

Standing left recurrent laryngeal neurectomy for prospective evaluation of laryngeal hemiplegia evaluated by a high-speed treadmill test

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OBJECTIVE

To describe left recurrent laryngeal neurectomy (LRLn) performed under standing sedation and evaluate the effect of LRLn on upper respiratory tract function using a high-speed treadmill test (HST). We hypothesized that (1) unilateral LRLn could be performed in standing horses, resulting in ipsilateral arytenoid cartilage collapse (ACC); and (2) HST after LRLn would be associated with alterations in upper respiratory function consistent with dynamic ACC.

ANIMALS

6 Thoroughbred horses.

METHODS

The horses were trained and underwent a baseline HST up to 14 m/s at 5% incline until fatigue. Evaluation included; airflow, pharyngeal and tracheal pressures, and dynamic upper respiratory tract endoscopy. Trans-laryngeal impedance (TLI) and left-to-right quotient angle ratio (LRQ) were calculated after testing. The following day, standing LRLn was performed in the mid-cervical region. A HST was repeated within 4 days after surgery.

RESULTS

Standing LRLn was performed without complication resulting in Havemayer grade 4 ACC at rest (complete paralysis) and Rakestraw grade C or D ACC (collapse up to or beyond rima glottis midline) during exercise. Increasing treadmill speed from 11 to 14 m/s increased TLI ($P < .001$) and reduced LRQ ($P < .001$). Neurectomy resulted in an increase in TLI ($P = .021$) and a reduction in LRQ ($P < .001$).

CLINICAL RELEVANCE

Standing LRLn induces laryngeal hemiplegia that can be evaluated using a HST closely after neurectomy. Standing LRLn may be useful for future prospective evaluations of surgical interventions for laryngeal hemiplegia.

Keywords: larynx, horse, airway, recurrent laryngeal nerve

Recurrent laryngeal neuropathy (LRLN) is a common performance-limiting condition of athletic horses that causes progressive atrophy of the cricoarytenoideus dorsalis (CAD) muscle and subsequent dynamic arytenoid cartilage collapse (ACC).¹⁻³ The reduction in rima glottis cross-sectional area often increases as speed increases, due to more negative airway pressures.⁴

High-speed treadmill studies are commonly used, both clinically and in a research setting, to

assess respiratory function and metabolic alterations in exercising horses utilizing speeds up to 14.5 m/s.⁵⁻⁹ Ergospirometry is a diagnostic tool used to continuously measure respiration and gas metabolism during exercise. The procedure can be performed to obtain objective data on ventilatory variables predictive of performance. In humans, VO_2 peak (the highest value oxygen consumption obtained during testing) is considered the best indicator of performance,¹⁰ and as such a number of respiratory masks have been developed in an attempt to accurately measure this in equine athletes.¹¹⁻¹³ Unfortunately, due to technical difficulties surrounding in field application of respiratory masks, their use is currently confined to larger clinical and research institutions.¹³

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Left recurrent laryngeal neurectomy (LRLn) has previously been performed under general anesthesia as a means of inducing laryngeal hemiplegia when evaluating the effects of laryngeal dysfunction and potential treatments.^{4,8,14-17} Standing surgical procedures are desirable in equine clinical practice due to the relatively high risk associated with anesthesia in horses compared to other species.^{18,19} As such, there has been a push to perform surgical procedures standing where possible. Furthermore, they often require fewer personnel and are cheaper to perform compared to under general anesthesia.²⁰ To date, there is little quantitative information on the short-term postoperative effect of LRLn on a high-speed treadmill test (HST) reaching 14 m/s. Additionally, the LRLn surgery has not been described as a standing procedure. Therefore, the objectives of the present study were to (1) describe LRLn performed in standing sedated horses and (2) quantify the early postoperative effect of LRLn on upper respiratory function during HST.

We hypothesized that: (1) LRLn could be performed safely and easily in standing horses, resulting in exercising ACC; and (2) HST within 4 days post-LRLn would be associated with alterations in upper respiratory function consistent with exercising ACC.

