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THE EFFECT OF A TONGUE-TIE ON UPPER AIRWAY MECHANICS AND NASOPHARYNGEAL DIMENSIONS IN HORSES

Ву

Cornelis J. Cornelisse

A THESIS

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ABSTRACT

THE EFFECT OF A TONGUE-TIE ON UPPER AIRWAY MECHANICS AND NASOPHARYNGEAL DIMENSIONS IN HORSES

By

Cornelis J. Cornelisse

The objectives of my research were to 1) determine the effect of a tongue-tie on upper airway mechanics in exercising horses, and to 2) determine the effect of a tongue-tie on nasopharyngeal dimensions and the position of the hyoid apparatus in anesthetized horses.

In the first experiment, five Standardbreds performed an incremental treadmill exercise test with and without a tongue-tie while tracheal and nasopharyngeal pressures and airflow were measured. In the second experiment, five horses were anesthetized and a computer tomographic study of the upper airway was performed with and without a tongue-tie on each horse.

The tongue-tie had no effect on upper airway mechanics in exercising horses and it did not alter nasopharyngeal dimensions or hyoid position in anesthetized horses.

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Dedication

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LIST OF ABBREVIATIONS

CSA Cross-sectional area

CT Computed tomography

DDSP Dorsal displacement of the soft palate

DV Dorso-ventral

HR_{max} maximal heart rate

OSA Obstructive sleep apnea

INTRODUCTION

The more demanding and intensive use of the horse in a variety of sports has caused owners and trainers to become increasingly frustrated with poor performance or, perhaps more correctly stated, performance not meeting the owners' and trainers' expectations. A variety of unproven methods, as well as using non-prescribed substances, have emerged in an attempt to improve the performance of these horses. Unfortunately, despite the widespread use of and belief in some of these methods, scientific evidence for efficacy or non-efficacy, is generally lacking. Adding to the confusion with regard to the effectiveness of these "treatments" is the fact that numerous methods are used to treat multiple conditions. Most importantly, when these types of treatments are selected, the cause of the poor performance frequently remains undiagnosed, or is not seriously pursued at all. The application of a tongue-tie, where the tongue is pulled out of the horse's mouth and tied to the lower jaw during racing, is an example of such a remedy.

History of the tongue-tie

The history and the use of the tongue-tie is poorly documented. There is little information on the frequency of its use, its presumed success, or its potential side effects. The tongue-tie is used after, or in conjunction with various bits as well alterations in tack in an attempt to keep the tongue in place. The oldest report on the use of a tongue-tie

dates from 1889 by George Flemming, a veterinary surgeon of the British army, in his book *Roaring in Horses* (1). In chapter two, entitled "Various causes of noisy respiration," he states that:

Some horses, also which are not Roarers, are in the habit of making a noise when pulling at the bridle in galloping, owing to their opening of the mouth and retracting the tongue so as to force back the soft palate to much an extent that it interferes with the passage of air between the nasal passages and the larynx. So annoying is this noise sometimes to the rider that to get rid of it the tongue is tied down to the floor of the mouth by a piece of wide tape (15).

This description of a respiratory noise during exercise is a good description of the noise horses make during episodes of dorsal displacement of the soft palate (DDSP), one of the presumed indications for use of a tongue-tie.

Methods of applying a tongue-tie

Different variants of tying the tongue have been developed. One method is to pull the tongue out to the lateral canthus of the mouth and tie it to the ring of the halter or the bridle. This method has been abandoned since, although rare, accidental catching of the tongue on external objects resulted in severe hemorrhage and the loss of the tongue. The current method used is to pull the tongue as far out of the mouth as possible and subsequently tie the tongue to the mandible, such that the tongue can be seen protruding through the incisors. Usually a leather, gauze, or elastic strap is used to tie the tongue. It is positioned around the tongue at the level of the base of the frenulum. This method of tying the tongue is currently an accepted and frequently used procedure on racetracks

in the United States. Although no data are available regarding side effects, based on the absence of reports from the veterinary profession as well the silence from a much more animal-rights-conscious public, it seems that the tongue-tie has no major side-effects. This might partly be due to the fact that it is applied shortly before the start of the race. Subsequently, with racing times of only a few minutes, the length of time that the horse wears the tongue-tie likely does not exceed 15-20 minutes.

The upper respiratory tract and poor performance

Upper respiratory tract problems are a significant cause of poor performance in the athletic horse (2,3,4). With the development of the standing and treadmill endoscopic exams of the upper airway of the horse, a better evaluation of the functional anatomy of upper respiratory tract is possible. The advent of high-speed treadmill upper respiratory tract evaluations has improved our diagnostic potential when evaluating horses with poor performance attributable to upper respiratory tract obstruction.

Poor performance in horses with upper respiratory tract disease results from complete or partial obstruction of the upper airway, impairing ventilation during strenuous exercise (5,6). Dorsal displacement of the soft palate is one such disease that causes respiratory obstruction and decreased minute ventilation. Unfortunately, despite the recent advances in diagnostic technology, the etiologies of many obstructive pharyngeal diseases are still obscure. Consequently, numerous scientific treatments for these disorders, as well as the use of combinations of them, have had disappointing success rates, at best averaging around 65% (7-10). Therefore, people are still using other "solutions" whose effectiveness is solely based on personal empiric or historical data.

One of the commonly applied and accepted preventive "treatments" for racehorses with a history of poor performance is the tongue-tie. Data documenting the effectiveness as well as the mechanism of action of the tongue-tie are lacking. However, there are increasing amounts of comparative data from human and animal studies on the role and function of the tongue musculature in stabilizing the upper respiratory tract. Specifically, research focusing on the human disease, obstructive sleep apnea (OSA), has improved the understanding of the role of the tongue in stabilizing the upper airway. These studies include clinical studies on human patients as well as more basic research using laboratory animal models.

Aim of the thesis

The aim of this thesis is to investigate the effectiveness of the tongue-tie in horses, and to compare this research to the other studies that have documented the importance of the tongue muscles in breathing and airway stability. This information may validate or refute the use of the tongue-tie in today's racehorses. First, I will describe the anatomy of the tongue and its function during breathing in other species. Then I will present two studies: the effect of a tongue-tie on upper airway mechanics, and the effect of a tongue-tie on nasopharyngeal dimensions and hyoid apparatus position.

CHAPTER 1

LITERATURE REVIEW

The tongue

Anatomy

The tongue is a multifunctional organ that participates in a variety of tasks. The tongue functions during prehension of food, swallowing, tasting food, lapping and grooming of the coat, panting for heat loss, and vocalization (11). Parts of the tongue have specialized functions in order to perform these varied tasks.

The tongue lies within the oral cavity and forms the floor of the oropharynx. The nasopharynx is dorsal to the oropharynx. The soft palate forms the roof of part of the oropharynx and the floor of part of the nasopharynx. The tongue is attached to the larynx and the hyoid apparatus by muscular connections (29,30,36). The hyoid apparatus forms the bony support for the larynx and pharynx and consists of several small bones that interconnect via moveable articulations (29,30,33). Positional changes of the tongue, like protrusion and retraction, can affect the conformation and position of the hyoid apparatus (39-41). This in turn can influence the shape and dimensions of the nasopharynx. To better understand why tongue position can affect nasopharyngeal stability and dimensions it is important to discuss the precise anatomy of the tongue and hyoid apparatus first.

Ontogeny

The tongue is formed by the merger of several swellings (premordia) that start to develop from the floor of the mouth and pharynx around the fourth week in the developing embryo (12,13). The oral part of the tongue is formed from the median (Tuberculim impar) and lateral swellings, which are part of the first branchial arch or the mandibular arch. These swellings merge and form the apex and body of the tongue. The lingual sulcus is the remnant where the two lateral swellings have joined. Since this part of the tongue is derived from the mandibular arch its epithelial lining is of ectodermal origin. The root of the tongue is formed from the second branchial arch, a swelling in the pharyngeal floor, with additional contributions from the third and fourth arches. This swelling splits into a cranial part (called copula), which fuses with the median and lateral swelling, and subsequently forms the root of the tongue. The caudal part will form the epiglottis. The epithelial covering of this pharyngeal part of the tongue is of endodermal origin. Between the median swelling and the copula is the point of origin for the thyroid diverticulum, which will eventually become the thyroid gland.

