the respiratory cycle is an area of research interest in this patient group.

DIAGNOSIS

Dynamic obstructions by definition require the upper airways to appear normal at rest, and that abnormal function is revealed during ongoing exercise. Thus, visualization of the larynx *during* exercise is required for a diagnosis in both species, allowing the clinicians to determine which structures are implicated, as well as when and in what sequence they become involved (Morris and Seeherman, 1991; Lane et al., 2006b; Roksund et al., 2017).

In equines treadmill endoscopy protocols have been in use for more than three decades (Morris and Seeherman, 1991). All apply the same principles of running the horse at the trot or gallop, until symptoms are seen or to fatigue, with an appropriately positioned endoscope in place during the entire test (Lane et al., 2006a). Most protocols include a method to determine the level of exertion, such as a heart rate monitor, ECG or gas flow analysis. Some centers include periods of free head carriage and periods of poll flexion to mimic real race or riding conditions, as recent articles report that head position can induce or aggravate many disorders (Strand et al., 2012). Pressure readings from the trachea and pharynx have become important tools at University clinics, to allow objective, precise measurement of the degree of inspiratory and/or expiratory airflow obstruction (Nielan et al., 1992; Ducharme et al., 1994). This has allowed the development of computational flow dynamics models, and consequently a better understanding of pathological processes and treatment planning (Rakesh et al., 2008a).

During the last decade overground endoscopes with GPS tracking have been developed, which allows the videoendoscopic test to be performed in the field (Franklin et al., 2008). Although overground endoscopy cannot be standardized as a treadmill test can, it does allow testing in the same environment in which symptoms occur. This allows evaluation of cases that are not suitable for, cannot come to the treadmill or do not get symptoms during treadmill testing.

The major differentials and co-morbidities for exertional dyspnea in the equine are lower airway problems, cardiac disease and poor fitness. Bronchoalveolar lavage is routinely performed as part of a respiratory performance workup to determine lower airway inflammatory disease status. ECG recordings during treadmill testing allow evaluation of any arrhythmias that may be present. It should be noted, however, that arrhythmias are commonly reported in racehorses and their clinical significance varies (Jose-Cunilleras et al., 2006; Allen et al., 2016).

In humans the continuous laryngoscopy exercise (CLE) test was similarly developed to study the visual presentation of the larynx during ongoing exercise in patients complaining of symptoms that on close questioning appeared to originate in the upper airways. The CLE test as described by Heimdal et al. (2006), is a complete incremental cardiopulmonary exercise test; including tidal flow volume loops, breath-by-breath gas analyses, ECG, video of the upper torso and head, and sound recordings, combined with an appropriately positioned endoscope in place for the entire test, with all data synced into one single file to allow storage and subsequent analyses (Heimdal et al., 2006). A number of modes of exercise can be used, but the CLE test is most often performed on a treadmill or an ergometer cycle controlled via software to produce a standardized exercise test. As with equine patients, replicating real life conditions where symptoms occur is useful and as such, laryngoscopy during rowing, stair climbing and even swimming have been described (Panchasara et al., 2015; Walsted et al., 2017). The benefit of this is the recreation of the conditions in which symptoms occur; the disadvantage is that these modes of exercise are harder to standardize, which poses a problem when evaluating effects from interventions or when conducting research.

As with equine patients, it is fundamental to view the larynx throughout the complete exercise test and recovery. This allows identification of the structures that are implicated, when and in what sequence they become involved and as such, a full visual representation on how the situation evolves; information that collectively is critical for appropriate treatment. For example, failing to observe excessive supraglottic tissue collapse prior to a glottic obstruction may result in patients receiving conservative treatment that may not be efficient, excessive supraglottic tissue obstruction is usually better treated by surgery (although the glottic component also needs to be addressed). Likewise in equines, failure to diagnose aryepiglottic fold collapse (MDAF) being secondary to failure of full arytenoid abduction and thus only treating the MDAF, will not result in full clinical improvement and return to performance. Thus, for both equines and humans, video laryngoscopy performed throughout an exercise test is by definition required to make a definitive diagnosis, and also imperative to institute appropriate treatment, although so far only in equines are these images supplemented by objective airway pressure measurements.

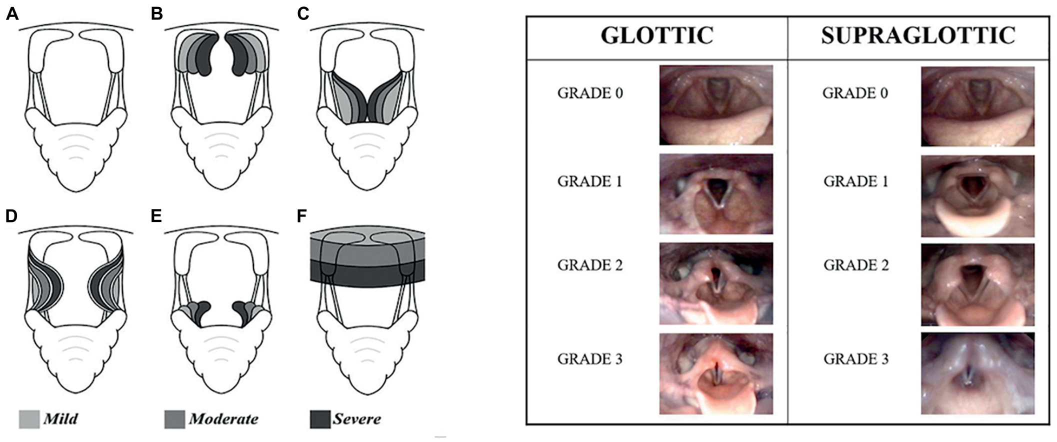

FIGURE 1 | (Left) The equine grading system. Schematic illustration of **(A)** normal larynx, **(B)** arytenoid cartilage collapse, **(C)** vocal fold collapse, **(D)** medial deviation of aryepiglottic folds, **(E)** collapse of margins of epiglottis, and **(F)** pharyngeal roof collapse. Light gray: mild, gray: moderate, and dark gray: marked. With permission from K. Olstad. (Right) The human grading system according to Maat et al. (2009) illustrated by endoscopic photographic images during exercise from the larynx showing the different grades of laryngeal obstruction at the glottic and supraglottic levels.

GRADING OF SEVERITY OF OBSTRUCTION

Grading systems for the degree of obstruction caused by luminal collapse or medialization of the various structures in the upper airway (mainly the larynx), have been developed independently for both humans (Maat et al., 2009) and equines, and are surprisingly similar. These systems are to a large extent based on subjective visual assessments (**Figure 1**).

In equines, tracheal pressure measurements, first described by Nielan et al. (1992), have provided an objective measure for research for three decades (Nielan et al., 1992). Veterinary experiences with equine dynamic upper airway obstructions are currently guiding the development of better diagnostic tools for EILO in humans. The larynx functions as the entrance valve to the lower airway. Thus, techniques for tracheal and pharyngeal pressure measurements throughout a full CLE test in order to determine the normal and excessive pressure drops during high intensity exercise over this so vitally important valve, have been requested in a recent literature review (Halvorsen et al., 2017). Encouraged by the successes obtained by veterinarians in objectively quantifying obstructions in equines (Ducharme et al., 1994; Rehder et al., 1995; Holcombe et al., 1999), it is hoped that a better understanding of these pressure relationships will facilitate development of similar computer-based simulation models for humans as for horses. The first translaryngeal pressure measurements in humans during an exercise test have recently been performed, and proved to be feasible and tolerable as an objective outcome measure for obstruction of ventilation over the larynx (Fretheim-Kelly et al., 2019). Thus, application of equine veterinary research and practice has great potential to aid the understanding of the pathophysiology and treatment of EILO, and thereby contribute to the provision of quantifiable data that can be used to guide therapy.

PATHOPHYSIOLOGY UNDERLYING DYNAMIC UPPER AIRWAY COLLAPSE

The inciting cause of collapse varies and has not been determined for humans. In equines, a number of forms of dynamic collapse seem to be due to anatomic/functional phenotypes for which certain breeds seem predisposed (Woodie et al., 2005; Strand et al., 2012). However, all result in an initial local narrowing of the airway, which with further exercise often worsens. These pressure/flow/size relations are described mathematically by the Bernoulli principle and Hagen–Poiseuille's formula. In simple terms, the narrower the lumen of a tube, the greater the velocity of the same volume of air compared with a tube of greater diameter, this in turn results in more negative transmural pressures. Thus, any structure that reduces the upper airway diameter will result in an increase in airflow velocity past this point, thereby increasing the lumen "collapsing pressures." Susceptible structures are drawn into the airway, further reducing the lumen diameter and exacerbating the cycle (Rakesh et al., 2008b; Strand et al., 2009).

SPECIFIC PATHOLOGIES

In humans, laryngeal obstruction is divided into supraglottic (structures rostral to the vocal folds) and glottic (at the level of the vocal folds) (Christensen et al., 2015). There is no such division in the equine. In the following paragraphs, each specific structure that is subject to collapse and thus potentially can obstruct the airflow, will be described and the treatment options discussed. Although each structure is described separately, most upper airway collapse involves multiple structures, this is termed "complex" airway collapse (Tan et al., 2005; Lane et al., 2006a; Strand and Skjerve, 2012).

SUPRAGLOTTIC COLLAPSE (HUMAN) VERSUS MEDIAL (AXIAL) DEVIATION OF THE ARYEPIGLOTTIC FOLDS (MDAF) (EQUINE)

The aryepiglottic folds (AEF) are found both in humans and equines, and are membranous tissues that run from the epiglottis to the arytenoid cartilages, serving a protective function during swallowing (Negus, 1937). In both equines and humans they are supported by the corniculate cartilages and in humans and some equines by the cuneiform cartilages (Negus, 1937; King et al., 2001). In equines a number of mechanisms have been suggested to lead to MDAF; e.g., failure of full abduction of the arytenoids, lifting of the epiglottis, a structural weakness of the tissue, an excess of fold tissue or stretching of the fold tissue secondary to other upper airway obstructions, resulting in laxity (Franklin, 2008). The result is displacement of the folds axially (medially) during inspiration, as the lax tissue cannot withstand the increasingly negative pressure created by the increasing airflow induced by the increased ventilation of exercise (King et al., 2001). This is supported by research using computer modeling, that shows that the AEF contralateral to the unilateral laryngeal collapse is subject to more negative airway pressures compared with horses free from pathology (Rakesh et al., 2008a). Histological examination has shown that collapsing AEFs have focal inflammation and edema similar to that seen in cases of laryngomalacia in humans (McCluskie et al., 2006). It has not been established if these histological changes are causative or a result of trauma induced by the airflow during collapse (McCluskie et al., 2006). In humans with EILO, there is virtually no knowledge on basic pathophysiology; however, the aerodynamic principles described in horses are one of the main causal theories currently under investigation (Halvorsen et al., 2017). The larynx is protected from inspiratory collapse by a rigid cartilage skeleton, particularly the cricoid cartilage, and by muscular actions that provide support for the glottis and supraglottic structures. Reidenbach (1998) suggests that a possible reason for the inward collapse of the AEF may be insufficient anchorage to the cartilage skeleton of the larynx. A flaccid, edematous, swollen and/or superfluous mucosa of the arytenoids and of the AEF may contribute by disturbing the airflow, thereby inducing change from laminar to turbulent flow at an earlier stage. A retroflex or omega-shaped epiglottis may contribute in a similar way or represent an obstruction by itself (Belmont and Grundfast, 1984). Airway pressure readings in humans with EILO may provide an objective answer to this, as they have done in horses. Other theories include laryngeal hypersensitivity, neurological reflex arc alterations, and psychological stresses; however, currently all theories lack supporting evidence. Given the complex functions of the larynx, it is likely that the pathophysiology of EILO is multifactorial, and that the etiology varies between individuals, as seems to be the case in horses (Tan et al., 2005). It seems likely that an anatomical or physiological anomaly predisposes the AEFs to collapse at the increasing negative intraluminal pressures induced by the greater airflow of exercise. This theory finds some support by findings in equines of significant associations between severity of deviation of the AEF and increasing number of upper airway abnormalities detected (Tan et al., 2005; Strand and Skjerve, 2012). **Figure 2** shows the endoscopic view of MDAF and supraglottic EILO.

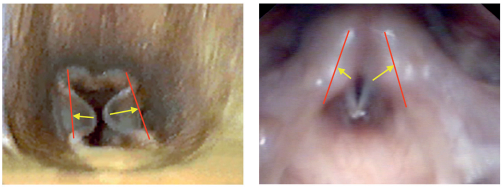

FIGURE 2 | Endoscopic photographic images during exercise of: **(left)** marked medial deviation of the aryepiglottic folds in an equine. **(Right)** Grade 3 supraglottic EILO in a human. Red lines denote expected line of aryepiglottic fold in a normal subject. Yellow arrows denote degree of collapse to normal.