Materials and Methods

Animal ethics was acquired (IACUC approval) and 6 retired Thoroughbred racehorses (4 geldings and 2 mares, 4 to 10 years, 473 to 526 kg) were enrolled in the study. Horses were housed in small (6 m X 6 m) individual outdoor pens each with a shelter, and maintained on a standard feeding protocol for the duration of the study. In all horses, physical examination and resting upper respiratory tract endoscopy (URTE) confirmed normal laryngeal anatomy and function (Havemeyer grade 1 or 2.1) with no evidence of gross tracheal contamination or mucous. Before testing, the horses were trained for 6 weeks on the treadmill to obtain fitness, using the protocol outlined by Nagahisa et al.²¹ Specifically, the horses were trained 3 days/week on a treadmill inclined at 5%. Initially, the training session consisted of warm-up (2 m/s for 1 min and 4 m/s for 2 min), cantering (7 m/s for 1 min), and gallop (9 m/s for 2 min). Over the 6-week training duration, the speed of time the horses were galloped at was increased from 9 m/s to 12 m/s. A respiratory mask similar to that of the ergospirometer was worn by the horses during the final week of training to allow acclimatization to its application. Subsequently, horses underwent a HST during which measurements of the following parameters were made: trans-laryngeal pressure, translaryngeal air flow, and dynamic upper respiratory tract examination. After HST, trans-laryngeal impedance (TLI) was calculated by dividing inspiratory trans-laryngeal pressure by inspiratory translaryngeal air flow (mmHg/L/s). Horses were assessed at rest, 11, 12, 13, and 14 m/s (all at 5% incline), and 2 minutes post-HST.

HST protocol

Horses were walked in hand for 5 minutes and warmed up on the treadmill for 3 minutes; walk (2 m/s) for 30 seconds, trot (4 m/s) for 2 minutes, canter (9 m/s) for 30 seconds, on a 5% incline. Subsequently, the treadmill was stopped, and the horses were instrumented with the airtight face mask, endoscope, and trans-laryngeal catheters via the left nostril. At the start of testing the horses were galloped at 11 m/s and the speed increased by 1 m/s every 30 seconds to 14 m/s. Horses were held at 14 m/s until they displayed signs of fatigue and could no longer maintain the speed needed to keep up with the treadmill, at which point testing was terminated. The baseline HST was performed 24 hours before LRLn and repeated within 4 days postoperatively (2 to 4 days).

Instrumentation of horses for HST

All instrumentation was performed as per Durando et al.⁷ After warm-up, a custom-designed airtight face mask was applied to the horse. An equine ergospirometer (Quadflow Equine Spirometry, QF, RobacScience Pty Ltd) mounted to the mask was used to measure airflow.¹¹ Trans-nasal pharyngeal and tracheal catheters (2m 1/8 X 0.8 mm, PFA tubing, Swagelok) connected to a differential pressure transducer (DP-45, Validyne Engineering Sales) were used to determine trans-laryngeal pressure^{11,12} and a 1 m trans-nasal endoscope used to obtain dynamic USTE video images (Olympus GIF-H180 video endoscope and EVIS EXERA II 180 Video System, Austvet Endoscopy). Both catheters for the differential pressure transducer were passed into the pharynx through the rostral aspect of the mask and secured with adhesive tape. The ends of the pharyngeal and tracheal catheters were positioned level with the tip of the endoscope and caudal to the larynx at the level of the third tracheal ring, respectively. The trans-laryngeal catheters were flushed with 50 mL of air every 30 seconds to clear any fluid. The endoscope was positioned within the pharynx level with the guttural pouch ostia and fixed in place for the duration of the HST.