The swellings are derived from migrating myotomes under the floor of the mouth. The origin of these myotomes is the occipital somites and they develop in the striated tongue-musculature, which becomes more prominent around the seventh week. The body of the tongue will start to develop papillae of several kinds (fungiform, filiform, vallate, foliate) as well as taste buds between the eighth and twelfth week. The root of the tongue is the site for the development of the lingual tonsil and occurs near term. Eventually a tongue will develop that will fit the shape of the mouth and have a mobile apex and body and a fixed root.

The intrinsic and extrinsic tongue muscles develop from the myotomes of the occipital somites. The intrinsic muscles originate and insert directly within the tongue. They consists of dispersed striated fibers that run longitudinally (longitudinalis m.), transversely (transversus m.), and vertically (verticalis m.) throughout the tongue, blending with the extrinsic tongue muscles and fat (11,14). Simultaneous contraction of the transverse and vertical intrinsic fibers will shape and stiffen the tongue (11). The longitudinal fibers shorten the tongue (15). Studies performed on cat tongues showed that the intrinsic tongue muscles are composed entirely of the type II fiber (15).

The muscles of the tongue

There are four pairs of extrinsic tongue muscles (11,14). They are called extrinsic since they have bony attachments outside the tongue. The genioglossus is a fan-shaped muscle that lies within and parallel to the median plane of the tongue. It originates from the medial surface of the mandible just caudal to the symphysis and its fibers radiate rostrally toward the tip of the tongue, dorsally to the body of the tongue, and caudally toward the root of the tongue. Contraction of genioglossus results in retraction of the tip, depression of the body, and forward movement (protrusion) of the root of the tongue (11,14,20). Genioglossus is the major tongue protrudor. The hyoglossus muscle originates from the lingual process of the basihyoid bone and runs cranially and dorsally, lateral to the genioglossus, toward the median dorsal plane. Contraction of hyoglossus results in tongue depression and retraction (11,14,20). Styloglossus is a long, thin muscle that lies along the lateral part of the tongue and originates from the stylohyoid bone near its articulation with the ceratohyoid bone. Styloglossus inserts at the tip of the tongue with its paired muscle and when it contracts it causes tongue retraction. In the cat these

extrinsic muscles are mostly composed of type II fibers; however, about 20-25% of the fiber are type I muscle fibers (15). A recent study on human genioglossus muscle characteristics reported 48.45% to 55.3% type IIA muscle fibers in snorers and 18.3% to 11.6% type IIB muscle fibers in patients suffering from OSA (16). The differences between snorers and OSA patients were statistically significant and indicated that these muscles adapt their fiber type to increased nocturnal and diurnal workload (16). The percentage of type I muscle fibers in genioglossus was the same for snorers and OSA patients (16).

The innervation of the tongue musculature

The ontogeny of the tongue also explains the sensory and motor innervation of the tongue (12,13). The sensory innervation develops at the border between ectoderm and endoderm, which is in front of the vallate papillae. The rostral two-thirds of the lingual epithelium receives sensory innervation from the nerves of the first and second branchial arches, which are the lingual branch of the trigeminal nerve and the facial nerve, respectively. The caudal one-third of the lingual epithelium is innervated by the glossopharyngeal and the vagus nerves, the nerves of the third and fourth branchial arches. The body and the root of the tongue are demarcated by a V-shaped groove, called terminal sulcus, just behind the vallate papillae. Like all structures derived from the occipital somites, the striated tongue musculature is innervated by the hypoglossal (XII) nerve (17–22). This includes the intrinsic and extrinsic muscles of the tongue. The distal part of the hypoglossal nerve divides into a medial and lateral branch. Recent studies in humans as well several animal species have revealed that the medial branch innervates the genioglossus muscle, and thus promotes protrusion of the tongue (18–22). The lateral

branch innervates the tongue retractor muscles, hyoglossus and styloglossus. The intrinsic tongue muscles are innervated by the medial and lateral branches of the hypoglossal nerve as well. The longitudinalis muscle is innervated by the lateral branch while the transversus and verticalis muscles are innervated by the medial branch (18).

The hyoid apparatus: anatomy and ontogeny

The extrinsic tongue muscles insert on the basihyoid bone. Contraction of these muscles alters the position of the basilyoid, as well as the conformation of the pharynx. The hyoid apparatus consists of a series of bony rods, joined together and forming a structure that suspends the tongue, larynx, and pharyngeal structures from the skull (29,30,33). These bones are formed from the mesoderm of the second (Reichert's cartilage) and third branchial arch (31,32). The cartilage of the second branchial arch divides into four fragments: tympanohyal, stylohyal, ceratohyal, and hypohyal. In humans, the tempohyal and stylohyal ossify and form the styloid process of the petrous part of the temporal bone. The ceratohyal degenerates into the stylohyoid ligament. The hypohyal undergoes endochondral ossification and becomes the smaller cornu and cranial part of the hyoid body. The smaller cornua are connected at the junction of the body and the greater cornua by fibrous tissue or occasionally synovial joints. The ventral cartilage of the third branchial arch becomes the greater cornu and the caudal part of the hyoid body through enchondral ossification. In early life the greater cornu is connected to the body by cartilage and later ossifies. Some literature suggests that the third branchial cartilage entirely accounts for the formation of the hyoid body.

Contrary to the development in humans, the styloid process and ceratohyal merge and ossify together forming the stylohyoid in horses (33). The proximal part of this

stylohyoid bone is cartilaginous and articulates with the petrous part of the temporal bone in a moveable joint. Distally, the stylohyoid bone articulates with the ceratohyoid bone, which is actually the smaller cornu of the basihyoid bone. Often in younger animals a small cartilaginous sesamoid bone, the epihyoid, is present and fuses with the stylohyoid later in life. Consequently, in older animals this articulation can become more rigid (33). The greater cornus of the basihyoid bone in the horse becomes the thyrohyoid bones that articulate with the larynx (33). The hyoid apparatus of other species like the dog and the cat closely mimics that of the anatomy of the horse (33).

The relationship between tongue, hyoid apparatus, and upper airway stability and dimension

Anatomically the tongue is fixed to the floor of the mouth and the bones of the hyoid apparatus. Due to this position, as well as its connections to the hyoid apparatus, the tongue affects the conformation and stability of the upper airway. During breathing, the upper airway is subject to pressure changes generated by the diaphragm and intercostal muscles. In certain types of upper airway obstructive diseases, the negative pressures generated during inspiration can cause nasopharyngeal collapse, and increased resistance, and impair ventilation. This phenomenon is more pronounced during exercise. Nasopharyngeal pressures generated during intensive exercise in the horse can be as low as -17.5 ± 2.1 cm H_2O (21). Therefore the structures forming and supporting the pharynx must be able to resist and counteract these collapsing forces. In 1947, Mitchinson and Yoffey demonstrated radiographically in people that the hyoid bone moves anteriorly during large breaths, suggesting that the muscles attached to the hyoid bone are active during breathing (38). Subsequent research focused on the muscles that

were anatomically able to influence the position of the hyoid, such as the genioglossus and geniohyoid muscles. In simulating the action of the genioglossus and geniohyoid muscle, Brouillette and colleagues demonstrated that anterior traction of the basihyoid bone in cadaveric rabbit heads resulted in more negative critical closing pressures. The critical closing pressure is the negative pressure that results in complete collapse or occlusion of the airway. In most species, the segment of the airway that collapses first is the nasopharynx, just cranial to the caudal edge of the soft palate. Similarly, endoscopic enlargement of the oropharynx was produced by increased levels of tension on sutures placed at the base of the tongue in cadavers of human infants, which mimicked genioglossus and geniohyoid muscle activity (40). Part of the enlargement in the oropharynx was due to the outward movement of the lateral and posterior pharyngeal wall, suggesting that the force applied to the tongue and hyoid bone was reflected in positional changes of the pharyngeal wall. The authors hypothesized that these effects were due to 1) passive stretching of the constrictor muscles, causing thinning of their mass; and 2) pulling the constrictor taut, correcting for any inward bowing (40). Similarly, ventral traction on the basilyoid bone in anesthetized dogs resulted in reduced pharyngeal resistance during inspiration and expiration, while ventral and lateral distraction of the pharyngeal walls was observed (41).