Treatment of MDAF is in principle similar in horses and in the corresponding scenario of supraglottic collapse in humans; i.e., mild cases in horses are treated with rest and incremental return to training (King et al., 2001). In humans, mild cases are offered information, biofeedback and breathing control techniques. Cases with more severe collapse are offered biofeedback, or surgical removal of excess aryepiglottic tissue in appropriate highly motivated patients (Maat et al., 2007). In equines, surgery is the primary treatment option for moderate to severe cases (King et al., 2001). Transendoscopic laser excision of the aryepiglottic fold in horses is done under standing sedation and local anesthetic, the excessive membranous AEF tissue is either unilaterally or bilaterally removed with laser excision (King et al., 2001). In humans there is no international consensus on surgical techniques, at the authors' institution the supraglottoplasty is performed under general anesthesia; releasing incisions to the AEF and removal of excessive tissue around the cuneiform cartilages with laser or microlaryngeal scissors (Maat et al., 2011). For both equines and humans, surgical treatment is reported to have a better subjective outcome compared with conservative treatment (King et al., 2001; Maat et al., 2011). Patients and trainers report a faster return to training and competition and a greater improvement in symptoms. In both equines and humans, it should be noted that conservative treatment is more time consuming, and requires greater efforts and compliance (King et al., 2001; Maat et al., 2011). Importantly, the effect of placebo after surgery should not be underestimated, and controlled trials utilizing sham treatment are certainly warranted (Maat et al., 2011).

LEFT LARYNGEAL NEUROPATHY

In equines and humans' failure of full abduction of the arytenoid cartilage may occur unilaterally or bilaterally; however, only unilateral failure will be reviewed here, as bilateral failure is uncommon and results in dyspnea at rest and is as such not a dynamic condition which is the focus of this review (Lane et al., 2006b).

The most common equine cause of left sided failure of arytenoid abduction is the condition "recurrent laryngeal neuropathy," caused by distal neuronal axonopathy of the recurrent laryngeal nerve (Dixon et al., 2001). In more advanced cases, this failure of abduction may be seen during resting laryngoscopy, which makes it a non-dynamic condition. It is important to view the larynx during exercise in "early" cases, as the grade of collapse can improve as well as worsen during exercise, due to variations in the recruitment of muscle fibers with corresponding variations of the clinical course (Dixon et al., 2001; Lane et al., 2006a).

In humans, damage to the left recurrent laryngeal nerve during surgery for patent ductus arteriosus, mediastinal, and head and neck surgery is a recognized surgical complication and the leading cause of left sided laryngeal hemiplegia (Røksund et al., 2010; Butskiy et al., 2015; Siu et al., 2016). This condition corresponds with equine "recurrent laryngeal neuropathy" grade 4 (end stage), as this implies complete disruption of motor innervation to the posterior cricoarytenoid (PCA) muscle (**Figure 3**). In equines, most notably thoroughbreds, this is a severe performance limiting upper airway inspiratory obstruction that requires surgical intervention to restore racing performance (Beard and Waxman, 2007). The surgery most commonly used is the "tie back" with ventriculocordectomy (Beard and Waxman, 2007). This fixates the left arytenoid in an abducted position with removal of one or both vocal folds and laryngeal ventricules. Work with computational fluid dynamics models has shown the optimal degree of abduction is 88% of maximal glottic cross sectional area to allow maximal ventilation without aspiration (Rakesh et al., 2008a). This results in a mild permanent narrowing of the airway, which leads to an increase in the air velocity through this position of the airway, causing a disproportionately greater negative pressure past the arytenoid which pulls the ipsilateral vocal fold into the airway (Rakesh et al., 2008a). Removal of the ipsilateral vocal fold prevents this secondary collapse. Other treatments that have been used include other surgical techniques for permanent fixation or removal of the left arytenoid (Williams et al., 1990), and more recently re-innervation and electrostimulation of the dorsal PCA (Fulton et al., 2003), which have proven successful and returns the function of the arytenoid to normal. Unfortunately, re-innervation takes too long in racing horses to presently be a viable treatment. Furthermore, early detection of denervation before muscle loss is fundamental to return to function in this treatment model (Fulton et al., 2003).

In humans, the aim of treatment is different to that in equines. The priority of the treatment is to decrease the risk of aspiration and to improve vocalization (Butskiy et al., 2015), as opposed to achieving maximal ventilatory capacity without aspiration. Consequently, the aim of treatment is to fix the vocal fold in a relatively adducted position. There are four major approaches to achieve this; injection laryngoplasty, thyroplasty, arytenoid adduction, and reinnervation of the thyroarytenoid muscle (Butskiy et al., 2015; Siu et al., 2016). All four approaches are considered to provide equally good outcomes (Butskiy et al., 2015; Siu et al., 2016). However, these outcomes are necessarily based on the treatment aims, as such, the significant airway obstruction that results from medialization of the vocal fold, especially during increased activity, is not considered. A treatment such as re-innervation of the PCA muscle, that has been successfully applied in equines and restored near normal function, could be a possible future option in humans. Based on what has been learnt from equine research, and that re-innervation has already been used successfully in bilateral vocal fold paralysis in humans, this technique shows future promise for hemiplegia patients with viable PCA muscle (Li et al., 2013).

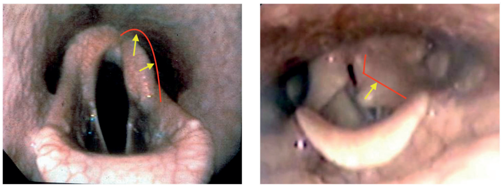

FIGURE 3 | Endoscopic photographic images during exercise of: **(left)** left-sided recurrent laryngeal neuropathy in an equine. **(Right)** Left laryngeal hemiplegia in a human. Red lines denote expected position of arytenoid cartilage in a normal subject. Yellow arrows denote degree of collapse to normal.

Other types of malpositioning of the arytenoids that result in airway obstruction include medial luxation of one arytenoid apex under the other (Dart et al., 2005). In equines this is termed Ventromedial Arytenoid Displacement (VMAD) (Dart et al., 2005). This is hypothesized to be due to the transverse arytenoid muscle not being able to support the axial aspect of the arytenoid (Barakzai et al., 2007). One study of VMAD noted post-mortem that the transverse arytenoid ligament was excessively wide. It is clear that further research is needed to determine the pathophysiology of this disorder (Barakzai et al., 2007).

In humans, scissoring of the corniculate cartilages has been anecdotally described, seemingly with a similar visual appearance to VMAD. VMAD results in obstruction of airflow through the most posterior part of the glottic opening. Computational flow dynamics models have shown that the main course of airflow is through the posterior portion of the glottis, above the vocal process (Rakesh et al., 2008a; Frank-Ito et al., 2015). So, although malpositioning of the arytenoids does not visually appear to represent a significant obstruction, it may indeed prove to be, as it obstructs the main channel of airflow. Further research is needed to confirm or refute this in clinical cases. Airway pressure measurements are currently being utilized to evaluate all types and degrees of upper respiratory tract obstruction in equines, including

VMAD; hopefully providing information that will aid our understanding of the significance of dynamically malpositioned arytenoids also in humans.

VOCAL CORD DYSFUNCTION IN HUMANS AND EQUINES – COMBINED SUPRAGLOTTIC AND GLOTTIC COLLAPSE IN HUMANS VERSUS DYNAMIC LARYNGEAL COLLAPSE IN EQUINES

Vocal cord dysfunction (VCD) is a controversial human diagnosis, and for the purpose of this discussion we will consider it only in response to exercise as a trigger. In equines, vocal fold collapse in absence of collapse of other structures is uncommon but has been described and experimental studies have shown that cricothyroid muscle dysfunction due to superior laryngeal nerve damage is the underlying mechanism (Holcombe et al., 2006). This results in a lack of tension on the vocal folds, which results in a passive drawing into adduction when the ventilatory volume increases. In humans, VCD may occur as the only or the primary obstruction but more commonly occurs secondary to supraglottic (i.e., aryepiglottic fold) collapse (Roksund et al., 2009; Roksund et al., 2017), giving a visual impression similar to what is labeled DLC in equines (**Figure 4**).

Dynamic laryngeal collapse occurs during poll flexion (flexion of the head relative to the neck) and results in passive collapse of the vocal folds, arytenoid cartilages, and in many cases the AEF (Strand et al., 2009). The underlying mechanism seems related to phenotype since innervation to the larynx and associated muscles is normal (Fjordbakk et al., 2015). The clinical phenotype is an equine with a rostral positioned larynx relative to the mandible accompanied by a narrow intermandibular space (Fjordbakk et al., 2013). This results in the larynx being compressed by the hyoid apparatus during head/neck flexion, preventing full abduction of the arytenoid cartilages during exercise (Fjordbakk et al., 2013).

In both humans and equines, VCD and DLC cause inspiratory obstruction at the glottic level, frequently causing near complete obstruction of the glottis, characterized by a loud stridor (Tan et al., 2005). Glottic obstruction appears to cause human patients the most distress, and cases associated with panic attacks often have a glottic component (Leo and Konakanchi, 1999). A noteworthy possible difference between glottic collapse in equines versus humans, is that the vocal folds are drawn in passively in equines, due to lack of maintenance of arytenoid abduction (Holcombe et al., 2006; Fjordbakk et al., 2008). Contrary, in humans, active contraction of the vocal folds is the most common cause of glottic obstruction (Leo and Konakanchi, 1999). In humans, treatment is aimed at regaining normal control of the vocal folds and preventing inappropriate contraction during inspiration. This can be achieved by biofeedback, speech therapy, inspiratory muscle training (IMT) and in some refractory cases botox (Roksund et al., 2017). In horses, biofeedback and speech therapy are not possible due to the required need to communicate the techniques to the patient. IMT is currently in the initial stages of trialing. The most common treatment in equines has been surgical resection of the vocal folds (Beard and Waxman, 2007). This removes some of the obstructing tissue with tolerable side effects, as domesticated equines do not rely on vocal communication (Beard and Waxman, 2007). However, in cases of DLC it has not proven effective, indicating that the arytenoid cartilage collapse is the main cause of obstruction (Fjordbakk et al., 2008).

EPIGLOTTIC RETROFLEXION (RETROVERSION)

Epiglottal retroflexion (ERF) arises when the apex of the epiglottis retroflexes and thereby covers the rima glottis, causing an obstruction of the entrance to the larynx (**Figure 5**) (Ahern, 2013; Roksund et al., 2017). A collapse of margins of epiglottis can also occur in equines. This condition is rare in humans and uncommon in equines (Tan et al., 2005; Ahern, 2013). In humans, it is often associated with an epiglottis that is omega shaped or with a high resting position and can be seen as a form of supraglottic collapse. The range of retroflexion varies from an epiglottis that fails the anterior rotation normally seen as exercise intensity increases, thereby disrupting airflow, to the epiglottis completely retroflexing into the rima glottis. In equines, the

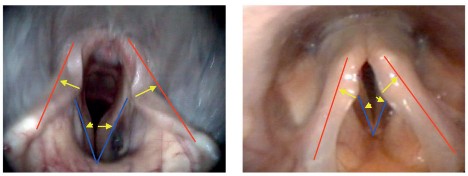

FIGURE 4 | Endoscopic photographic images during exercise of: **(left)** moderate dynamic laryngeal collapse in an equine. **(Right)** Grade 3 combined supraglottic and glottic EILO in a human. Red lines denote expected line of aryepiglottic fold in a normal subject. Blue lines denote expected line of vocal fold in normal subject. Yellow arrows denote degree of collapse to normal.

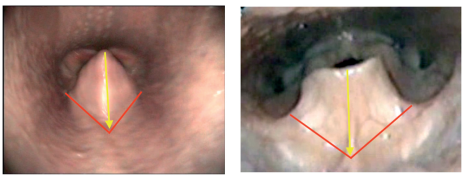

FIGURE 5 | Endoscopic photographic images during exercise of: **(left)** epiglottic retroversion in an equine. **(Right)** Epiglottic retroversion in a human. Red lines denote expected margins of epiglottis in a normal subject. Yellow arrows denote degree of collapse to normal.

condition has been associated with neuromuscular dysfunction of the hyoid musculature and with damage to the hypoglossal nerve, and consequently hypoepiglotticus muscle paralysis or dysfunction (Ahern, 2013). In a clinical commentary by Ahern (2013), the most common cause of epiglottic retroflexion in equines was trauma to the hypoglossal nerve as a complication of previous upper respiratory tract surgery, other causes included idiopathic and upper respiratory tract infection.

Treatment is based on preventing or limiting retroflexion of the epiglottis into the rima glottis. In humans, the epiglottis is sutured to the tongue base (epiglottopexy). This is not possible in equines due to the conformation of the soft palate in this species. In equines, injection of polytetrafluoroethylene into the epiglottic base, "subepiglottic augmentation," has been described and was successful in one case (Tulleners and Hamir, 1991). Another described technique is injection of substances to induce friction in the articulation of the epiglottis with the larynx, but this technique has not been critically evaluated in published clinical trials. In both equines and humans, resection of a portion of the apex of the epiglottis can also be performed to reduce the level of obstruction (Ahern, 2013). Currently, prognosis for treatment in both species is unknown, as there are too few cases to compare outcomes (Ahern, 2013).

CONCLUSION

Equines and humans are the predominant species that compete at speed and as such are susceptible to airway disorders that are induced at high exercise intensities. As this review demonstrates, dynamic obstructions of the larynx are a common cause of exertional dyspnea and reduced exercise performance in both humans and equines. The underlying anatomy, physiology and the visual appearance of a number of types of dynamic collapse are seemingly comparable in the two species. As such, the equine may provide a good model for human disease, advancing our understanding of pathophysiology and treatment in humans, while allowing equines "early access" to potential future cutting-edge diagnostics and treatment developed in human medicine.