Data acquired from the respiratory mask was recorded and analyzed using Powerlab/Labchart software (Powerlab/Labchart, ADInstruments). Calibration was performed before each HST as previously validated.¹¹ Trans-laryngeal impedance (mmHg/L/s) was calculated by dividing the translaryngeal pressure differential by the airflow post-HST. Dynamic URTE video recordings were reviewed and used to obtain representative digital images of the larynx at each speed during HST and the left-to-right quotient angle ratio (LRQ) was measured to assess the degree of ACC. Measurements were performed using Image J Software (Image J) as previously described,²² where results approaching an LRQ of 1 indicate increasing rates of abduction. Collapse of the left corniculate process beyond the midline (exercising grade D) was conveyed using a negative value.

Surgical technique

Twenty-four hours after baseline testing a standing LRLn was performed. Sedation was achieved using a combination of detomidine (0.01 to 0.016 mg/kg bwt IV) and butorphanol (0.01 mg/kg bwt IV) and boluses were repeated as necessary. The neurectomy was performed through a routine mid-cervical approach (**Figure 1**). After infiltration of local anesthetic (Vetacaine, 2% mepivacaine hydrochloride, 20 mg/mL, Ilium) around the proposed incision site a 5 cm incision was made ventral and parallel to the jugular vein through the skin and subcutaneous tissue. A combination of sharp and blunt dissection was used to isolate the left recurrent laryngeal nerve deep into the carotid artery. Identification of the recurrent laryngeal nerve was verified using direct digital stimulation whilst observing characteristic arytenoid abduction twitches on resting endoscopy. After confirmation, a 4 cm section of the nerve was sharply excised. The subcutaneous tissue and skin were closed using an absorbable suture and a sterile dressing was applied over the surgical site. Surgical time was recorded to the closest minute and was defined as the time of first incision until the completion of dressing application. The horses received procaine penicillin (22 mg/kg IM) and phenylbutazone (4.4 mg/kg IV) perioperatively. Postoperatively, a complete physical examination was performed every 6 hours, and the horses were monitored for peri- or postoperative complications.

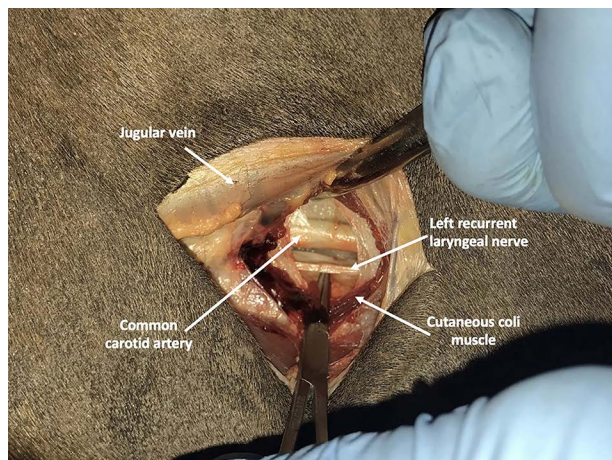


Figure 1—Digital photograph of the standing left recurrent laryngeal neurectomy (LRLn) surgical site showing isolation of the left recurrent laryngeal nerve.

Statistical analysis

Data distribution was assessed by a Shapiro-Wilk test. Normally distributed data are reported as mean \pm SD and nonnormally distributed data are reported as median and range. The effects of speed and LRLn were assessed by a 2-way-repeated measures ANOVA. A paired t test was used to assess the effect of LRLn on the total distance traveled during HST. Statistical analysis was performed using commercially available software (GraphPad Prism 9,

GraphPad Software, Inc; MedCalc Software bvba) and $P \leq .05$ was considered statistically significant.

Results

Standing LRLn was performed without intra-operative or postoperative complications in all horses and none of the horses demonstrated clinical signs of pain associated with the surgery. Median surgical time was 29 minutes (range = 22 to 45 minutes) with the length of the procedure reducing in time as experience increased. On baseline HST all horses demonstrated grade A exercising laryngeal function (normal). Postneurectomy, a resting grade 4 (Havemeyer resting laryngeal function) and an exercising grade C ($n = 5$) or D ($n = 1$) (Rakestraw exercising laryngeal function) ACC was seen in all horses in URTE.²³ Both pre- and post-LRLn, increasing speed from 11 to 14 m/s increased TLI ($P < .001$) (**Table 1**).