Continued supporting evidence that muscular activity influences the position and shape of the hyoid apparatus and the pharynx was produced in studies where specific nerves and muscles were stimulated during negative pressure challenge to the upper airway. Electrical stimulation of the genioglossus muscle in an isolated upper airway preparation in dogs resulted in more negative critical closing pressures (42). Endoscopically, this selective electrical stimulation was correlated with increased

nasopharyngeal dimensions (42). Similarly, the critical closing pressure was found to be linearly related to the peak electrical activity of the genioglossus muscle. Specifically, as electrical stimulation of the genioglossus muscle increased, the critical closing pressure became more negative (42). Also, sectioning of the hypoglossal nerve, the motor supply to genioglossus, abolished the electromyographic activity of the genioglossus muscle during negative pressure application to the upper airway and resulted in less negative critical closing pressure in an anesthetized rabbit model (39). The conclusions from these studies indicate that 1) electrical stimulation of the genioglossus muscle stabilizes and dilates the pharynx, and 2) impaired genioglossus function resulted in a more collapsible pharynx. Therefore, contraction of upper airway muscles, such as the genioglossus, is important to maintain upper airway patency during breathing.

The respiratory activity of the tongue musculature

Regulation of the respiratory activity

A multitude of studies have shown that the genioglossus muscle has phasic respiratory electromyographic activity (22,46–50,86). The onset of this activity as well as its peak activity occurs several msec before the onset of the diaphragmatic activity (52,62). This allows the upper airway to be supported before the initiation of negative pressure in the airway, preventing collapse (47,51,62). The activity of the upper airway muscles is closely regulated in a manner that can fulfill the demands of the working respiratory system, maintaining the balance between upper and lower respiratory tract activity.

The upper airway muscle activity is regulated and tuned by three mechanisms that involve mechanoreceptors, stretch receptors, and chemosensitive receptors.

Mechanoreceptors

Mechanoreceptor regulation involves pressure and flow receptors in the mucosa of the upper airway, especially the nasal and supraglottal mucosa. Mechanoreceptors in the nasal mucosa are innervated by branches of the trigeminal nerve (V), while receptors in the laryngeal mucosa are innervated by branches of the superior laryngeal from the vagus nerve (X). Studies in several species using isolated upper airway models have shown that increased negative upper airway pressure results in increased upper airway muscle activity, including the genioglossus muscle (52,58,59-64). Sectioning of the vagus and trigeminal nerves abolished this increased activity (51,61,62,64). Topical anesthesia of the nasal, pharyngeal, and laryngeal mucosa also decreases the electromyographic activity of upper airway muscle response to negative pressure stimulation, but does not abolish the response completely (65,73-75).

Stretch receptors

This is due to the presence of drive or stretch receptors, which are also mechanoreceptors, deep to the mucosal layer. These receptors are sensitive to changes in muscle length and position (66). These stretch receptors have been found in the extrinsic tongue muscles (genioglossus, styloglossus, and hyoglossus) and intrinsic tongue muscles (70–72).

Hering and Breuer reported in 1868 that inflation of the lung led to a decrease in respiratory frequency, which is well known as the Hering-Breuer reflex. This effect is due to the activation of stretch receptors in the smooth muscle of the bronchial wall (66). Activation of the receptors leads to suppression of the central medullary respiratory centers via the vagus nerve. Consequently, this leads to decreased activity of the

diaphragm and intercostal muscles. Transection of the vagus nerve abolishes the reflex. This vagally mediated reflex also depresses the activity of the upper airway muscles. Bilateral vagotomy results in increased peak electromyographic activity and prolonged phasic inspiratory genioglossus activity in dogs (76), and increased hyoglossal muscle activity in the rat (86). Therefore, lung inflation has a strong inhibitory effect on the respiratory-related activity of the upper airway nerves and muscles, including the hypoglossal nerve and the extrinsic tongue muscles (86).

Chemoreceptors

Central and peripheral chemoreceptors control respiratory drive (80,81). The central receptors in the respiratory centers measure CO₂ fluctuations in the cerebrospinal fluid, while glomus cells in the carotid bodies and aortic arch are sensitive to hypercapnia as well hypoxia of arterial blood. Consequently, stimulation of these receptors will modulate the intrinsic rhythm of the cells in the medullary respiratory centers. This in turn will affect the activity of the primary respiratory muscles: the diaphragm and intercostal muscles (80–82). Chemoreceptor modulation is also an important activator of the accessory respiratory muscles in the upper airway (53). Normo- and hyperoxic hypercapnia causes increased activity of the genioglossus muscle in cats, dogs, rats, and humans, and the styloglossus and hyoglossus muscles in rats (22,63,83,85,86). Similarly, severe poikilocapnic hypoxia resulted in increased genioglossus, styloglossus, and hyoglossus activity in rats and people (22,85–86).

The physiologic role of the tongue in upper airway stability and dimension Introduction

I have reviewed the anatomy of the tongue and emphasized the effect of the tongue muscles on the hyoid apparatus and their combined action on the nasopharynx. This review has clarified the function of the tongue muscles, especially the genioglossus, in nasopharyngeal dilation and stability. Based on this information the tongue muscles can be considered accessory or secondary muscles of respiration, and dysfunction of these muscles could potentially play a role in upper airway obstruction, such as OSA and DDSP in horses.

Indeed, in an endoscopic study on human patients suffering from OSA and control patients, Ferguson and colleagues found that voluntary tongue protrusion resulted in a significant increase in the cross-sectional area (CSA) of the upper airway at the level of hypopharynx (area just in front of the glottis), the oropharynx, and the velopharynx (portion of the nasopharynx where the soft palate forms the floor) compared to these areas when the tongue is in a neutral position (87). Similarly, with the aid of fluoroscopy, Kobayashi et al. measured a small but significant increase in the retroglossal space (space from the caudal margin of the soft palate to the tip of the epiglottis) in human subjects with a laryngectomy during a resistive breathing protocol and correlated this with increased genioglossus EMG activity (88). The increase was attributed to forward movement of the posterior part of the tongue during inspiration (88).

Co-activation

The results of the majority of the studies discussed thus far have focused on the effect of tongue protrusion and genioglossus activity. Genioglossus muscle activity has

mostly been studied selectively, disregarding the activity of the antagonistic muscles, the hyoglossus and styloglossus.

The most current research has focused on the physiological role of the tongue as a whole, investigating genioglossus, hyoglossus, and styloglossus activities. Genioglossus, styloglossus, and hyoglossus are co-activated during breathing and have increased peak activity during inspiration in anesthetized rats breathing a hyperoxic hypercapnic gas mixture (22,86). Similar results were obtained in studies on awake human patients breathing a hypoxic hypercapnic gas mixture (85). In these experiments it was also found that the activity of genioglossus, hyoglossus, and styloglossus muscles precedes inspiratory flow (85). Interestingly, the net effect of simultaneous activity of these muscles was actually tongue retraction, not tongue protrusion. This occurred because the retractor muscles generate 10 times more force than the protrudor muscle (19). These results were comparable with the results of whole hypoglossal nerve stimulation, in which tongue protrudor and retractor were simultaneously activated and tongue retraction occurred (19,20,23).