AUTHOR CONTRIBUTIONS

ZF-K and TH involved in searching, writing, and compiling the manuscript. ZF-K, TH, OR, J-HH, and ES provided concept of the study and funding. HC, MV, CF, ZF-K, TH, J-HH, ES, and OR involved in reviewing, editing, and writing of the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

II Feasibility and tolerability of measuring trans-laryngeal pressure during exercise

The Laryngoscope

Feasibility and Tolerability of Measuring Translaryngeal Pressure During Exercise

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Objectives/Hypothesis: To determine if simultaneous tracheal and supraglottic pressure measurement performed during a continuous laryngoscopy exercise (CLE) test is possible, tolerable, and feasible, and if so, whether measurements can be used to determined airflow resistance over the larynx, thus providing an objective outcome measure for the CLE test, the gold standard for diagnosing exercise-induced laryngeal obstruction.

Study Design: Explorative descriptive clinical study.

Methods: A CLE test was performed with the addition of two pressure sensors (Mikro-Cath 825-0101; Millar, Houston, TX) placed at the epiglottic tip and at the fifth tracheal ring. To place sensors, laryngeal anesthesia and a channel scope were required. Tolerability and feasibility was determined by a Likert score and subjective indication from subjects and operators. Adjustments to the technique were made to increase tolerability. The pressure data were continuously collected and analyzed for artifacts, drifts, frequency response, and used with flow data to calculate translaryngeal resistance.

Results: All subjects (n = 7) completed all procedures. Two main areas of concern were identified regarding tolerability: application of topical anesthesia to the larynx and nasal discomfort due to the added diameter of the laryngoscope. Protocol adjustments improved both. Pressure data were obtained from all procedures in all subjects, were consistent, and followed physiological trends.

Conclusions: Continuous measurement of the translaryngeal pressure gradient during a CLE test is possible, feasible, and tolerable. A CLE test with direct measurement of the translaryngeal pressure gradient might become a valuable tool in the objective assessment of respiratory function, and normal values should be established in health and disease.

Key Words: Translaryngeal resistance, exercise test, exercise-induced laryngeal obstruction, exertional dyspnea.

Level of Evidence: NA

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INTRODUCTION

Exercise-induced dyspnea is a common patient complaint. Symptoms are sometimes due to poorly controlled asthma and arise from obstruction of the intrathoracic airways, a condition labelled exercise-induced bronchoconstriction (EIB).[1] Alternatively, symptoms may arise from obstructions of extrathoracic airways, most often involving the laryngeal structures and if so labelled exercise-induced laryngeal obstruction (EILO).[2] Despite EILO being a common disease, with a prevalence of 5% to 7% in the general adolescent population,[3,4] and as high as one in three in predisposed groups,[5,6] our understanding of the role played by the larynx during exercise in health and disease is at an early stage, with large knowledge gaps.

Airway scientists have recently agreed on a standardized way to establish an EILO diagnosis, based on visual images obtained from continuous laryngoscopy performed during ongoing exercise (continuous laryngoscopy exercise [CLE] test).[7,8] Grading systems are based on relative changes in laryngeal aperture size from rest to peak exertion during the CLE test. These verified grading systems all involve subjective decision making, and their reproducibility has been questioned.[9] Objective outcome measures are needed to disentangle diagnostic confusions between EILO, EIB, and asthma,[10,11] and to improve clinical decision making in relation to treatment of EILO, especially for cases where irreversible surgery is being considered.

Upper respiratory tract obstruction is also a common problem in exercising horses, causing poor performance

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and dyspnea as in humans, tracheal pressure readings are being used to substantiate observations.[12,13] In veterinary medicine, pressure readings have informed surgical decision making and provided objective outcome measures for research for decades.[14,15]

We hypothesized that measuring airway pressures during exercise in humans will inform our understanding of upper airway mechanics, clinical decision making, and provide an objective outcome measure. Pressure measurements in the upper airway have only been made in humans at rest.[16] The aim of this study was therefore to address if tracheal pressure measurement performed during a CLE test is possible, feasible, and tolerable, and if it can be used to determine airflow resistance over the larynx in exercising humans.

MATERIALS AND METHODS

Study Design and Subjects

We performed an explorative, descriptive clinical study to develop a feasible and tolerable test protocol for measuring translaryngeal pressure gradients in exercising humans. Test subjects (n = 7) were recruited from the pediatric and ear, nose, and throat departments staff of Haukeland University Hospital, Bergen, Norway, completing 11 CLE tests. Participants had unknown EILO status, but all were familiar with the CLE test. No subject was examined within 2 weeks of a respiratory tract infection. A general physical examination, including height and weight, was performed.

The study was approved by the Regional Committee on Medical Research Ethics of the Western Norway Health Region Authority (2017/636/REK vest).

Lung Function Measurements

Baseline lung function parameters were determined by a spirometer (JAEGER Vyntus; CareFusion, Höchberg, Germany) in accordance with guidelines of the European Respiratory Society,[17] recording forced vital capacity and forced expiratory volume in the first second.

Preparations for Pressure Recordings and CLE Test Protocol

A 12-lead portable electrocardiograph device was attached to the subject. Nostrils and nasal cavity were anesthetized with 4% lidocaine. An endoscopic video camera system (Visera, CLV-S40; Olympus, Tokyo, Japan) was connected to a fiberoptic laryngoscope (ENF-V2; Olympus) in a sterile plastic cover with work channel, which was advanced through a hole in a modified facemask (Hans Rudolph, Inc., Kansas City, MO) through the nasal cavity to the oropharynx. Lidocaine (4%) was used to anesthetize the vocal folds and proximal trachea by a dripping technique through the work channel. The laryngoscope was fixed to the headset. Two pressure sensors (Mikro-Cath 825-0101; Milar, Houston, TX) were introduced through the work channel. The first was positioned approximately at the first tracheal ring. The second was positioned at the epiglottis tip. The sensors were secured to the headset and connected to a data-acquisition box (Powerbox 8/35; ADInstruments, Oxford, United Kingdom), and data were collected and stored on a MacBook Pro laptop (Apple Inc., Cupertino, CA) using LabChart 8.0 software (ADInstruments). Data acquisition was set at 40 Hz. A video camera and microphone were placed in front of the subject to document external images and sounds, and the ergospirometry unit was attached to the facemask.

The Maximum Voluntary Ventilation Maneuver and the CLE Test

The CLE test, including spirometry and maximum voluntary ventilation (MMV) procedure, were performed as described previously,[8] with the pressure transducers as an added element. Gas exchange parameters were recorded using the breath-by-breath method. Subjects ran on a treadmill (Ergo ELG70; Woodway, Weil am Rhein, Germany), to individual experience of exhaustion using a modified Bruce protocol with 90-second incremental intensity steps. Gas exchange variables were recorded (JAEGER CPX; Vyntus, Hochberg, Germany).

Collection of Pressure Data and Calculation of Translaryngeal Resistance

Pressures were continuously measured. Pressure traces were visually evaluated for evidence of interference. Maximum inspiratory translaryngeal resistance was determined during the MVV maneuver, walk-to-run transition, and at exhaustion (the last 10 seconds of CLE test). An average of 10 consecutive breaths at these time points were noted. Translaryngeal resistance was calculated by the following equation: [$R_L = P_T - P_E / AF$] where R_L is laryngeal resistance (cm H_2O/Ls^{-1}), P_T is tracheal pressure reading (cm H_2O), P_E is epiglottic pressure reading (cm H_2O), and AF is airflow in Ls^{-1} as determined by minute ventilation divided by 60, then multiplied by inspiratory ratio. Data validation was addressed by assessing the relationships between pressure data curves and flow curves, artifacts, drifts, and frequency responses. Tolerability was determined by subjective reporting from subjects during and after the test, and by Likert score 1 to 5 (see Supporting Information, Appendix 1, in the online version of this article) and observation of the laryngeal mucosa posttesting for signs of irritation. Feasibility was determined by subjective reporting from the operators and time taken to perform the test compared to a standard CLE-test.

Revisions of the Test Setup

After four test subjects had been examined, the setup was adjusted. Two percent topical lidocaine was used to anesthetize the vocal fold area, administered via an Olympus spray-tip catheter (PW-6C-1; Olympus) producing a mist of lidocaine instead of droplets. One milliliter was sprayed as the test subjects expressed a long /e/ (closed vocal folds) and 1 mL with the vocal folds abducted. Further doses were given as required, judged by the test subject eliciting a glottic closing reflex or not when the sensors tip came into contact with the laryngeal inlet.

The original laryngoscope requiring use of a sterile plastic cover with work channel was exchanged for a laryngoscope with a built-in work channel (ENF P3; Olympus). Nasal spray (xylometazoline 0.5 mg/mL hydrochloride) was introduced to reduce nasal cavity edema, allowing easier insertion of the laryngoscope.

The tracheal pressure sensor was advanced to approximately the fifth tracheal ring, minimizing the risk of accidental displacement into a supraglottic position, and ensuring the sensor be placed below any laryngeal jet effect.

The first four test subjects then repeated the test protocol according to these adjustments, so that all seven test subjects performed their examinations according to this revised protocol.

RESULTS

Seven subjects (Table I) completed 11 tests, with four subjects running both the primary and revised protocols. Data were collected from all subjects in all tests;

TABLE I.
Demographics of the Test Subjects.

Subject No.	Age, yr	Sex	FEV_1	Previously Performed a Standard CLE Test
1	61	Male	4.24	Yes
2	58	Male	5.02	Yes
3	40	Female	3.17	Yes
4	43	Female	3.00	No
5	41	Male	4.62	No
6	59	Male	4.11	No
7	56	Male	4.00	Yes

CLE = continuous laryngoscopy exercise; FEV_1 = forced expiratory flow in first second.

however, three of the primary protocol tests had issues with pressure catheter positioning or test performance leading to unreliable data. Subjects 2 and 3 did not run to exhaustion, and thus their CLE test data (but not their pressure data per se) where unreliable. Subject 4's tracheal catheter came out of position during MVV, determined by the pressure data not being consistent with the catheter being in a tracheal position.

Tolerability

There were variations in reported degree of discomfort (Table II), with two main areas of concern: insertion of the laryngoscope with a plastic cover and application of topical lidocaine to the laryngeal aperture. The subjects' feedback from the first setup of tests (n = 4) informed protocol revisions, after which tolerability improved, and all test subjects completed all planned examinations thereafter. The listed protocol amendments improved the average total Likert scale score from 2.4 to 1.7. Nasal discomfort during insertion of the laryngoscope and application of topical lidocaine to the laryngeal aperture area nevertheless remained the main causes of discomfort. Greatest improvement to tolerability was made by misting the lidocaine through a spray catheter instead of dripping, reducing Likert scale scores by one point in all individuals. Changing from a scope with plastic cover to a scope with a built-in work channel improved the average Likert scale score by 0.75. Having the tracheal pressure sensor positioned at the fifth tracheal ring did not cause any more discomfort than having the sensor at the more rostral position.

TABLE II.
Subjective Discomfort While Performing the Test.

Subject No.	Protocol 1	Protocol 2
1	3/3/1	2/2/1
2	2/3/1	2/2/1
3	3/3/2	2/2/1
4	3/3/2	2/2/1

Likert scores (1 = no discomfort to 5 = intolerable) addressing subjective discomfort (only four subjects completed both the original and the revised protocols). The first number indicates insertion of scope; the second number the application of lidocaine to the laryngeal aperture; and the third number denotes running with the continuous laryngoscopy exercise test with sensors in situ.

Feasibility

Feasibility in terms of timing improved rapidly with team experience, and testing was running relatively smoothly after having tested six subjects. The additional time required compared to a standard CLE test was approximately 10 minutes and linked to topically anesthetizing the larynx and sensor placement. Pressure measuring equipment requires minimal additional setup. The test can be performed with one doctor and one assistant as is protocol for the CLE test today; however, involving a third person makes testing easier. Using a scope with a built-in work channel improved feasibility, as it was easier to maneuver the scope to the correct position for application of topical lidocaine and to guide correct placement of pressure sensors.

Validation

Data acquisition at 40 Hz produced pressure curves with minimal interference while ensuring maximum and minimum pressures were recorded (Fig. 1). Pressure readings became more negative during inspiration and more positive during expiration, as airflow volumes increased, closely following the flow curves. Pressure measurements obtained from the sensors placed at the epiglottic and tracheal regions were temporally aligned (Fig. 1). There was no sign of temperature drift or damping of the pressure curves. Pressure readings obtained throughout the exercise tests from two different days from the same test subject were similar (Fig. 2). Having the tracheal pressure sensor at the first tracheal ring made it vulnerable to supraglottic displacement, especially at high airflow volumes, and advancing the sensor to the fifth tracheal ring prevented this.

DISCUSSION

This study has demonstrated that translaryngeal pressure measurements can be obtained while performing a standard CLE test in motivated, well-informed adult individuals. Added nasal discomfort due to the larger laryngoscope and the anesthetizing procedure of the laryngeal aperture and upper tracheal area constituted the main tolerability concerns. Pressure measurements could reliably be recorded throughout the maximal exercise test when the tracheal sensor was positioned at the fifth tracheal ring. Readings corresponded well to changes seen in airflow and rate of breathing, with no signs of drift, damping, or interference at 40 Hz acquisition. No adverse events occurred, and all participants completed all procedures. Overall setup required only minor extra equipment and time to complete, as compared to a standard CLE test. We consider the described setup to be tolerable and feasible for research, but its reproducibility and validity needs to be confirmed in properly designed studies before it can be applied in a clinical context.