Table 1—The effect of LRLn on peak inspiratory flow (PIF), peak inspiratory pressure (PIP), translaryngeal impedance (TLI), and left to right quotient angle ratio (LRQ) pre- and postoperatively at 14 m/s during high-speed treadmill test (HST).

Variable	Pre-LRLn	Post-LRLn	P-Value
PIF (L/s)	-65.12 ± 10.87	-36.70 ± 8.35	0.0045*
PIP (cmH ₂ O)	-46.36 ± 8.70	-65.94 ± 3.98	0.0004*
TLI (cmH ₂ O/L/s)	0.74 ± 0.22	1.88 ± 0.36	0.0042*
LRQ	0.94 ± 0.03	0.10 ± 0.07	0.0008*

Values are reported as mean \pm SD.

* $P \leq .05$.

After LRLn there was an increase in inspiratory TLI ($P = .021$) (**Figure 2**) resulting from both decreased inspiratory flow (IF) ($P = .005$) and increased inspiratory pressure (IP) ($P < .001$). At maximal speed, the mean TLI before LRLn was 0.74 ± 0.22 cmH₂O/L/s, which rose to 1.88 ± 0.36 cmH₂O/L/s after LRLn. After LRLn there was also a reduction in

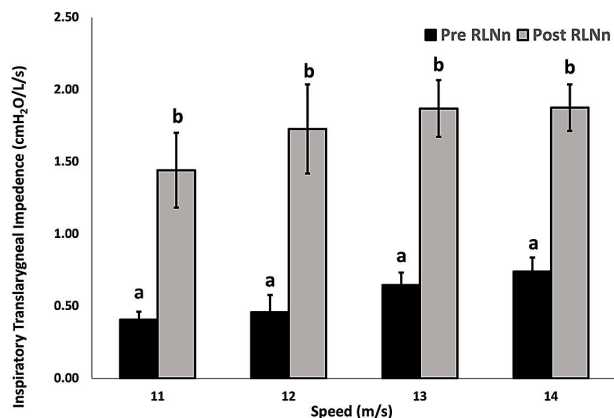


Figure 2—Effect of standing left recurrent laryngeal neurectomy (LRLn) on inspiratory translaryngeal impedance (TLI) (mean \pm SEM) during high-speed treadmill test (HST) pre- and post-LRLn. Different letters (a, b) indicate $P < .05$.

LRQ ($P < .001$; pre-LRLn 0.94 ± 0.03 , post-LRLn 0.1 ± 0.16) (**Figure 3**). The mean total distance traveled during HST decreased after LRLn, but this change was not significant (pre-LRLn 2004 ± 295 m, post-LRLn 1668 ± 206 m; $P = .099$). Of the 6 horses, 4 traveled a lesser total distance during HST postoperatively compared to preoperatively. The remaining 2 horses traveled the same distance.

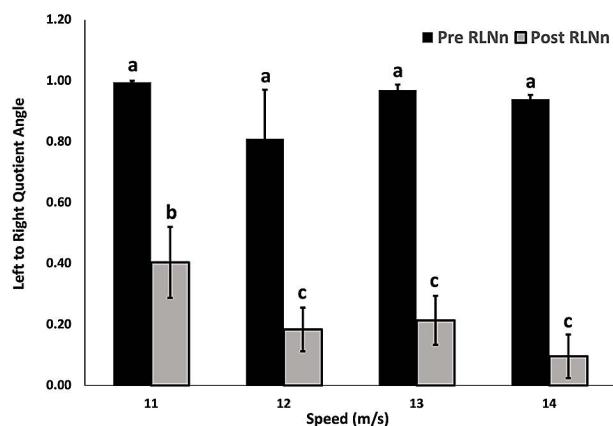


Figure 3—Effect of standing left recurrent laryngeal neurectomy (LRLn) on left-to-right quotient angle (LRQ) during high-speed treadmill test (HST) pre- and post-LRLn. Different letters (a, b, c) indicate $P < .05$.