Indeed, several studies found that direct electrical stimulation of the genioglossus muscle, or stimulation via the medial branch of the hypoglossal nerve, resulted in visible tongue protrusion with increased inspiratory flow, decreased nasopharyngeal resistance, and no changes in pharyngeal closing pressure (20,21,24). Contrary to this, the direct stimulation of the hypoglossus muscle, or its stimulation via the lateral branch of the hypoglossal nerve, resulted in tongue retraction, which was associated with decreased inspiratory flow and no changes in nasopharyngeal resistance (20,21,24). However, simultaneous stimulation of tongue protrudor and retractor muscles resulted in net tongue retraction with increased inspiratory flow, more negative pharyngeal closing pressure,

and no change in nasopharyngeal resistance (20,23). Most of these studies used experimental settings in which the mouth was sealed. Interestingly, in a recent rat study, genioglossus muscle stimulation with an open-mouth model resulted in increased inspiratory airflow that was more pronounced than with the mouth sealed, and did not cause a significant change in the nasopharyngeal critical closing pressure. Co-stimulation of tongue protrudor and retractors, on the other hand, was not influenced by the open mouth model and resulted in similar increases in inspiratory flow and decreases in nasopharyngeal critical closing pressure. Thus it appears that protrudor activation as well as co-stimulation of tongue protrudor and retractor muscles can improve inspiratory airflow, but due to different mechanisms. Co-activation of these muscles results in decreased pharyngeal closing pressure, indicative of improved airway stability, while protrudor muscle stimulation results in increased pharyngeal dimensions, mostly of the oropharynx. Therefore, genioglossus activity likely increased the size of the oropharynx to a greater degree than the nasopharynx.

Several studies support these findings. Unpublished observations by Fregosi et al. using magnetic resonance imaging in a selective nerve stimulation protocol in anesthetized rats suggested that co-stimulation of these muscles depressed the tongue and enlarged the retroglossal space (20,86). Considering that the tongue-retractors produce 10 times more force than the tongue protrudor (19), voluntary tongue protrusion resulted in increased oro- and hypopharyngeal CSA in a computed tomography (CT) study by Edmonds et al. in patients suffering from OSA (27). Ferguson et al. found a similar effect on oro- and hypopharyngeal CSAs with an endoscopic study in healthy volunteers although there was also a significant influence on the velopharyngeal diameters (87). Co-activation of the tongue musculature during a resistive breathing protocol in

laryngectomized patients in a CT study by Kobyashi et al. resulted in increased retroglossal space enlargement (88). Therefore, these studies support the idea that tongue musculature activity and position influences the oropharynx and retroglossal space more than the nasopharynx in humans. More important is the subsequent finding by Hida et al. that direct whole hypoglossal nerve stimulation resulted in a 25% decreased compliance in the isolated canine upper airway (26), while transcutaneous submental electrical activation (which results in similar effects as whole hypoglossal stimulation) completely abolished the collapse of the oro- and nasopharynx due to negative pressure applied to the airway in the study by Edmonds et al. on his OSA patients (27). This supports the idea that increased rigidity and stability of the upper airway is due to activity of the tongue musculature.

This stability can be additionally increased with activity of other secondary muscles of respiration. Especially the muscles of the sterno-thyro-hyoid group have been shown to have phasic inspiratory activity (45,47). Their action results in airway elongation and decreased pharyngeal closing pressures (41,43-45). Additional tongue protrusion has shown to enhance this effect (43). Indeed, in exercising horses the effect of sternothyrohyoid myectomy resulted in increased translaryngeal and tracheal inspiratory pressures and resistance, indicating decreased stability of the upper airway (89).

CHAPTER 2

THE EFFECT OF A TONGUE-TIE ON UPPER AIRWAY FUNCTION IN EXERCISING HORSES

Abstract

Objective

The objective of this study was to determine the effect of a tongue-tie on upper airway mechanics in exercising horses.

Animals

5 Standardbreds were used in this study.

Procedure

Peak inspiratory and expiratory tracheal and pharyngeal pressures and airflow were measured while horses exercised on a treadmill with and without a tongue-tie. Respiratory frequency was also measured. Horses ran at speeds that corresponded to 50%, 75%, 90%, and 100% of maximal heart rate. The tongue-tie was applied by pulling the tongue forward out of the mouth as far as possible and tying it at the level of the base of the frenulum to the mandible with an elastic gauze bandage.

Inspiratory and expiratory tracheal, pharyngeal, and translaryngeal impedance, minute ventilation, and tidal volume were calculated. Data were analyzed with a two-way

ANOVA for repeated measures. Results were considered significant if P < 0.05. For the post-hoc comparison of significant data the Student-Newman-Keuls test was used.

Results

We were unable to detect a significant difference between groups for inspiratory or expiratory tracheal or pharyngeal impedance, peak pressure, peak expiratory flow, tidal volume, respiratory frequency, or minute ventilation. Horses that ran with a tonguetie had significantly (P < 0.0009) higher peak inspiratory flows than horses that ran without a tongue-tie. In the post-hoc comparison this effect was significant at 4 m/s, HR_{50} , and HR_{90} .

Conclusion

Application of a tongue-tie did not alter upper respiratory mechanics in exercising horses.

Clinical relevance

Application of a tongue-tie may be beneficial in exercising horses with certain kinds of obstructive upper airway dysfunction but does not improve upper airway mechanics in normal horses.

Introduction

Many racehorses with a complaint of poor racing performance run with their tongues tied in an attempt to improve upper airway function. Historically, reported use of a tongue-tie dates from as far back as 1889 when it was recommended "to tie the

tongue to the floor of the mouth to get rid of annoying respiratory noise during exercise" (1). However, although today's application of a tongue-tie still focuses on respiratory noise in conjunction with poor performance, many horses are run with their tongues tied to improve racing performance. Frequently, the cause of poor performance has not been diagnosed in these horses. This broad and ill-defined application of the tongue-tie in racehorses makes it difficult to determine if, indeed, tying the horse's tongue affects the horse's performance and upper airway function. As well, there is no objective scientific data regarding the influence of a tongue-tie on performance or upper airway mechanics in horses.

Studies in other species have shown that activity of extrinsic tongue muscles, especially the genioglossus muscle, may be important in upper airway stability and patency (20,21,23,24,39,42,87,88). The genioglossus muscle is an extrinsic tongue muscle that is responsible for tongue protrusion (14). Based on comparative literature on the function of the genioglossus muscle, we hypothesized that horses running with their tongues tied would have improved upper airway mechanics. Therefore, the purpose of this study was to determine upper airway mechanics in exercising horses with and without a tongue-tie.

Material and methods

Horses

Five Standardbred horses were studied. Four horses were geldings and one horse was a mare. Horses were 5 to 11 years old and weighed 486 to 527 kilograms. The study was approved by the All-University Committee for Animal Use and Care. Horses were maintained on pasture and vaccinated against tetanus, equine influenza, equine

rhinopneumonitis, EEE/WEE and *Streptococcus equi*. Physical examination of the horses, as well an endoscopic exam of the upper respiratory tract at rest and while exercising on a treadmill revealed no abnormalities. Prior to the experiments, the horses were trained to run on the treadmill. The speeds corresponding to maximal heart rate (HR_{max}) for each horse were determined during an incremental exercise test and heart rate was determined with a telemetric ECG system^a. The speeds corresponding to HR₅₀, HR₇₅, and HR₉₀ were subsequently interpolated from these data (90).

Instrumentation

One hundred and fifty cm long polyethylene (polyethylene tubing, 2.15 mm ID, 3.25 OD)^b sidehole catheters were used to measure tracheal and nasopharyngeal pressures. Tracheal and pharyngeal pressures were measured by use of differential pressure transducers^c and recorded on a respiratory function computer. The transducers were calibrated before each experiment with a water manometer.

Airflow was measured with a 15.2 cm-diameter pneumotachograph^d, which was fitted on a fiberglass face mask. The resistance of the pneumotachograph was 0.04 cm H_2O L⁻¹ s⁻¹ at 90 Ls⁻¹, while the combined resistance of face mask plus pneumotachograph was 0.05 cm H_2O L⁻¹ s⁻¹ at 90 Ls⁻¹ Before each experiment the pneumotachograph was calibrated with a rotameter^e capable of measuring flows up to 90 Ls⁻¹.

Tongue-tie

A tongue-tie was applied by pulling the tongue out of the mouth as far as possible. Subsequently, at the level of the base of the frenulum, the tongue was tied to the mandible with an elastic gauze bandage. The catheters for tracheal and pharyngeal

pressures were passed through the right naris and under endoscopic guidance. The tracheal catheter was positioned at the level of the upper proximal third of the cervical trachea and the nasopharyngeal catheter was positioned at the level of the opening of the right guttural pouch. The face mask was fitted on the horse's heads with a rubber shroud and adhesive to ensure that the face mask was airtight. The pneumotachograph was then attached to the mask.