Tolerability and Feasibility

Tolerability was predominantly affected by the laryngoscope diameter causing nasal discomfort on

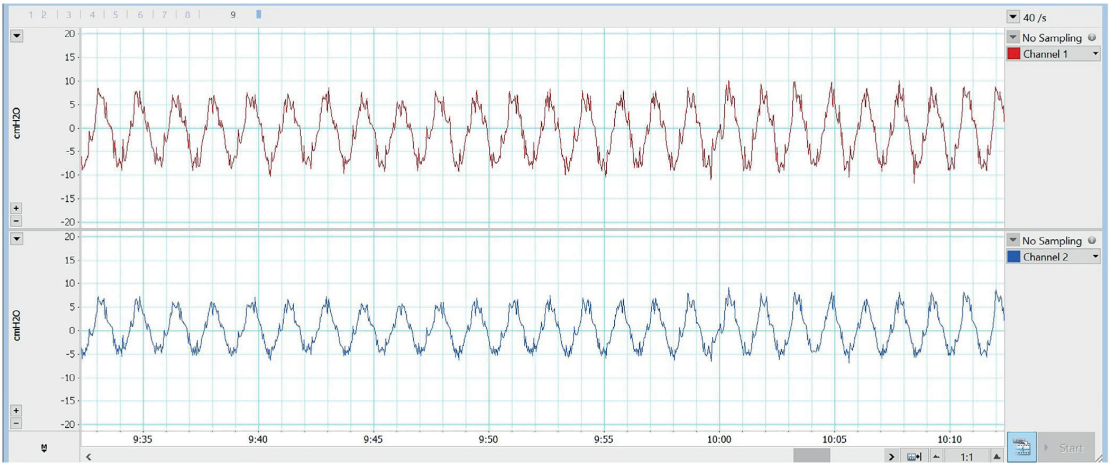

Fig. 1. Example of pressure tracing from the end of a continuous laryngoscopy exercise test, with the blue line depicting the epiglottic and the red line the tracheal pressure readings. At 40 Hz, maximum and minimum data are recorded, as seen by the even consecutive peaks. There is minimal background interference as seen by tracings with minimal secondary displacements. The epiglottic and tracheal tracings are temporally aligned, indicating that the tracings are well correlated. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

insertion and by application of topical anesthesia to the laryngeal aperture. Modification to the original methodology improved tolerability of both concerns. Application of topical lidocaine to the laryngeal aperture was reported to be the most unpleasant part of the procedure by all participants. Altering the strength from 4% to 2% and misting as opposed to dripping improved tolerability. Further improvement may be achieved by a lidocaine nebulization, which has been extensively described in the literature for awake bronchoscopy and intubation.[18,19] Injection through the cricothyroid membrane is described for in-office laryngeal proceedures[20] and has been reported as providing better patient comfort than lidocaine nebulization for awake intubation.[21] Future testing will determine if these methods improve tolerability for tracheal pressure sensor placement without interfering with the very object of the procedure. A further benefit of the revised protocol was a more subject tailored approach with less lidocaine being used, leading to less stimulation of mucosal secretion, better visualization of the larynx, fewer swallow episodes and less subject discomfort.

A laryngoscope with a work channel reduces its diameter and facilitates introduction through the nasal cavity and improves control over the placing of the scope and the introduction of the sensors. However, the use of a channel scope increases both labor and costs, as cleaning between uses becomes significantly more laborious and costly than if using a scope in a protective sleeve. However, in most hospital environments the equipment and expertise needed to clean and store channel scopes is readily available.

Validity

40 Hz acquisition rate was chosen based on personal experience, published literature regarding equines, and resting pressure measurements in humans.[13,16] This acquisition rate ensured that maximum and minimum pressures were recorded, which may not have occurred at lower rates, while still keeping artifacts from probe movement and background noise at a low level. There was no evidence of damping by mucus or increasing humidity. The pressure traces obtained formed curves with consistent maximum and minimum readings over a period of unchanged breathing as would occur if only ventilation affected pressure. No drift from baseline with time suggests that significant temperature drift did not occur. The manufacturer (Millar Inc.) reports a ± 1 cm H_2O drift with temperatures between 25°C and 40°C, but such drifts did not occur here as airway temperature was relatively stable within the controlled laboratory environment and in the subjects' upper airways over the short testing time. Movement artifacts were minimal, and there were no obvious erroneous readings or outliers, except those that could be accounted for by speaking, coughing, and swallowing. This contrasts findings made at rest by Baier et al.,[16] who reported catheter whip, where the catheter made contact with the airway wall causing false pressure readings. Catheter whip may have been prevented in this study by the pressure sensors being supported in the work channel. In the Baier et al. study, the catheter was placed unsupported through the nostril opposite to the scope. In equines, whose airway pressures are much greater, the catheter is protected in a special plastic cover. Such protection was not deemed necessary in this study, as it seemed unlikely that the catheter would be exposed to the same magnitude of pressure change.

A concern with this methodology is that application of topical lidocaine might affect larynx function during exercise. Baier et al.[16] reported that topical lidocaine did not alter total respiratory system resistance. However, topical anesthesia of the equine airway has been reported to influence upper airway pressure measurements during exercise,

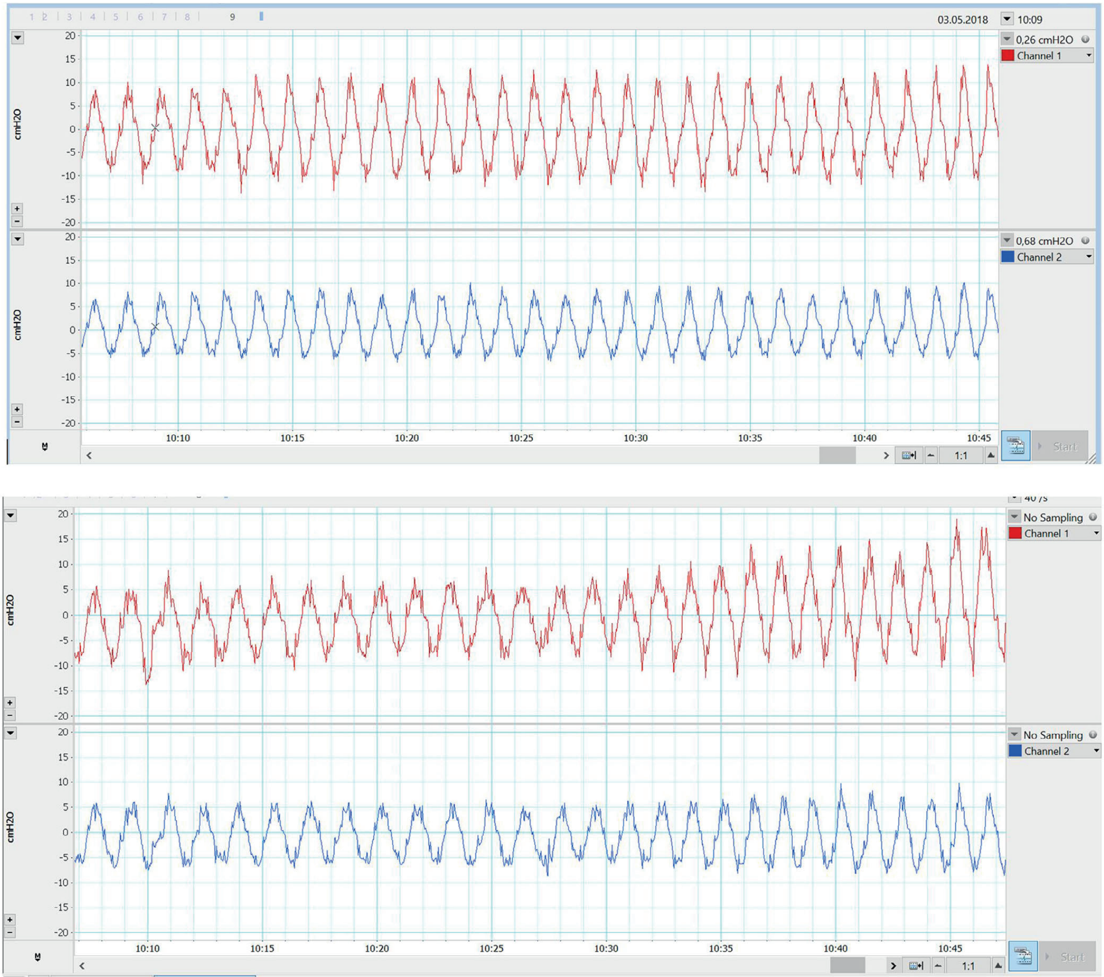

Fig. 2. Two separate tracings during the same time period from two different continuous laryngoscopy exercise tests, from the same individual, illustrating the similarities. The blue lines depict the epiglottic and the red lines the tracheal pressure readings. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

although predominantly in the pharyngeal region.[22] Considering the anatomical differences between the equine and human oropharynx, notably the relationship between the soft palate and the epiglottis, the pharyngeal region is less likely to play a significant role for human laryngeal resistance, although further research is needed to confirm this. Obviously, these issues need to be understood before the test can be applied in clinical and research contexts in relation to patients suffering from EILO.

Resistance Calculations

Previous research in exercising equines and in humans at rest has shown that pressure traces are relatively similar in all individuals. However, during this research, it became evident that this was not the case during exercise in these test subjects. Pressure patterns during MVV were relatively similar in most individuals, but during exercise different trace patterns where seen, likely reflecting different breathing strategies adopted by individuals to increase their minute ventilation. Some subjects preferentially increase tidal volume, whereas others increase breathing frequency. The time ratio of inspiration to expiration also varied. Variations in pressure patterns became more evident with increasing exercise intensity. This affects the resistance calculations, as the total minute ventilation was used to determine flow at any given time. If the individual uses a short time on inspiration at the expense of expiration, this will result in an increased flow rate during inspiration and thus a higher translaryngeal resistance. The data from this and future studies will take into account and be analyzed with these parameters to give more accurate resistance calculations and determine how different breathing strategies affect airway mechanics. Resistance was greater at walk-to-run transition than at the end of the CLE test, when flow was greatest, in all but two subjects. This is most likely explained by the observation on laryngoscopy that healthy individuals do not fully abduct the arytenoids until they are at running speeds. The laryngeal inlet area is smaller at walk-to-run than at the end of the CLE test, resulting in the increased resistance measured. These responses need to be explored in detail and linked with clinical data and images in future studies. The magnitude of inter subject variability for translaryngeal resistance readings in this study seems reasonable and consistent with variability in total airway resistance in studies using esophageal sensors to determine pleural pressure.[23]

Utility

The need for an objective and absolute outcome measure for the CLE test has been highlighted as a major research priority in recent European Respiratory Society/European Laryngological Society/American College of Chest Physicians statements.[2,7] Currently, only relative, subjective grading scales of laryngeal aperture size during increasing exercise intensity are available. This is an unsatisfactory measure particularly for irreversible clinical decision making (surgery) and research. This article describes a method for objectively assessing the resistance to airflow over the larynx during maximal treadmill exercise, applied as an add-on option to a standard CLE test. The method opens for direct and real-time comparisons between translaryngeal pressure drops obtained before and after treatments, and also as a comparison with visual changes of the laryngeal aperture as observed during a CLE test. The method also enables real-time translaryngeal resistance measurements in direct conjunction with observations of the patients' symptoms and with their cardiorespiratory parameters, as these variables are obtained throughout a CLE test.

CONCLUSION

Translaryngeal resistance measurements can be done in exercising humans, and thus hold potential to provide objective, continuous, numerical and verifiable data to describe laryngeal function during exercise. The method appears feasible and tolerable and provides reliable translaryngeal pressure measurements. If the larynx is viewed as the entrance valve to the airway tree, future access to such data can become as important to respiratory medicine as transvalvular pressure gradients are in today's cardiology.

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III Reliability of trans-laryngeal resistance measurements during maximal exercise

1. TITLE

Reliability of trans-laryngeal airway resistance measurements during maximal exercise

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6. RUNNING TITLE

Trans-laryngeal resistance during exercise

7. CONFLICTS OF INTEREST

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

8. AUTHORS CONTRIBUTIONS TO THE STUDY

Zoe Louise Fretheim-Kelly conceptualized and designed the study and the data collection instruments, collected and organized the data, was responsible for the resistance calculations, drafted the initial manuscript, and reviewed and revised the final manuscript.

Mette Engan performed data collection, carried out the statistical analyses, and revised the manuscript.

Hege Clemm conceptualized and designed the study and the data collection instruments and reviewed and revised the final manuscript.

Tiina Andersen conceptualized and designed the study and the data collection instruments and reviewed and revised the final manuscript.

John-Helge Heimdal conceptualized and designed the study and the data collection instruments and reviewed and revised the final manuscript.

Eric Strand revised the analyses and reviewed and revised the final manuscript.

Thomas Halvorsen conceptualized and designed the study and the data collection instruments and reviewed and revised the final manuscript.

Ola Røksund conceptualized and designed the study and the data collection instruments, performed data collection, revised the statistical analyses, reviewed and revised the manuscript.

Maria Vollsæter conceptualized and designed the study and the data collection instruments, coordinated and supervised data collection, organized data, revised the statistical analyses, and reviewed and revised the final manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

10. ABBREVIATIONS

EILO	Exercise Induced Laryngeal Obstruction
CLE test	Continuous Laryngoscopy Exercise test
EIB	Exercise Induced Bronchoconstriction
COPD	Chronic Obstructive Pulmonary Disease
CPET	Cardiopulmonary Exercise Test
CR	Coefficient of Repeatability
ICC	Intraclass Correlation Coefficient

11. WORD COUNTS: 2991

ABSTRACT

Objective

Exercised induced laryngeal obstruction (EILO) is an important cause of exertional dyspnea. The diagnosis rests on visual judgement of relative changes of the laryngeal inlet during continuous laryngoscopy exercise (CLE) tests, but we lack objective measures that reflect functional consequences. We aimed to investigate repeatability and normal values of trans-laryngeal airway resistance measured at maximal intensity exercise.

Methods

Thirty-one healthy non-smokers without exercise related breathing problems were recruited. Participants performed two CLE tests enabling verified positioning of two pressure sensors, one at the tip of the epiglottis (supraglottic) and one by the fifth tracheal ring (subglottic). Airway pressure and flow data were continuously collected breath-by-breath and used to calculate trans-laryngeal resistance at peak exercise. Laryngeal obstruction was assessed according to a standardized CLE-score system.

Results

Data from 26 participants (16 females) with two successful tests and equal CLE scores on both test-sessions were included in the trans-laryngeal resistance repeatability analyses. The coefficient of repeatability (CR) was 0.62 $cmH_2O/l/s$, corresponding to a CR% of 21%. Mean (SD) trans-laryngeal airway resistance ($cmH_2O/l/s$) in participants with no laryngeal obstruction ($n = 15$) was 2.88 (0.50) in females and 2.18 (0.50) in males. Higher CLE scores were correlated with higher trans-laryngeal resistance in females ($r = 0.81$, $p < 0.001$).

Conclusions

This study establishes trans-laryngeal airway resistance obtained during exercise as a reliable parameter in respiratory medicine, opening for more informed treatment decisions and future research on the role of the larynx in health and disease.

KEYWORDS

CPET, exercise test, exercise induced laryngeal obstruction, larynx, exercise induced bronchoconstriction

LEVEL OF EVIDENCE : 3

The larynx plays a key role in modulating total airway resistance, and we have therefore investigated the reliability of trans-laryngeal airway resistance in exercising humans. Trans-laryngeal airway resistance was higher in females than in males at peak exercise, and the repeatability was adequate and independent of the magnitude of the resistance. This is the first study to establish trans-laryngeal airway resistance as a reliable parameter in human respiratory medicine, opening for more informed treatment decisions and future research on the role of the larynx in health and disease.

INTRODUCTION

Exercise related breathing problems are common complaints in patients of all ages. Symptoms can be due to a range of different and often overlapping disorders, challenging our diagnostic skills in clinic, and complicating phenotyping in research. Within respiratory causal pathways, we tend to aim work-up at conditions in the peripheral airways, particularly asthma and exercise induced bronchoconstriction (EIB) or its irreversible counterpart, chronic obstructive pulmonary disease (COPD). However, the larynx is evidently heavily involved in exertional dyspnea, both as the independent disease entity: exercise induced laryngeal obstruction (EILO) [1-4], or as a contributing part of the bronchial obstruction in asthma [5,6] and COPD [7]. These "upper versus peripheral airway interactions" are debated, exemplified by recent exchanges of opinions in the literature [8].

We have previously suggested that similar respiratory symptoms might be differentially interpreted by health care providers with different backgrounds, and that apparent disagreements might reflect a heterogeneous pathophysiology [9]. This situation is probably accentuated by the fact that most tools for evaluation of upper airway patency involve some degree of subjective assessment. EILO diagnostics relies on visual judgment of *relative* changes of the laryngeal inlet as these appear on endoscopic images during exercise [4,10]. It is not given that the same degree of relative closure produces the same obstruction to ventilation nor the same sense of dyspnea in different persons. This contrasts asthma and COPD, where diagnostic evaluations are supported by reproducible numerical data from spirometry. Thus, there is a need in respiratory medicine for numerical data that describe functional consequences of the relative laryngeal narrowing we can observe endoscopically or by using imaging techniques.

In veterinary medicine, airway pressure readings in equines have informed treatment decisions and research for years [11,12]. Based on this experience, we have confirmed that trans-laryngeal airway pressure can be measured during high intensity exercise also in humans [13]. As a first step to establish trans-laryngeal airway resistance as a valid parameter in respiratory medicine, reliability must be determined. Thus, the aims of this study were to investigate repeatability of trans-laryngeal resistance measured during maximal intensity treadmill exercise in healthy, non-smoking volunteers, and to use these data to indicate normative ranges for males and females.

METHODS

Study design and participants

In this explorative study, participants were recruited from staff, students or associates of Haukeland University Hospital and Western Norway University of Applied Sciences, Bergen, Norway; all completing two tests within two weeks. All were healthy non-smokers with no reports of exercise related breathing problems, and symptoms of respiratory tract infections should not have been reported during the two weeks prior to tests.

The study was approved by the Regional Committee on Medical Research Ethics of the Western Norway Health Region Authority (2017/636/REK vest). Written informed consents were obtained from all participants.

Continuous laryngoscopy exercise (CLE) test and the pressure recordings

Trans-laryngeal airway resistance was measured continuously during the CLE test, which is a complete maximal cardiopulmonary exercise test (CPET) performed with a flexible laryngoscope positioned in the epi-pharyngeal area introduced via a transnasal route [10]. Trans-laryngeal resistance was calculated breath-by-breath based on airway pressure recordings obtained from two sensors placed above and below the larynx, and inspiratory airflow measured at the mouth [13].

Briefly, a 12-lead portable ECG was attached. The nostrils and nasal cavity were anesthetized with lidocaine 4 %. An endoscopic video camera system (Olympus Visera, CLV-S40, Tokyo, Japan) was connected to a fiberoptic laryngoscope with work channel and diameter of 4.9 mm (Olympus ENF-VT3, Tokyo, Japan). The laryngoscope was advanced through a hole in a modified facemask (Hans Rudolph, Inc., Kansas City, MO, USA) through the nasal cavity to the oropharynx (Figure 1 – photo of test situation). Lidocaine 4 % was used to anesthetize the vocal folds and proximal trachea, administered via an Olympus Spray tip catheter PW-6C-1 producing a mist. One ml was sprayed as the participant expressed a long "e" (closed vocal folds) and one ml with the vocal folds abducted. Further doses were given as required. Two pressure sensors (Mikro-Cath 825-0101, Milar, Houston, USA) were introduced through the work channel, the first positioned approximately at the fifth tracheal ring and the second at the tip of the epiglottis (Figure 1 and Supplemental file 1 video). The laryngoscope was fixed to a custom-made headset. The sensors were secured to the headset and connected to a data acquisition box (Powerbox 8/35, ADIInstruments, Oxford, UK), with the data collected and stored on a MacBook Pro, Apple laptop using LabChart 8.0 software. Data acquisition was set at 40 Hz. Participants ran on a treadmill (Woodway PPS 55 Med Weil am Rhein, Germany) to individual experience of exhaustion using a modified Bruce protocol with 60 second incremental intensity steps. CPET variables (minute ventilation (VE), heart rate, peak oxygen consumption (peak VO_2)), were recorded using a Vyntus SentrySuite Cardiopulmonary Exercise (CPX) unit powered by SentrySuite software (Vyaire Medical GmbH, Leibnizstrasse, Hoechberg, Germany).

Collection of pressure data and calculation of trans-laryngeal airway resistance

Pressures were continuously measured throughout the CPET. All reported variables and those used to calculate resistance were recorded close to peak exercise. Trans-laryngeal airway resistance was calculated breath by breath, based on corresponding pressure and flow values, using the following equation and given as the average of 10 consecutive breaths:

Trans-laryngeal airway resistance was calculated by the following equation:

$[R_L = P_T – P_E / AF]$ where

R_L is Laryngeal airway resistance ($cmH_2O/l/s$)

P_T is Tracheal pressure reading (cmH2O)

P_E is Epiglottic pressure reading (cmH2O)

AF is inspiratory Airflow in liters/second (l/s) as determined by breath-by-breath data from the CPX unit.

Endoscopic evaluation

Three experienced clinicians viewed the endoscopic video feeds. CLE scores were noted at the end of test as described by Maat et al [14]. Briefly, supraglottic and glottic levels were scored separately, grade 0 being no obstruction, grade 1 mild obstruction, grade 2 moderate and grade 3 severe obstruction. For the purpose of this study, the expression 0/1 denotes obstruction grade 0 glottic and grade 1 supraglottic at peak exercise, and these were the maximum scores allowed in order to be defined as “no laryngeal obstruction” [15]

Statistics

Data were reported as means with standard deviations (SD) and differences with 95 % confidence intervals (CI). The coefficient of repeatability (CR) for trans-laryngeal resistance defines the value below which the absolute difference between two replicate measurements is expected to be found with 95% probability [16,17]. Briefly, the CR is calculated as follows: The variance of the two observations from each subject is calculated by determining the difference between the two measures, squaring the value, and dividing by two. The square root of this value gives the within subject standard deviation (S_w). The CR can then be calculated: CR= 2.77 x S_w and accounts for both random and systematic errors. Both CR and CR % (CR as a percentage of the pairwise mean) were reported. CR is directly related to 95% limits of agreement (LoA) [16]. A plot of the SDs of the mean differences between repeated tests were made to visualize the relationship between the repeated tests, where the 95% LoA between the two tests were expressed as +/- 1.96 SD of the differences [18]. One-sample t-test versus zero was used to examine for systematic bias between the two tests. The differences between tests were regressed on the average to test for proportional bias, i.e., whether the differences were influenced by the numerical magnitude of the measurement [19].

A preliminary normal range for trans-laryngeal resistance was calculated based on data from participants with normal laryngeal findings (here defined by CLE-scores 0/0-1) reported as the mean value with SD and 95 % CI. Stratified by gender, the Kendall's Tau-b correlations coefficient (r) was used to measure the correlation between the trans-laryngeal resistances across the CLE categories.

The criterion for statistical significance was set at $p < 0.05$. Statistical calculations were performed using the statistical software SPSS version 25 (IBM SPPS Statistics, Armonk, NY, USA) and MedCalc version 19.5.3 (MedCalc Software Ltd, Osted, Belgium).

RESULTS

Participants

Altogether, 31 participants were recruited, their characteristics are given in Table 1. All participants had a structurally normal larynx at rest. The overall distribution of CLE scores in the complete group of participants is depicted in Figure 2. For repeatability calculations, we used the trans-laryngeal resistance data from 26 participants (16 females) with two successful tests and equal CLE scores on both occasions (Table 2). To indicate normal values, we used the resistance data from the 15 subjects (6 females) with CLE scores rated as 0/0-1 (Table 3).

Repeatability

There was no difference in trans-laryngeal resistance, maximal heart rate (HR), peak oxygen consumption (VO_2peak) and minute ventilation (VE) measured on the two repeated tests (Table 2). For trans-laryngeal resistance, the CR was 0.62 cmH_2O/l/s, corresponding to a CR% of 21 %. No constant or proportional bias were found for repeated measurements of trans-laryngeal resistance, visualized in the difference plot (Figure 3).

Trans-laryngeal airway resistance

Mean (SD) trans-laryngeal resistance (cmH_2O/l/s) in participants with CLE score (0/0-1) was 2.88 (0.50) for females and 2.18 (0.50) for males, with a mean (95% CI) difference between females and males of 0.71 (0.13 to 1.28) cmH_2O/l/s, $p = 0.02$ (Table 3). Higher CLE scores were associated with higher trans-laryngeal resistance in females ($r = 0.81$, $p < 0.001$). The same did not apply in the male participants, however, acknowledging that only four males had higher CLE scores than 0/0-1 (Figure 2).

DISCUSSION

This is the first study to report trans-laryngeal airway resistance measured repeatedly in humans during high intensity exercise. The repeatability was excellent and independent of the magnitude of laryngeal obstruction as defined by CLE scores. Trans-laryngeal resistance was higher in females than males. In females, there was a positive association between trans-laryngeal resistance and laryngeal obstruction as graded by CLE score, a finding we could not reproduce in males.

Laryngeal structure versus function

The larynx represents the single most important point of resistance of the airway tree, accounting for approximately 25% of total airway resistance during resting mouth breathing [20,21]. The effort to overcome this resistance requires 12-30% of the total respiratory work, increasing with higher airflow and turbulence [21]. Most human bodily proportions exhibit some form of population distribution, and it seems reasonable to assume that this is also the case for laryngeal dimensions relative to body size and gender. The significance of this variation in health and disease is surprisingly poorly understood, given the critical role the larynx plays in the airways.