Experimental design

The horses were run on a high-speed treadmill with or without a tongue-tie in a randomized cross-over design. There were 2-4 days between trials. Horses warmed-up on the treadmill at 4 m/s for 3 minutes and then ran at the speeds corresponding to HR₅₀, HR₉₀ and HR_{max} for 60 s at each speed. Pressures and flow measurements were collected on the respiratory computer.

Data analysis

An average of 10 consecutive breaths was calculated for peak inspiratory and expiratory tracheal and pharyngeal pressure and flows at each speed. Inspiratory and expiratory tracheal and pharyngeal impedance (Z) was calculated as peak pressure divided by peak flow. The difference between peak tracheal pressure and peak pharyngeal pressure during inspiration or expiration divided by the corresponding peak flow was calculated to determine the translaryngeal inspiratory and expiratory impedances. Respiratory computer calculated tidal volume by integrating airflow. Respiratory frequency was determined by counting the number of breaths during the last

30 s at each speed. Minute ventilation was calculated as tidal volume \times respiratory frequency per minute.

Data were analyzed by a two-way ANOVA for repeated measures. Post-hoc pair wise comparisons were made for significant data using the Student-Newman-Keul's test. The level of significance was set at P < 0.05.

Results

Peak inspiratory flow was significantly higher in horses exercising with a tonguetie than those without a tongue-tie (P < 0.009). In the post-hoc comparison this was significant at the speeds 4m/s, HR_{50} , and HR_{90} (Table 1). We were unable to detect a significant difference in any of the other variables measured or calculated for horses running with or without a tongue-tie.

Table 1: Upper airway mechanics measurements in horses running on a treadmill with (TT) or without (NTT) a tongue-tie (mean \pm std.)

	NTT/ TT	SPEED					
Measure- ment		4 m/s	HR _{so}	HR ₇₅	HR ₉₀	HR ₁₀₀	
FLOW _{IN}	NTT	34.3 ± 6.8	39.1 ± 7.5	51.7 ± 6.3	58.9 ± 3.5	62.7 ± 5.8	
	TT	38.8 ± 6.4*	44.6 ± 9.3*	55.0 ± 7.0	63.4 ± 4.6*	64.9 ± 4.0	
FLOW _{EX}	NTT	28.9 ± 3.6	37.5 ± 6.1	45.9 ± 3.9	50.2 ± 3.8	54.4 ± 1.9	
	TT	34.6 ± 4.9	42.0 ± 4.4	49.0 ± 4.0	54.7 ± 3.9	55.1 ± 3.4	
	NTT	2.83 ± 1.1	3.6 ± 1.1	4.5 ± 1.2	4.8 ± 1.3	5.3 ± 1.3	
VT,	TT	2.2 ± 0.5	2.9 ± 0.8	3.9 ± 0.6	4.2 ± 0.6	4.5 ± 0.9	
_	NTT	79.3 ± 22.4	77.1 ± 16.1	73.5 ± 11.8	81.8 ± 10.6	83.1 ± 9.8	
F _{RESP}	TT	90.6 ± 11.4	85.6 ± 11.5	82.2 ± 7.6	85.0 ± 7.6	82.0 ± 14.0	
VENT	NTT	262 ± 120	310 ± 113	371 ± 114	417 ± 141	435 ± 133	
VENT _{MIN}	TT	191 ± 80	244 ± 82	307 ± 70	370 ± 86	403 ± 98	
D	NTT	14.0 ± 3.1	16.8 3.8	25.9 ± 7.6	30.8 ± 5.6	33.6 ± 5.9	
P _{TRIN}	TT	14.4 ± 2.7	18.9 ± 6.1	25.7 ± 7.0	33.4 ± 5.0	36.3 ± 5.5	
	NTT	7.4 ± 1.1	9.2 ± 0.7	11.4 ± 1.3	12.0 ± 1.0	12.8 ± 1.6	
P _{TREX}	TT	7.5 ± 1.0	10.3 ± 2.3	12.4 ± 2.7	12.7 ± 2.1	13.3 ± 2.0	
7	NTT	0.41 ± 0.12	0.44 ± 0.11	0.51 ± 0.16	0.53 ± 0.11	0.54 ± 0.11	
Z _{TRIN}	TT	0.37 ± 0.07	0.43 ± 0.13	0.47 ± 0.14	0.53 ± 0.11	0.56 ± 0.10	
_	NTT	0.24 ± 0.06	0.25 ± 0.04	0.25 ± 0.04	0.24 ± 0.02	0.24 ± 0.04	
Z _{TREX}	TT	0.22 ± 0.04	0.25 ± 0.06	0.26 ± 0.06	0.23 ± 0.03	0.24 ± 0.04	
P _{PHIN}	NTT	11.7 ± 3.0	13.9 ± 4.0	19.8 ± 4.7	21.7 ± 4.9	23.3 ± 6.9	
	TT	11.6 ± 3.2	13.7 ± 3.0	18.7 ± 4.4	21.5 ± 6.2	22.8 ± 6.8	
P _{PHEX}	NTT	4.6 ± 0.8	6.2 ± 0.8	8.2 ± 1.7	8.1 ± 1.2	9.2 ± 1.9	
	TT	5.4 ± 1.7	6.8 ± 1.1	8.3 ± 1.8	9.4 ± 2.1	9.6 ± 2.8	
Z _{PHIN}	NTT	0.34 ± 0.09	0.35 ± 0.09	0.39 ± 0.10	0.37 ± 0.08	0.37 ± 0.09	
	TT	0.15 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.17 ± 0.04	0.17 ± 0.04	
7	NTT	0.16 ± 0.06	0.17 ± 0.05	0.18 ± 0.05	0.16 ± 0.03	0.17 ± 0.04	
Z _{PHEX}	TT	0.15 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.17 ± 0.04	0.17 ± 0.04	

Table 1 (cont'd).

Measure-	NTT/ TT	SPEED					
ment		4 m/s	HR ₅₀	HR ₇₅	HR ₉₀	HR ₁₀₀	
Z _{TRLARIN}	NTT	0.07 ± 0.10	0.09 ± 0.09	0.12 ± 0.10	0.16 ± 0.12	0.17 ± 0.11	
	TT	0.07 ± 0.10	0.12 ± 0.11	0.13 ± 0.13	0.19 ± 0.15	0.21 ± 0.15	
Z _{TRLAREX}	NTT	0.10 ± 0.04	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	
	TT	0.07 ± 0.06	0.08 ± 0.07	0.09 ± 0.06	0.06 ± 0.03	0.07 ± 0.06	

T = tongue-tie; NTT = no tongue-tie; Flow_{IN} = peak inspiratory flow (L/s); Flow_{EX} = peak expiratory flow (L/s); VT₁ = tidal volume (L); F_{RESP} = respiratory frequency (breaths/min); VENT_{MIN} = minute ventilation (L); P_{TRIN} = peak tracheal inspiratory pressure (cm H₂O); P_{TREX} = peak tracheal expiratory pressure (cm H₂O); P_{TRIN} = Tracheal inspiratory impedance (cm H₂O/L/s); P_{TRIN} = peak pharyngeal inspiratory pressure (cm H₂O); P_{PEX} = peak pharyngeal expiratory pressure (cm H₂O); P_{PEX} = peak pharyngeal expiratory impedance (cm H₂O/L/s); P_{TRIN} = Pharyngeal expiratory impedance (cm H₂O/L/s); P_{TRIN} = Translaryngeal inspiratory impedance (cm H₂O/L/s); P_{TRIN} = Translaryngeal expiratory impedance (cm H₂O/L/s)

Discussion

In this study, we were unable to measure any significant effect of a tongue-tie on upper airway mechanics in exercising horses. There was a significant increase in peak inspiratory flow in horses with a tongue-tie compared to horses without a tongue-tie. This increase in airflow was most likely due to a change in breathing pattern with the tongue-tie. This alteration in breathing pattern with the tongue-tie may have been caused by discomfort or anxiety related to having the tongue tied out of the mouth. However, we were unable to detect a significant effect of a tongue-tie on inspiratory or expiratory tracheal or nasopharyngeal impedance, minute ventilation, tidal volume, or respiratory frequency. Based on these results, application of a tongue-tie in horses with normal airway function will not improve upper airway mechanics.