Resistance in a tube is proportional to the fourth power of the radius, and even minor laryngeal idiosyncrasies may have functional consequences that are clinically relevant. Thus, it is not surprising that laryngeal dimensions have been implicated in most major airway disorders. Already in 1991, Hurbis and Schild indicated that the normal laryngeal response to exercise was distorted in asthma [22]. Their findings have later been supported by studies applying imaging technology in patients with asthma and COPD [5-7,23,24]. Additionally, we have recently shown that non-invasive positive pressures applied to assisted cough in patients with respiratory insufficiency, inadvertently might infringe on laryngeal patency, leading to an unfortunate loss of therapeutic effect [25,26]. These studies all rest on rather crude imaging methods that address laryngeal structure and relative changes of size scored visually [6,10]. We lack methods to produce numerical *absolute* data that reflect the functional consequences of these visual observations. More refined methods are required to move this field of respiratory medicine forward, and to ensure that clinical decisions and estimates of treatment effects are based on valid variables.

Repeatability of trans-laryngeal airway resistance

We have previously shown that trans-laryngeal pressures can be measured during high intensity exercise [13]. This present study confirms the reliability of this technology outside an "inner circle" of enthusiasts, indicating that trans-laryngeal resistance can be measured with a repeatability not very different from other parameters widely used in respiratory medicine, like spirometry [27].

When questioned before enrollment, all our participants declined respiratory symptoms during exercise. Nevertheless, a surprisingly high proportion of females had CLE-scores at peak exercise which were classified as more than "no obstruction". Their CLE scores were remarkably stable, and their data was therefore included in the repeatability calculations. We found that repeatability was not influenced by increasing CLE-scores, nor by the magnitude of the trans-laryngeal resistance. We believe this increases the relevance of this study, indicating that trans-laryngeal resistance is a repeatable and valid parameter also in individuals with various degrees of laryngeal obstruction. There was no bias that suggested learning effects from repeated CLE-tests; the mean difference between two replicate tests did not differ from zero.

Trans-laryngeal airway resistance versus gender and relative laryngeal obstruction

Most studies on vocal cord dysfunction and ILO suggest a female predominance, at least after puberty. Anatomical studies suggest similar laryngeal structure in boys and girls before puberty, while a male advantage regarding size seems to develop during puberty [28,29]. This fits the prevalence data from two studies of EILO, with no gender difference in the study with 13–15-year-old participants, but a female predominance in the study with 14–24 -year-old participants [2,30]. When addressing ranges for trans-laryngeal resistance in our study, we based the calculations on participants with no signs of obstruction during the test (CLE scores 0/0-

1). The broad resistance ranges in our small population may partly reflect the low number of participants. However, these broad ranges are also consistent with the wide range of possible breathing patterns adopted at peak exercise, observed also with other respiratory parameters [31]. Trans-laryngeal resistance was 32% higher in females than in males in our adult population. This potential gender effect must clearly be tested in larger studies.

The number of participants with CLE scores exceeding 0/0-1 was too low to properly address relationships with the resistance measurements, and also not the aim of this study, as only subjects without exercise related respiratory symptoms were enrolled. Nevertheless, in females with CLE scores exceeding 0/0-1, trans-laryngeal resistance was clearly outside the proposed "normal range" and also increasing with increasing CLE-scores. On further questioning following the CLE-test, inspiratory noise typical in EILO was reported by most of these subjects, but they had never considered this abnormal. This finding is supported by Norlander et al., who conclude there is a need for more research and clearer criteria for claiming pathology in this area of respiratory medicine [32]. Given these unexpected circumstances, data from these females who had not considered their laryngeal obstruction a respiratory problem cannot be used to indicate which level of laryngeal resistance is likely to be found in typical EILO-patients. However, we propose that these findings represent an incipient *functional* validation of the observed *structural* laryngeal changes in EILO [14].

Trans-laryngeal resistance tended to be lower in males, independent of their CLE-scores, *also* in the few males with CLE scores exceeding one. Regrettably, too few observations prevent statistical handling of this gender aspect. Nevertheless, one may speculate if males have a larger "laryngeal reserve capacity" for airflow, allowing some degree of inward displacement of laryngeal structures without leading to higher airflow resistance. These speculations support the notion that EILO symptoms are more common in females than males.

Strengths, limitations and weaknesses of the study

This study opens a novel research area in human respiratory medicine; real-time functional assessment of the larynx during high-volume ventilation. The strengths of the study were a highly experienced staff with decades of experience in EILO research and clinical work, a well-equipped exercise laboratory, and interdisciplinary collaboration between specialists from a wide range of disciplines.

The main limitation was the overall low number of participants, which prevents firm conclusions, particularly as regards normal ranges and gender differences. Also, our adult population above 24 years of age does not reflect the typical EILO patient, who most frequently present as a teenager [9]. Explorative research in healthy subjects under the age of 16 years presents greater ethical issues, particularly when testing a method that may be judged slightly unpleasant, and we therefore started with an adult population. However, we clearly need studies with broader participant demographics to establish normal values.

Introducing a pressure transducer below the vocal folds requires the use of topical lidocaine, which may alter the laryngeal response to exercise. It is currently not established how and to what extent local anesthetics affect upper airway mechanics; but it has been suggested that laryngeal hyposensitivity is associated with an increased risk of EILO [33]. Our finding that participants with no recollection of respiratory symptoms during exercise in fact did develop laryngeal obstruction during testing, may support this theory. We need a better understanding of potential effects of lidocaine on upper airway functions to fully exploit this method in clinical work and research.

Future perspectives

Airway pressure measurements have informed clinical work and treatment decisions in equine upper respiratory tract surgery for years [11,12]. We acknowledge that widespread use of this method in humans currently is limited by lack of commercially available equipment that can ensure smooth implementation in routine work. We also need to establish some very basic information; e.g. normal ranges relative to body size, ventilatory volume and gender. The applicability of this technology during the extreme scenario of high intensity exercise, suggest its feasibility also in patients at rest. We predict that trans-laryngeal resistance somehow will become integrated in future advanced diagnostic evaluations in respiratory medicine, and perhaps also guide our ambition to optimize treatment tools that rest on non-invasive application of positive pressures.

CONCLUSION

We conclude that trans-laryngeal airway resistance can be measured at peak exercise in "average people", and that the technology appears robust in terms of applicability and repeatability. We found that females had higher resistance than males, and that higher degrees of laryngeal obstruction in females were associated with higher resistance. The study opens a novel research area in human respiratory medicine; i.e. real-time functional assessment of structural laryngeal response patterns as these can be observed by endoscopy or imaging techniques.

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Legend figure 1:

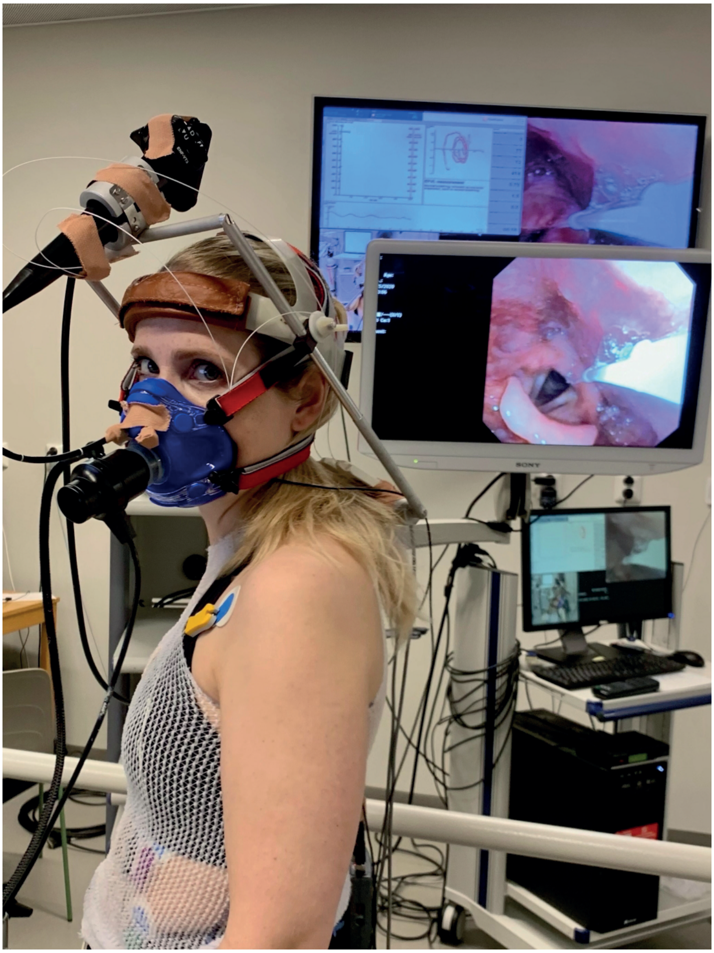
CLE-test with pressure measurement. Equipment set up.

Legend figure 2:

Error bars for the mean trans-laryngeal resistance with 95% confidence interval according to the continuous laryngeal exercise (CLE) score and gender. The first and the second digit in the CLE score category corresponds to the glottic and supraglottic score at maximal exercise, respectively.

Legend figure 3:

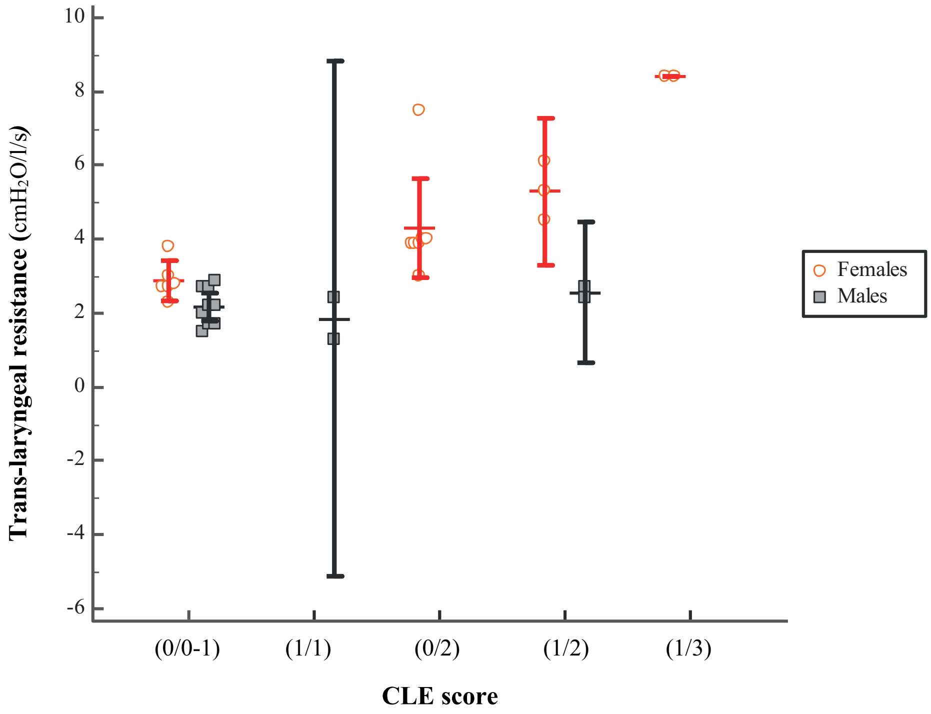
Agreement between trans-laryngeal resistance obtained from 26 subjects examined twice at peak exercise during a maximal exercise test on treadmill. The horizontal lines depict the mean difference between the trans-laryngeal resistance obtained in test 1 and test 2, whereas +/- 1.96 standard deviations of this difference represent the 95% limits of agreement between the two tests. The 95 % confidence intervals for the mean, the upper limit of agreement and the lower limit of agreement are indicated by vertical lines. The mean difference was 0.07 $cmH_2O/l/s$, the upper limit of agreement was 0.69 $cmH_2O/l/s$ and the lower limit of agreement was -0.55 $cmH_2O/l/s$.



Legend figure 1: CLE-test with pressure measurement. Equipment set up.

548x730mm (96 x 96 DPI)

Figure 2



10
8
6
4
2
0
-2
-4
-6
Trans-laryngeal resistance (cmH2O/l/s)
(0/0-1)
(1/1)
(0/2)
(1/2)
(1/3)
CLE score
Females
Males

Figure 3

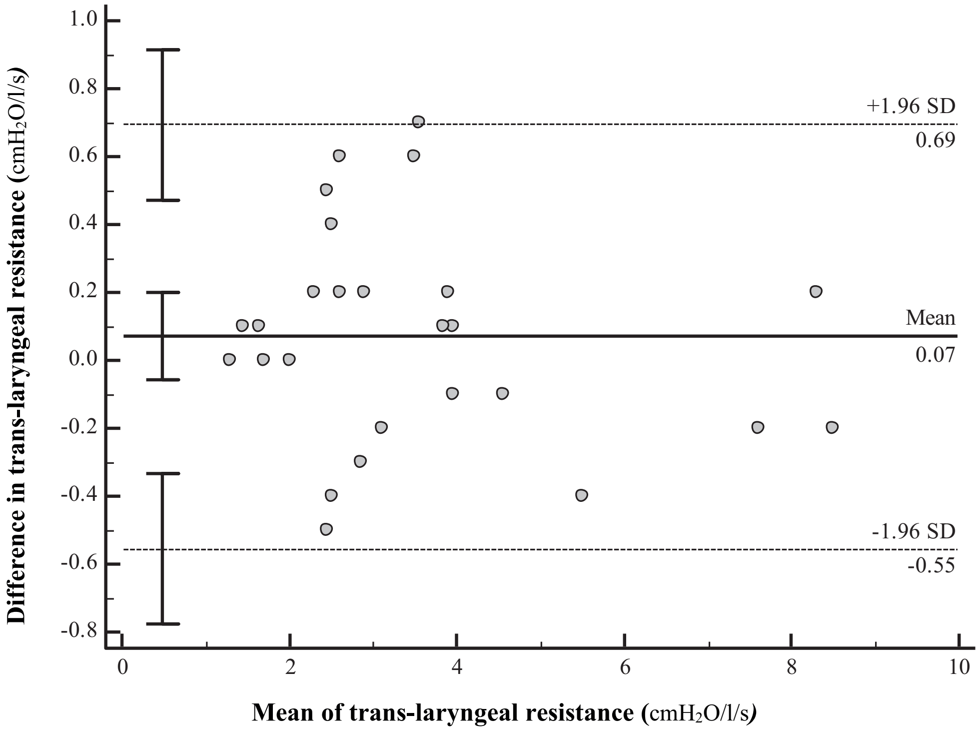

1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
+1.96 SD
0.69
Mean
0.07
-1.96 SD
-0.55
0
2
4
6
8
10
Difference in trans-laryngeal resistance (cmH2O/l/s)
Mean of trans-laryngeal resistance (cmH2O/l/s)

Table 1 Characteristics of the 31 participants

	Females n = 18		Males n = 13	
	mean	SD	mean	SD
Age, *years*	32.4	7.4	39.2	11.5
Weight, *kg*	64.6	7.6	85.2	7.5
Height, *cm*	166.9	6.4	183.5	5.3
BMI, kg/m^2	23.2	2.5	25.3	2.0
Heart rate, *beats/min*	182	7	180	11
Minute ventilation, *l/min*	106	14	153	22
Peak VO_2, *ml/kg/min*	44.2	6.4	45.7	8.0

Abbreviations: BMI: body mass index; SD: standard deviation; VO_2: oxygen consumption

Table 2 Comparison of trans-laryngeal resistance and ergospirometry data obtained during two repeated treadmill exercise tests for the 26 participants attending the repeatability study

	Difference*	
Measurements	mean	95% CI
Trans-laryngeal resistance, *$cmH_2O/l/s$*	0.07	-0.06, 0.20
Heart rate, *beats/min*	-0.1	-1.4, 1.1
Minute ventilation, *l/min*	-1.9	-5.1, 1.3
Peak VO_2, *ml/kg/min*	-0.3	-1.3, 0.7

Abbreviations: VO_2: oxygen consumption

*) Paired difference

Table 3 Trans-laryngeal resistance at peak exercise in participants with CLE score (0/0-1) *

	mean	SD	95% CI
Trans-laryngeal resistance, *$cmH_2O/l/s$*			
Females (n = 6)	2.88	(0.50)	2.35, 3.41
Males (n = 9)	2.18	(0.50)	1.79, 2.56

Abbreviations: CLE; continuous laryngoscopy during exercise; SD: standard deviation

s) The trans-laryngeal resistance is reported only for participants with CLE score (0/0-1) representing no glottic obstruction and no or mild supraglottic obstruction (0-1) at peak exercise.

IV A bitless bridle does not limit or prevent dynamic laryngeal collapse

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GENERAL ARTICLE

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A bitless bridle does not limit or prevent dynamic laryngeal collapse

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Abstract

Background: Bits have often been incriminated as a cause of upper respiratory tract obstruction in horses; however, no scientific studies are available to confirm or refute these allegations. Clinical signs of dynamic laryngeal collapse associated with poll flexion (DLC) are induced when susceptible horses are ridden or driven into the bit.

Objective: To determine whether use of Dr Cook's™ Bitless Bridle, instead of a conventional snaffle bit bridle, would reduce the severity of DLC in affected horses measured objectively using inspiratory tracheal pressures.

Study design: Intervention study using each horse as its own control in a block randomised order.

Methods: Nine Norwegian Swedish Coldblooded trotters previously diagnosed with DLC were exercised on two consecutive days using a standardised high-speed treadmill protocol with either a conventional bridle with a snaffle bit, or Dr Cook's™ Bitless Bridle. Head and neck position, rein tension, inspiratory tracheal pressure measurements, and laryngeal videoendoscopy recordings were obtained. A heart rate greater than 200 bpm, and similar degrees of poll flexion/head height, had to be achieved in both bridles for the individual horse's data to be included for comparison.

Results: Seven horses' data met the inclusion criteria. The change in mean inspiratory tracheal pressure between free and flexion phases in the bitless bridle (−15.2 ± 12.3 cmH_2O) was significantly greater (*P* < .001) than in the snaffle bit bridle (−9.8 ± 7.9 cmH_2O). Mean inspiratory pressure during the free phase was significantly (*P* < .001) more negative with the snaffle bit bridle (−32.3 ± 6.3 cmH_2O), vs the bitless bridle (−28.5 ± 6.9 cmH_2O). Mean pressures in flexion phase, snaffle bridle (−42.1 ± 10.8 cmH_2O), vs bitless bridle (−43.7 ± 15.6 cmH_2O) where not significantly different between bridles (*P* = .2).

Main limitation: Small sample size due to difficulty recruiting suitable clinical cases.

Conclusions: This study could not provide any clear evidence that the effect of a snaffle bit in a horse's mouth influences the development or severity of DLC. Instead,

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head and neck angles induced by rein tension seem to be the key event in provoking DLC in susceptible horses.

KEYWORDS
horse, dynamic laryngeal collapse, upper respiratory tract, bit, bitless bridle, tracheal pressure measurement

1 | INTRODUCTION

Upper respiratory tract (URT) obstruction is a common cause of poor performance in racehorses[1-3] and a major cause of lost earnings and wastage from training.[1,4] Bits have been blamed for directly causing or worsening the severity of URT collapse,[5-7] and, since there are no studies to confirm or refute these allegations, bits have become a controversial welfare issue in horses.[7] Dr. Cook's™ cross-under Bitless Bridle (The Bitless Bridle Inc.) was developed to address these purported airway issues but has not been objectively tested in horses with different forms of URT collapse.

Dynamic laryngeal collapse (DLC) associated with poll flexion is a form of URT collapse that is only evident when affected horses are exercised "on the bit."[8] It is characterised by bilaterally symmetric vocal fold collapse, with some degree of concurrent arytenoid cartilage collapse that can result in moderate to severe inspiratory obstruction.[8,9] Horses have been documented to collapse physically during racing due to this form of airway obstruction. The disorder quickly resolves when horses go off the bit and exercise with a more extended head and neck carriage. Dynamic laryngeal collapse is the most frequently diagnosed dynamic URT obstruction in Norwegian Swedish Coldblooded Trotters (NSCT),[10] but has also been documented in other breeds, and in disciplines that require horses to exercise in high poll flexion.[11,12]

The jointed snaffle, a bit commonly used in harness racing, acts by exerting pressure on the bars of the horse's mouth and the tongue.[13,14] The jointed snaffle has a squeezing action on the mouth with little pressure being applied to the tongue with the head in a neutral position. However, with progressive poll flexion the snaffle acts increasingly on the lower jaw and tongue.[15] Dynamic laryngeal collapse occurs in poll flexion and it could be considered that the bits' changing action on the tongue in this position induces the disorder via direct interactions between the tongue, hyoid apparatus and larynx.

The aim of the study was to determine whether use of Dr Cook's™ Bitless Bridle, instead of a conventional bridle with snaffle bit in the horse's mouth, would reduce the severity of DLC in affected horses as measured objectively using inspiratory tracheal pressures. Our hypothesis was that in NSCT horses diagnosed with DLC, use of Dr Cook's™ Cross-under Bitless Bridle (The Bitless Bridle Inc.) would result in less negative inspiratory pressures as a sign of decreased airway obstruction in poll flexion compared with a bridle with a jointed snaffle.

2 | MATERIALS AND METHODS

2.1 | Horses

NSCTs diagnosed with DLC using high speed treadmill videoendoscopy (HSTV) that presented to our institution between October 2011 and May 2018 were prospectively included in the study. The horses were racing fit, had no clinical evidence of concurrent disease or lameness, and had not undergone previous surgery for URT obstruction.

2.2 | Standardised exercise protocol

The horses were exercised in regular racing tack (conventional bridle with snaffle bit or Dr Cook's™ Cross-under Bitless Bridle (The Bitless Bridle Inc.), light harness, checkrein and long reins). A standardised treadmill exercise protocol developed for evaluation of the URT in harness racehorses was conducted.[9] Briefly, the exercise protocol consisted of one minute of exercise in free head carriage, followed by a minute in poll flexion, achieved by gathering and driving the horse "into the bit." This was followed by the horse running alternating phases of one minute of free head carriage, and one minute of poll flexion until the horse showed signs of fatigue, failed to engage the bit or completed four phases in total. Treadmill speed was set at 8.5 m/s and at a 1.5% incline for all tests. For inclusion in data analysis the horse had to: achieve and maintain a heart rate of greater than 200 beats per minute; have a similar head position (height) as determined by ratio 1 and degree of poll flexion measured as detailed below; and be subjected to similar rein tension with the bitless bridle during the flexion phase as with the snaffle bit bridle. Horses were allocated by block randomisation to run with either the bitless bridle or the snaffle bit bridle on the first day, and the alternate bridle on the second day.

2.3 | Rein tension

Rein tension was measured by attaching "Super Samson"™ spring balances (Salters, range 0-25 kg, Salters, FKA Brands Ltd.) inline between the reins and leather grips the driver was holding. During free head carriage the driver applied no force to the reins. During the poll flexion phases the force (weight) applied to the left and right rein was recorded at 20, 30 and 40 seconds after the start of the flexion phase. An average of these 3 measurements was used to determine the rein tension applied during each flexion phase.

2.4 | Head position

A digital video camera (Everio HD, JVC, JVK/Kenwood UK Ltd.) was placed on a stand to the left of the treadmill at a consistent measured spot, marked by tapes on the floor, to capture the horses' head, neck and thorax. White self-adhesive markers were placed on the bridle at the base of the left ear, at the level of the bit, and additionally on top of the left withers (Figure 1). Post processing of the film in a media player (VLC, http://www.videoLAN.com) by pausing and taking one still frame shot was used to obtain 3 still images at 20, 30 and 40 seconds from each minute of the exercise test. The still frames were used to determine an average for each minute of the following parameters: The angle between the withers marker, base of ear (poll marker) and bit (α); and the vertical distance from the sidebar to the poll marker divided by the vertical distance from the withers marker to the sidebar (ratio 1) (Figure 1) as reported previously.

2.5 | Videoendoscopic evaluation

Videoendoscopic (Karl Storz, Karl Storz Endoscopes AS) images were digitally recorded. Recordings from the videoendoscopic examination were later reviewed in real-time and slow motion by two blinded Diplomates of the European College of Veterinary Surgeons with more than 10 years of experience in evaluating equine upper respiratory tract disorders. Dynamic laryngeal collapse was graded by consensus as previously described.[17] Grading was by numerical value (0-3) with arytenoid cartilage collapse (ACC) and vocal fold collapse (VFC) being graded separately, where 0 is normal position and 3 is marked collapse of the structure. The grade given for each of the abnormalities was determined by the most severe grade persisting for 10 seconds or more.

FIGURE 1 A horse on the treadmill showing placement of markers and measurements made. Red lines show "poll flexion angle"; the angle between the two red lines gives the angle of poll flexion in degrees. Green lines show ratio of poll to withers height, calculated by dividing length of poll to treadmill bar height in mm by length of withers to treadmill bar height in mm

2.6 | Tracheal pressure

Tracheal pressure was measured using either "Samba" (Vastra Frolunda, Samba Sensors AS) digital (horse 1-4) or "Millar" (Millar Inc.), analogue (horse 5-9) pressure measurement equipment. The respective pressure sensor was inserted through a 150 cm polyethylene catheter (Baxter Scientific Products) with six side holes in the distal eight cm; the sensor was placed level with the fourth hole from the sealed tip and connected to the control unit. The polyethylene catheter was then placed under videoendoscopic guidance via the right nasal passage so that the catheter tip was 30 cm distal to the larynx in the trachea. The catheter was sutured in place to the external nares. Pressure tracings were continuously recorded in "LabChart Pro version 7" (ADInstruments, ADInstruments Ltd.) on a laptop computer. Peak inspiratory tracheal pressure was determined for each minute of the exercise test by averaging the values for 10 consecutive breaths during the last 15 seconds of each phase of the test.

2.7 | Heart rate

Heart rate was measured by placing a heart rate monitor (Polar, PolarElectro Ltd.) behind the girth at the level of the heart. Data were remotely sent to a pulse watch allowing real time recording of heart rate in beats per minute (bpm). Heart rate was recorded at 20, 30 and 40 seconds into each phase of the exercise test, these 3 values were used to give an average for that phase.