Many racehorses perform with their tongues tied out of the mouth in an attempt to improve upper airway function and, therefore, performance. Often the reason for the poor performance is unknown and the tongue-tie is used as a palliative measure. To date, no scientific data was available concerning the effect of the tongue-tie on upper airway mechanics of the horse. However, research in other species has shown that genioglossus muscle activation, the major muscle that protrudes the tongue, dilates and stabilizes the upper airway (20,21,23,24,42,87,88).

A basic understanding of the tongue anatomy is helpful to understand why the genioglossus muscle is an important airway dilating muscle and the possible rationale, or lack thereof, for using a tongue-tie in horses with nasopharyngeal instability. Intrinsic and extrinsic muscles control the position and action of the tongue. The intrinsic muscles are located entirely within the tongue and they alter its shape and rigidity (14). The genioglossus and hyoglossus are two extrinsic tongue muscles that are innervated by different branches of the hypoglossal nerve (20,21). The genioglossus is a fan-shaped muscle that lies within and parallel to the median plane of the tongue (14). The genioglossus muscle originates from the medial surface of the mandible just caudal to the symphysis and is innervated by the medial branch of the hypoglossal nerve (14,20,21). A large tendon runs throughout the muscle. Muscle fibers radiate rostrally toward the tip of the tongue, dorsally, and toward the root of the tongue (14). The hyoglossus is a flat, wide muscle that lies in the lateral portion of the root of the tongue. The hyoglossus originates from the lateral aspect of the basihyoid bone and from the portions of the stylohyoid and thyrohyoid bones and is innervated by the lateral branch of the hypoglossal nerve (14,20,21). Contraction of the hyoglossus muscle retracts the tongue (14,20,21). Contractions of both the genioglossus and hyoglossus muscles depress the tongue (14,20,86). Contraction of the caudal genioglossus fibers protrudes the tongue, while the cranial fibers toward the tip of the tongue will retract the tip of the tongue (14).

The role of the genioglossus muscle in pharyngeal dilation and stabilization has been studied extensively in many species including humans. Many studies have shown that respiratory-related electromyographic activity recorded from the genioglossus muscle in humans and animals increases in response to hypoxia, hypercapnia, and airway occlusion (22,48-50,52,60,84-86). Electrical stimulation of the genioglossus muscle dilates and stabilizes the pharynx (24,42). As well, stimulation of the hypoglossal nerve dilates the pharynx, improves pharyngeal stability, and decreases the negative pressure needed to collapse the airway (20,21,23). Results of a study in people showed that active tongue protrusion by the subjects increased the CSA of the hypopharynx, oropharynx, and velopharynx as measured by endoscopy (87). This information led us to the hypothesis that pulling the tongue out of the horse's mouth and tying it would indeed dilate and stabilize the pharynx. However, passive traction on the tongue may be quite different from active contraction of the genioglossus muscle.

Recent information in rats and humans suggests that during intense breathing efforts, the genioglossus and hyoglossus muscles are co-activated (20,22,85,86). Hypoxia, hypercapnia, and airway occlusion caused parallel increases in drive to the tongue protrudor and retractor muscles (20,22,85,86). This activation consistently produced a net retraction force when the genioglossus and hyoglossus muscles were co-activated (20,22,23). Despite causing tongue retraction, co-activation of the protrudor and retractor muscles resulted in improved airflow mechanics and enhanced stability of the pharynx (20,23). These results seem somewhat contrary to previous studies that suggested that tongue protrusion was important in upper airway stability (24,87,88).

Indeed, contraction of the genioglossus muscle results in tongue protrusion and depression (14,20). Hyoglossus contraction also causes tongue depression, in addition to retracting the tongue (14,20). Therefore, tongue depression may be the critical force needed to dilate and stabilize the pharynx (20,86).

We measured the upper airway mechanics in exercising horses with normal upper airway function with and without a tongue-tie. We were unable to detect a significant difference in airway mechanics between these two groups. Our inability to show efficacy of the tongue-tie may relate to the normal airway function of the horses studied. We used intense exercise as a method of increasing respiratory effort in the horses. It is likely that the upper airway dilating and stabilizing muscles were functioning appropriately in these horses, since they had no evidence of upper airway abnormalities. If the genioglossus muscle was functioning appropriately, perhaps we should not have expected to detect a change in upper airway mechanics with the application of a tongue-tie. It may be useful to investigate the effect of the tongue-tie on horses with upper airway dysfunction. More likely, our inability to measure an effect of a tongue-tie on upper airway mechanics may be due to the mechanism of action of the genioglossus muscle during breathing. Application of a tongue-tie involves pulling the horse's tongue out of the mouth and tying it to the mandible or the bridle. This action is distinctly different from contracting the genioglossus muscle. The tongue-tie may protrude the tongue but not depress the tongue. Depression of the tongue may, indeed, be the critical action of the genioglossus and hyoglossus muscles in producing upper airway stability and dilation.

In conclusion, application of a tongue-tie did not improve upper airway mechanics in normal, exercising horses.

CHAPTER 3

A COMPUTED TOMOGRAPHIC STUDY OF THE EFFECT OF A TONGUE TIE ON HYOID APPARATUS POSITION AND NASOPHARYNGEAL DIMENSIONS IN ANESTHETIZED HORSES

Abstract

Objective

The objective of this study was to determine the effect of tongue protrusion on the position of the hyoid apparatus and upper airway dimensions in anesthetized horses.

Animals

Five adult horses were used in this study.

Procedure

Position and configuration of the hyoid apparatus as well as the dimension of nasopharynx were studied in anesthetized horses with or without a tongue-tie by means of CT in a cross-over design. The position and configuration of the hyoid apparatus, as determined by length D and angles α and β within the hyoid apparatus, were analyzed from computer-generated three-dimensional (3-D) images. The dimension of the nasopharynx was analyzed from standardized sagittal CSAs and dorso-ventral (DV)

diameter at several levels of the upper airway. In a similar way the CSA of the oropharynx was analyzed.

Data analysis and statistics

Hyoid apparatus length and angles were tested between groups for significance with wilcoxon signed rank test and paired t-test respectively. The DV-diameters and CSAs were tested with a two-way repeated ANOVA. A significance level of p=0.05 was chosen.

Results

There was no significant difference in length D and angles α and β of the hyoid apparatus, indicating no change in its position and configuration. Similarly, the nasopharyngeal DV diameters as well the naso- and oropharyngeal CSAs at all levels of the standardized slices were not significantly different with or without tongue-tie.

Conclusion

Tongue-tie does not influence the position of the hyoid apparatus and upper airway dimensions in anesthetized horses.

Introduction

Many of today's racehorses with a complaint of poor racing performance are raced with a tongue-tie in an attempt to improve upper airway function. The use of a tongue-tie has been reported as far back in 1889 in order "to get rid of annoying

respiratory noise during exercise" (1). However, to date no objective data are available regarding the effect of a tongue tie on upper airway dimension and stability in the horse.

The tongue is anatomically closely related to the nasopharynx structures. Important is the fact that the tongue is attached to the hyoid apparatus, which consists of a series of bony rods that articulate together and form a structure that suspends the tongue, larynx, and pharyngeal structures from the skull (30,33). Some of the articulations have a degree of movement and therefore can change the position and configuration of the hyoid apparatus. Since many pharyngeal structures are connected to the hyoid apparatus, this could result in changes in upper airway dimensions (39,40,41). The tongue itself consists of several muscles that are attached to the hyoid apparatus. The genioglossus muscle is the major tongue protrudor while the hyoglossus and styloglossus muscles are considered tongue retractors (14,36). Thus activity of these muscles could result in a position and or configuration change of the hyoid apparatus and subsequently change nasopharyngeal dimensions (39-41).

Indeed, recent research in several other animal species as well as in human beings actually supports a partial role for the tongue in maintaining upper airway patency and stability. These studies were able to demonstrate increased upper airway dimensions and/or improved dynamic respiratory parameters with voluntary tongue protrusion, selective genioglossus stimulation or co-stimulation of all tongue musculature indicating a role for the tongue in airway patency stability (20,21,25,28).

Based on these recent comparative data in human beings and other animal species we studied the effect of a tongue-tie on the position and configuration of the hyoid apparatus and nasopharyngeal dimensions by means of CT in anesthetized horses. We hypothesized that the application of a tongue tie will result in changes in the position and

configuration of the hyoid apparatus, and subsequently result in an increase in DV diameters and sagittal CSAs of the nasopharynx.

Material and methods

Horses

Five adult horses were used in this study, which was approved by the All-University Committee on Animal Use and Care: one Standardbred mare, one Standardbred gelding, one Appaloosa gelding, one Quarter Horse gelding, and one Haflinger cross gelding. Bodyweight varied between 335 to 502 Kg as did age from 2 to 25 years. The Haflinger-cross had a persistent tracheostomy from previous unrelated experiments. Horses were maintained on pasture for at least 30 days prior to study. All horses were vaccinated against equine influenza, rhinopneumonitis, EEE/WEE, and *Streptococcus equi*.

Anesthesia

After premedication with xylazine^a (0.5 mg/kg, IV), anesthesia was induced with ketamine^b (1.0 mg/kg, IV) and diazapam^c (0.1 mg/kg, IV). Anesthesia was maintained with an intravenous "Triple drip" of 5% Guaifenesin^d containing 0.5 mg xylazine^a/ml and 2.0 mg ketamine^b/ml, and delivered at a rate of 2 ml/kg/h. When the anesthesia depth was considered to light the infusion rate was increased and/or a 500 mg intravenous bolus of thiopental^c was given to archive satisfactory anesthesia depth. Oxygen was supplied via tracheal insufflation at a rate of 10 L/min. For this a nasally inserted tube with an outer diameter of 8 mm was positioned into the proximal third of the trachea.

Position and imaging

For imaging, the horses were positioned in sternal recumbency on a cushioned CT-table with the head and neck in extended position. Side reins were fitted from the halter to a previous fitted thoracic girth in order to stabilize the head and neck. Similarly, ropes from the thoracic girth were tied to the table in order to stabilize the body. The poll of the head and the nasomaxillary bone were also taped to the table to secure lateral and forward stability. The anatomical region spanning from the lateral canthus of the eye to the atlanto-occipital condyl was studied with a CT-scanner. Slice diameter of the images was 5 mm, resulting in a total of 35-40 slices per run. The first CT-run was without the use of a tongue-tie, followed by one with the tongue-tie applied. For this purpose, the tongue was pulled out and forward as far as possible via the lateral canthus of the mouth, and tied to the table with an elastic gauze bandage. The bandage was fixed around the tongue at the level of the base of the frenulum.

Data analysis

Data were collected from sagittal CT slices as well as computer-generated 3-D images of the equine skull. The rami of the mandible were removed with computer assistance from the lateral 3-D view in order to visualize the position and configuration of the hyoid apparatus. Measurements from lateral 3-D view included the length from the acoustic external meatus to the basihyoid (length D) and the angles between the basisphenoid and the stylohyoid bone (angle α) as well stylohyoid bone and ceratohyoid bone (angle β) (Figure 1). Length D and angles α and β were measured with a ruler and protractor, respectively. Bony markers on a paraxial view served as landmarks to generate four standardized sagittal slices at several levels of the upper airway (Figure 2).

From these slices the DV diameter and CSA were compared between images without and with the application of the tongue-tie. For this the images were loaded into PhotoShop 5.0 where DV diameter and the CSA of the nasopharynx were colored with computer assistance. Subsequently, the DV length and CSA of the colored surface was calculated in pixel numbers with a special computer software program. Additionally, the CSA of the oropharynx was processed in a similar way because DV oropharyngeal diameters were too difficult to assess.

Statistics

Statistical significance with or without a tongue-tie for length D was analyzed with a Wilcoxon Signed Rank Test, while angle α and angle β were tested for statistical significance with a paired t-test. Similarly the DV diameters and sagittal CSA's between groups were tested for significance with a two-way ANOVA for repeated measures. For all tests a significance level of 0.05 was chosen.

Results

We were unable to detect an overall significant change in any of the parameters of the hyoid apparatus (length D, angle α and β) as well as in any of the sagittal DV and CSA measurements at several levels of the nasopharynx. Data regarding the hyoid apparatus (D,α,β) did not show any change while data regarding nasopharyngeal dimension (DV, CSA) varied per individual horse. For instance the nasopharyngeal CSA for slice 1 increased in three horses while it decreased in the other two. The overall effect for all slices was not significant in any of nasopharyngeal DV and CSA

measurements. Similarly, no significant change in the oropharyngeal CSA could be detected (Tables 2 and 3).

Table 2: Length and angles in the hyoid apparatus with or without a tongue-tie from 3-D CT images (range or SD)

Parameter	NTT	TT	<i>p</i> -value
Length D (in mm)	39.5 (39-44)	39 (38.6–44.0)	1.00*
Angle α (in °)	42.4 ± 9.71	42.1 ± 7.94	0.77**
Angle β (in °)	57.1 ± 4.07	59.2 ± 3.49	0.26**

Length D: Median/range of length between the external acoustic meatus and

basihyoid bone.

Angle α : Mean and SD of angle between the stylohyoid and basihyoid bone.

Angle β : Mean and SD of angle between the stylohyoid and ceratohyoid bone.

*: Wilcoxon Signed Rank Test

**: Paired t-test

Table 3: Mean and SD dorso-ventral diameters and cross-sectional area surfaces for nasopharynx and oropharynx at four different sagittal upper airway levels of the horse with or without a tongue-tie

Parameter	NTT	TT
DV (in pixel)		
Slice 1	93.2 ± 28.8	100.4 ± 24.5
Slice 2	106.3 ± 28.3	105.4 ± 21.8
Slice 3	124.6 ± 33.7	128.2 ± 31.8
Slice 4	146 ± 35.8	158.8 ± 33.3
NPH-CSA (in pixels)		
Slice 1	5598 ± 2458	5847 ± 2358
Slice 2	5893 ± 4347	5257 ± 4498
Slice 3	5523 ± 1495	5628 ± 1262
Slice 4	6877 ± 1593	7419 ± 1492
OPH-CSA(in pixels)		
Slice 1	647 ± 704	649 ± 674
Slice 2	880 ± 1107	664 ± 775
Slice 3	638.2 ± 719	762 ± 671
Slice 4	279 ± 584	384 ± 453

NTT:

No Tongue-tie

TT:

Tongue-tie

NPH-DV: NPH-CSA: Nasopharyngeal dorso-ventral diameter Cross-sectional area of the nasopharynx

OPH-CSA:

Cross-sectional area of oropharynx

Discussion

Earlier studies in several animal species as well as in human beings suggested a role for the tongue in upper airway patency and stability (39,40,42,41,47). This was largely attributed to the tongue's connection to a flexible hyoid apparatus. Studies in rabbits and dogs, mimicking the protruding action of the genioglossus muscle with the anterior or ventral traction on the basilyoid bone, resulted in more negative nasopharyngeal closing pressures (39,41). Indeed, in subsequent isolated airway studies the electrical stimulation of genioglossus muscle, or its innervation, all resulted in significant increased inspiratory flow as well decreased (i.e., more negative) nasopharyngeal closing pressures indicating improved stability (20,21,25,28). Similarly, voluntary tongue protrusion in human beings resulted in an endoscopically enlarged velopharyngeal CSA, while a resistive breathing protocol resulted in a small but significant fluoroscopic increase of the retroglossal pharyngeal space in laryngectomized patients (87,88). These increases were subsequently associated with the increased inspiratory genioglossus activity. However, most of these studies related forward movement of the tongue to airway dimension, thereby ignoring parallel activity of other airway muscles that could have contributed to increased CSA and/or pharyngeal stability. Recent research has found that during inspiration tongue protrudor and retractor muscles both show increased inspiratory activity, and that their effect results in tongue retraction as well as tongue depression (22,85). Subsequently, tongue protrudor and retractor coactivation has been shown to result in an increased inspiratory flow and decreased (i.e., more negative) pharyngeal closing pressure (20,21,28). Most of the experimental animal studies also used protocols in which the mouth was sealed. However, recently a study in the rat with a mouth-open model showed that selective protrudor stimulation only

resulted in increased inspiratory flow. Simultaneous protrudor and retractor stimulation with an open or sealed model was actually not affected and still resulted in the same increase in inspiratory flow and decrease (=more negative) closing pressures (20). The study concluded that during an open-mouth protocol, tongue protrusion at best results in increased oropharyngeal dimension with no effect on nasopharyngeal stability, and that co-stimulation of tongue protrudor and retractor muscles are more important in nasopharyngeal stability. Tongue protrusion in an anesthetized cat model with open mouth also failed to improve nasopharyngeal respiratory parameters, supporting this conclusion (43). This finding is important since the horse is an obligate nasal breather and in our study the mouth was not sealed.