2.8 | Data analysis

Summary statistics of tracheal inspiratory pressure (mean, standard deviation, minimum and maximum values) were calculated for each horse, separated by bridle type (snaffle or bitless) and time period (free or flexion). Linear mixed model analysis with each horse as a random effect and a compound symmetry (exchangeable) correlation structure were fitted to assess the impact of bridle on tracheal inspiratory pressure. Two models were fitted; with bridle as categorical variable (Snaffle free phase [S1], Snaffle flexion phase [S2], Bitless free phase [B1] and Bitless flexion phase [B2]) and with the difference between free and flexion phase for snaffle and bitless as dichotomous variable (S1-S2 vs B1-B2). Intraclass correlation coefficients (ICC) were calculated based on the variance estimates from the models to give an estimate of the level of clustering in the data:

$$\mathrm{ICC} = \sigma^2_{\mathrm{horse}} / \left(\sigma^2 + \sigma^2_{\mathrm{horse}} \right)$$

Models with and without the horse random effect were compared with likelihood ratio tests (LRT). Assumptions for linear mixed models were evaluated. Statistical software (STATA version 16, Stata Corp) was used for all analyses. Significance level was set at $P < .05$.

3 | RESULTS

The horses recruited for the study were between 4 and 7 years of age (mean 5.2 years), and included 4 mares, 4 stallions and one gelding. Tracheal pressure and laryngeal videoendoscopic recordings were obtained for all nine horses; however, only seven horses met all the inclusion criteria for comparisons to be made. One horse was excluded because it did not reach and maintain a heart rate of 200 bpm. Another horse was excluded because it did not achieve a similar degree of poll flexion in the bitless and snaffle bit bridles. The data on poll flexion angles, head height and rein tension are summarised for the seven included horses in Table S1. None of these seven horses had DLC when exercised in free head carriage (Table 1). Some degree of DLC occurred in all individuals when exercised in poll flexion, in both the snaffle bit and the bitless bridle (Table 1).

Only inspiratory pressure data recorded during the first (free) and second (flexion) exercise phase were analysed as inclusion criteria were met by only two horses in the third (free) and forth (flexion) phase due to horses fatiguing during the test. The linear mixed model (Table 2), considering all horses and controlling for repeated measurements, demonstrated that there was a difference between the inspiratory pressure means in the free phase, when DLC was not present, with the snaffle bit bridle causing greater obstruction −32.3 cmH_2O than the bitless bridle −28.5 cmH_2O ($P < 001$). In the flexion phase, DLC was present in all horses, and the difference in inspiratory pressure between the snaffle bit bridle – 42.1 cmH_2O and the bitless bridle −43.7 cmH_2O was not significant ($P = .2$). Examination of the difference in mean inspiratory pressure between the free and flexion phase in each type of bridle demonstrated that the bitless bridle was associated with greater head position-induced change in airway pressures than the snaffle bit bridle, with free to flexion differences of −15.2 and −9.8 cmH_2O respectively ($P < .001$).

Assumptions for linear mixed models were considered met. Results from the LRT gave $P < .001$, indicating that the random effect of horse was significant. ICC from the models were high (65% and 51%) indicating that there was larger variation between horses than within each individual horse.

Nasopharyngeal collapse and dorsal displacement of the soft palate were not observed in any of the included horses regardless of bridle type or whether tension was applied to the reins.

4 | DISCUSSION

This study did not provide any clear evidence that the action of a snaffle bit in a horse's mouth influences the development or severity of DLC. Instead, changes in head and neck angles induced by applying rein tension seem to be the key event in provoking DLC in susceptible horses. Poll flexion produces conformational changes especially regarding the relative positioning of the larynx and hyoid apparatus within the intermandibular space.[18]

As with previous investigations, we found that poll flexion increases peak inspiratory pressures in most horses relative to inspiratory pressures documented when exercised in free head carriage with the head and neck extended.[9,16] Head and neck position have been shown to have an effect on a number of dynamic URT obstructions including palatal instability, intermittent dorsal displacement of the soft palate, epiglottic retroversion, medial deviation of the aryepiglottic folds, DLC and dynamic nasopharyngeal collapse.[10,17,19,20] Exercise in poll flexion increases the severity of collapse in these URT disorders.[10,19,21]

It has been hypothesised that tack may play a role in the aetiology of dynamic airway collapse or can be used to prevent dynamic airway collapse. Woodie et al[22] demonstrated that an external device "the laryngohyoid support" could prevent dorsal displacement of the soft palate in experimentally induced cases. A modified check rein that prevents poll flexion has been shown to limit DLC in the research environment.[16] Thus, it was hypothesised that forms of tack that affect laryngeal position may play a role in the aetiology of DLC or help prevent it. The mechanism by which the snaffle bit was hypothesised to play a role in the development of DLC was by causing the tongue to be moved from its normal anatomical position, to alleviate the discomfort caused by the pressure of the bit on the tongue, and thus via the tongue's muscular attachments to the hyoid apparatus have an effect on laryngeal position.[7,23-26] For example, protraction of the tongue by the genioglossus muscle will also result in the basihyoid bone being pulled rostrally.[27] A more rostral larynx position has been associated with increased risk of DLC.[18] In addition, tongue position effects overall laryngeal and pharyngeal stability, so displacement from its normal anatomical position may have more generalised

TABLE 1 Summary of grade of arytenoid cartilage collapse (ACC) and vocal fold collapse (VFC) during the free head carriage phase and poll flexion phase for each horse

Horse		1	2	3	4	5	6	7
Free phase	S	0/0	0/0	0/0	0/0	0/0	0/0	0/0
ACC/VFC	B	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Flexion phase	S	2/3	2/1	1/1	1.5/3	1/1	1/0	1/2
ACC/VFC	B	2/2	1/0	1/2	2/2	1/0	1/0	2/2

Abbreviations: 0, normal; 1, mild; 2, moderate; 3, severe; B, bitless bridle; S, snaffle bit.

TABLE 2 Inspiratory tracheal pressures (mean ± SD) in cmH_2O recorded in seven horses exercising on a treadmill with head position free and flexed and with two different bridle designs (snaffle bit and bitless)

Horse nr.		1	2	3	4	5	6	7	All horses	P-value
Inspiratory pressure free phase	Snaffle bit bridle	−35.5 ± 6.0	−35.8 ± 3.6	−29.9 ± 2.6	−41.7 ± 3.6	−26.1 ± 2.5	−25.7 ± 2.2	−31.6 ± 2.2	**−32.3 ± 6.3**	
	Bitless bridle	−31.6 ± 3.6	−32.2 ± 3.6	−29.0 ± 2.8	−38.4 ± 2.8	−28.3 ± 3.1	−19.1 ± 3.1	−21.0 ± 2.2	**−28.52 ± 6.9**	***P* = .001**
Inspiratory pressure flexion phase	Snaffle bit bridle	−53.8 ± 3.7	−45.7 ± 2.3	−43.3 ± 4.5	−50.9 ± 10.1	−30.0 ± 2.9	−25.8 ± 2.0	−45.3 ± 4.0	−42.1 ± 10.8	
	Bitless bridle	−47.4 ± 2.6	−44.0 ± 4.6	−43.9 ± 4.3	−73.8 ± 9.8	−26.0 ± 3.7	−26.9 ± 2.7	−43.8 ± 2.3	−43.7 ± 15.6	*P* = .2
Difference between inspiratory pressure in free and flexion for each type of bridle	Snaffle bit bridle	−18.3	−9.9	-13.4	-9.2	-3.9	−0.1	-13.7	**−9.8 ± 7.9**	
	Bitless bridle	−15.8	−11.8	-14.9	-35.4	+2.3	-7.8	-22.9	**−15.2 ± 12.3**	***P* < .001***

Bold text denotes values that are significantly different.

*Tracheal inspiratory pressures were compared in mixed linear model analysis.

effects on upper airway stability.[25,26] The jointed snaffle bit has been shown to exert pressure on the tongue when increasing rein tension is applied[13] and the horse is gathered into poll flexion. However, as DLC of similar severity also occurred in the bitless bridle, it seems unlikely that the action of the bit on the tongue plays a role in this disorder. The bit has also been implicated in other URT disorders and has been proposed to affect ventilatory function and thus cause breathlessness;[5-7] however, no other scientific studies directly comparing respiratory responses to bits and bitless bridles have been reported. Since our study demonstrates that the bit has no major effect on the development or severity of DLC, it suggests that a change in the type of bit used is unlikely to provide necessary alleviation of clinical signs for horses affected with this performance limiting URT disorder. Other types of URT disorders such as nasopharyngeal collapse may be more affected by the action of bits and as such should be investigated in future studies in breeds predisposed to these disorders. In our relatively small study using horses preselected as having DLC, nasopharyngeal collapse and dorsal displacement of the soft palate was not observed in any of the horses regardless of bridle type, or whether there was tension applied to the reins.

Obtaining sufficient horses for clinical studies such as this is difficult. First, the horses must have the diagnosis, which should be readily inducible. Racing fitness is a criterion for inclusion due to the vigorous treadmill protocol conducted over two consecutive days. Also, the owners/trainers must be willing to allow their racehorses to participate. Additionally, precise replication of the testing protocol is essential. Therefore, we controlled for a number of parameters such as: heart rate, rein tension, head height and poll flexion angles to ensure similar testing conditions and followed a stringent exercise protocol (Table S1). ICC from the models were high (65% and 51%) indicating that there was a larger variation between horses than within each individual horse, and providing supporting evidence that there was not a large variation in tests for each individual horse, indicating variables were controlled. As there did not appear to be an effect of the jointed snaffle bit on tracheal inspiratory pressure in this small group of horses extending the study to recruit further horses was deemed unnecessary. The difference in mean inspiratory pressure between the two phases, free verses flexion could be interpreted as the bitless bridle caused greater obstruction than the snaffle bit bridle (Table 2). This observation is likely due to the snaffle bit bridle having a more negative baseline inspiratory pressure in the free phase compared with the bitless bridle, but both bits had similar inspiratory pressures during the flexion phase. This resulted in a greater difference in inspiratory pressure between the two phases in the bitless bridle giving the impression that the bitless bridle caused more obstruction. The more negative inspiratory pressure in the snaffle bit bridle during the free phase is an interesting finding and suggests that the presence of a snaffle bit caused mild URT obstruction in these horses. Dr Cook has hypothesised that the airtight lip seal is broken by the presence of a bit and this is an underlying cause in the development of URT pathology.[28] The loss of this seal and the resulting loss of sub-atmospheric pressure in the oropharynx potentially prevents the soft palate from lying firmly against the base of the tongue resulting in a more dorsal positioning of the soft palate and reduction in nasopharyngeal diameter.[28] This reduction in cross-sectional area of the nasopharynx would then result in greater resistance to airflow and more negative inspiratory pressures.[28] No abnormalities were noted on videoendoscopy in any of the horses during the free phase; however, the videoendoscope was positioned such that only the caudal nasopharynx and larynx were visible. The clinical significance of this snaffle-related obstruction is unknown. However, as inspiratory pressures were highly similar for the flexion phase in both bridles, when DLC was present, the snaffle bit does not appear to be associated with DLC pathogenesis. Further studies are needed regarding the snaffle bit´s effect on upper airway dynamics in order to determine its influence on airway mechanics and pathology.

One horse (#5) did not attain the expected drop or worsening of inspiratory pressure with the bitless bridle in poll flexion. Possible explanations for this could be unobserved heterogeneity which can complicate a study of this nature. This would include differences in daily variation in stamina, learning effects on day two to move more efficiently on the treadmill under testing, and in some horses' greater baseline fatigue on day two. By alternating the bridle and bit type used on the first day it was hoped to reduce the impact of these effects on our results.

A final source of error could be the use of the checkrein. We considered whether the bitless bridle test should be conducted without a checkrein; however, we chose to use the checkrein due to its importance in maintaining a similar head height and degree of poll flexion.[16] Had a checkrein not been used with the bitless bridle, it is possible that the horses would have exercised with a greater degree of poll flexion and a lower head carriage than with the checkrein present, causing a bias if the checkrein was used when exercising with the snaffle bit bridle. This would have made comparisons between groups impossible, as both head height and degree of poll flexion seem to be important factors in inducing DLC associated with poll flexion.[16] Thus, it was decided that all tack should be kept constant except for the presence or absence of a jointed snaffle bit. The check bit sits high in the mouth and exerts its pressure on the maxilla limiting the ability of the horse to drop its head and neck, and to potentially break into a gallop during training/racing. It is the action of the snaffle on the tongue and thus hyoid apparatus/larynx that was the theoretical mechanism by which the snaffle bit may induce DLC. The check bit has no action on the tongue and thus should not affect hyoid or larynx position via this mechanism.

The aim of this study was to determine if the jointed snaffle bit's increasing action on the tongue during periods of poll flexion induced or affected the severity of DLC in susceptible horses using objective inspiratory tracheal pressure as the outcome measure. The use of a jointed snaffle bit does not appear to be the inciting event in development of DLC in NSCT, nor does it appear to affect severity of obstruction when DLC is present. Therefore, a change in bit or bridle type will likely not provide improvement of airway function during racing in clinical cases affected with DLC.

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CONFLICT OF INTEREST

None of the authors have competing interests to declare.

AUTHOR CONTRIBUTIONS

All authors contributed to data collection, writing and proofing of the manuscript.

ETHICAL ANIMAL RESEARCH

All procedures were approved by the Faculty of Veterinary Science, Norwegian University of Life Sciences in accordance with national legislation for ethical animal research.

OWNER INFORMED CONSENT

Owners gave consent for their animals' inclusion in the study.

DATA ACCESSIBILITY STATEMENT

The data that supports the findings of this study is available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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8 Errata

Page 56 line 27 four-fold should read sixteen-fold

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