Our study did not find any significant change in position of the hyoid apparatus. A possibility for lack of change of the hyoid apparatus could be explained by the fact that the head-neck position was extended. This could have caused maximal caudal traction on the hyoid apparatus by the sterno-thyro-hyoid group and fixated the hyoid apparatus in a more caudal-ventral position. However, a mildly extended head-neck position most closely resembles the natural head carriage of horses under racing conditions, so applying a tongue-tie in the awake, non-relaxed horse would have had even less effect on the position and configuration of the hyoid apparatus. Because so many upper airway structures are attached to the hyoid apparatus, the lack of change in its conformation could also partly explain the lack of change of the CSA in the studied part of the nasopharynx. However, the research comparing the sealed versus the open-mouth protocols also suggests that tongue protrusion with an open mouth will more likely affect oropharyngeal dimensions. The lack of change in the oropharynx in our study is not clear. It is possible that anesthesia could have affected the muscle tone of the tongue,

subsequently resulting in an already relaxed and depressed tongue and ventrally deviated palate. Anesthesia however was not reported to be a problem in the variety of animal studies and in gallamine-induced muscle-paralyzed cats, rabbits, or dogs the airway did not became more collapsible (43,56,57). Our standardized sagittal slices focused on nasopharynx in the hyoid apparatus region, and thus relatively caudal to the body and attachments of the tongue (Figure 2). It is possible that changes in the oropharynx therefore happened more cranially and were missed on our standardized slices.

The most compelling explanation for lack of significant findings in our study is that the tongue-tie relies on passive traction of the tongue and not on muscle contraction. In all studies that reported increased naso-pharyngeal dimension or increased critical closing pressure with genioglossus contraction, the muscle was stimulated primarily or activated by electrostimulation of the hypoglossal nerve (19–28). In our study, the tongue was passively pulled out of the mouth in anesthetized horses. There is no reason to suspect that any of the tongue muscles or the hypoglossal nerve were stimulated by this passive action.

Based on these CT observations in combination with the literature, it can be concluded that tongue-protrusion as a result of a tongue-tie is not likely to influence nasopharyngeal dimensions in horses. This is supported by recent data in which application of a tongue-tie did not change upper airway dynamic parameters in normal horses during a treadmill exercise protocol (see chapter 2). However, it cannot be ruled out that tongue protrusion, especially with a closed mouth, could influence airway stability.

GENERAL CONCLUSION

Many horses on the racetrack today are run with a tongue-tie because of poor performance, presumably caused by upper airway collapse and instability during intensive exercise. The rationale behind the use of the tongue-tie is ill defined but relates to the tongue's close proximity to the part of the upper airway that is most prone to collapse and instability during exercise: the nasopharynx. For this reason, the tongue-tie is probably considered to be an upper airway dimension-enhancing device. Although the tongue-tie has been around for a long time, to date no scientific data are available regarding its effect on upper airway dimension and stability in the exercising horse.

However, research in human beings and several other species has revealed that the tongue musculature contributes to upper airway stability and dimension. This research found that the tongue protrudor muscle (genioglossus), as well that the tongue retractor muscles (stylo- and hyoglossus), should be considered accessory muscles of respiration. A variety of experimental settings have shown that activity of these muscles influences upper airway dynamic parameters, therefore suggesting an influence on upper airway stability and or dimension. Depending on the activity or co-activity of the tongue muscles these effects have been associated with tongue protrusion, tongue retraction, and depression or tongue retraction alone. An important factor of the tongue's influence on the upper airway is its attachment to a flexible hyoid apparatus. Since so many pharyngeal structures are connected to this hyoid apparatus, a change in the position of

the tongue could result in a change in the position and configuration of the hyoid apparatus, and thus a change in upper airway dimension and/or stability. Most recent research suggests that the co-activation of the tongue musculature is most likely to contribute to upper airway stability, regardless of whether the mouth is open or closed. Tongue protrusion alone, however, most likely influences oropharyngeal dimension, especially when the mouth is closed.

Based on the comparative literature the aim of this thesis was to investigate the effect of the tongue-tie on upper airway dynamic parameters and dimension in the healthy horse. The effect of the tongue-tie on dynamic respiratory parameters was studied in an incremental treadmill exercise protocol, while the influence on position and configuration of the hyoid apparatus as well the nasopharyngeal dimension was investigated by means of a CT study on anesthetized horses. The results from these two studies show that the tongue-tie does not influence dynamic respiratory parameters or cause a change in position and configuration of the hyoid apparatus in healthy horses. This coincides with lack of chance in the caudal nasopharyngeal dimensions, the part that is most prone to collapse during intensive exercise. No significant change in oropharyngeal dimensions was evident either. These two studies show that although the application of a tongue-tie mimics the activity of the genioglossus muscle, it appears that applying a tongue-tie does not influence upper airway dimension. Therefore it will not improve exercise performance. These studies do not rule out that a tongue-tie could result in increased stability of the upper airway, especially in horses with some form of collapse of the nasopharynx. Based on recent literature such an effect is only likely to occur in conjunction with simultaneous activity of the sternothyrohyoid musculature.

Thus application of a tongue-tie in healthy horses, or horses with lack of instability of the nasopharynx, will not benefit from a tongue-tie.

APPENDIX A

Footnotes to chapter 2 material and methods

^aDigital VHF Telemetry System, MI403A, Hewlett Packard, Palo Alto, CA

^bBaxter Scientific Products, McGraw Park, IL

^cD{-45-22, Validyne Engineering Sales, Norhtridge, CA

^dLaminar flow straightener element, Merriam Instruments, Grand Rapids, MI

^cModel FP-2-37-10/77, Fisher & Porter Co, Warminster, PA

APPENDIX B

Footnotes to chapter 3 material and methods

^aXylazine-100 injectable; The Butler Company, Columbus, OH

^bKetaset; Fort Dodge Animal health, Fort Dodge, IO

^cDiazapam 5mg/ml; Elkins-Sinn, Cherry Hill, NJ

^dGuaifenesin injection 50 mg/ml; Phoenix Scientific Inc, St Joseph, MO

'Pentothal; Abbott laboratories, North Chicago, Il

^fGeneral Electric CT-9800 scanner; GE Medical Systems, Milwaukee, WI

APPENDIX C

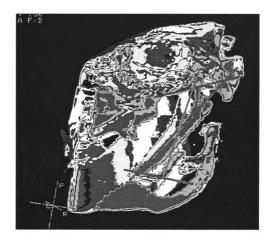


Figure 1: Measurement of length D and angles α and β for studying a change in position and configuration of the hyoid apparatus

Length D: The length from the acoustic external meatus to the basihyoid bone Angle α : Angle between the basisphenoid and the stylohyoid bone

Angle β : Angle between the Stylohyoid bone and Ceratohyoid bone

APPENDIX D

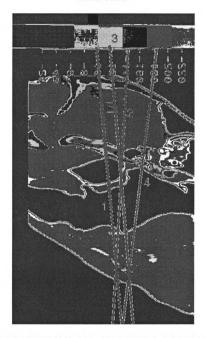


Figure 2: Overview of level of Slices 1 to 4 from a midsagittal view of the equine skull

Bony markers on a paraxial view serve as landmarks to generate four standardized sagittal slices at several levels of the upper airway. From these slices the dorso-ventral diameter (DV) and cross-sectional area (CSA) were compared between images without and with the application of the tongue-tie.

APPENDIX E





Figure 3: Example of dorso-ventral diameter and cross sectional area of the nasopharynx without (A) and with a tongue-tie (B).

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