Discussion

Although used in previous studies investigating recurrent laryngeal neuropathy,^{4,17,24–27} this is the first description of recurrent laryngeal neurectomy performed in standing sedated horses. The procedure was performed through a mid-cervical approach and successfully induced left ACC greater than or equal to exercising grade C. The surgical time in all horses was less than 45 minutes and there were no adverse effects associated with the procedure. Once isolated, digital stimulation with concurrent URTE provided clear confirmation of the recurrent laryngeal nerve reducing the risk of accidentally transecting an alternate nerve. In the author's experience standing LRLn was quick, safe, and simple to perform. Additional benefits include the avoidance of risks associated with general anesthesia and recovery (and particularly after this procedure, airway obstruction), fewer personnel required, and minimization of costs to perform the procedure. While these benefits could also be said for induction of LRLN using a local anesthetic solution, surgical transection provided a reliable, consistent, and immediate induction of severe left laryngeal hemiplegia. However, the most notable benefit compared to local blockade, is the induction of a long-term model that can evaluate the chronic effects of denervation, a particular benefit for longitudinal studies. Finally, a second benefit of neurectomy is that the histological changes would be evident and could be tracked whereas they wouldn't be temporarily induced using local blockade.

The time lag between pre- and postintervention HST assessment has been a limitation of prior studies using recurrent laryngeal neurectomy. If long enough, the period of time spent out of work will result in physiological changes consistent with detraining. Although slower than in human athletes, significant alterations in equine respiratory and musculoskeletal function occur within 4 to 12 weeks of training cessation.²⁸ It is also of note that horses with laryngeal hemiplegia are at increased risk of lower airway pathologies, including lower airway inflammation and exercise-induced pulmonary hemorrhage.⁹ In the present study, because both baseline and postneurectomy tests were performed within 4 days of one another there was minimal time for this to happen. Perhaps more importantly, it improves the likelihood that the horses will perform at equal intensity for pre- and postintervention HSTs, meaning the data represents changes in respiratory function that are solely attributed to ACC. Finally, the use of horses as their own controls eliminates variability associated with innate athletic ability and reduces the number of horses needed per trail.

In agreement with prior studies, there was a significant increase in TLI after LRLn, attributable to both increased inspiratory pressure and decreased inspiratory flow.^{4,15,17} Also of note, the distribution and change in LRQ during exercise was variable between individuals, highlighting another benefit of using individual horses as their own controls.

Although the study only included a small number of horses 4 traveled cumulatively less distance during HST before the HST had to be terminated due to fatigue. This was the expected outcome and is logically due to reduced respiratory function and build-up of lactic acid. Although further evaluation with a larger number of horses.

Although exercising ACC beyond the midline has been acknowledged in the literature and is now considered a grade D,²³ how to objectively measure the degree to which this occurs is yet to be defined. In the present study collapse beyond the midline was conveyed as a negative LRQ value. Alternatively, all ACC equivalent to or beyond the rima glottis midline could have been recorded as an LRQ of 0. However, this does not account for collapse progression at higher speeds. Another technique considered was rima glottis cross-sectional area (CSA).^{22,29,30} By using CSA (encompassing the dorsal rima glottis to the ventral aspect of the corniculate process), ACC beyond the midline is accounted for and, furthermore, difficulty visualizing the ventral aspect of the rima glottis during exercise is overcome. On the other hand, unlike LRQ which is a ratio, CSA measurements are affected by endoscopy movement and subsequent magnification. Even with precise placement, it is impossible to eliminate movement during exercise, which would result in both intra- and inter-horse variability.

Limitations of the study include the small population size ($n = 6$), the inability to measure peak $\dot{V}O_2$ accurately, and the possibility that some horses only reached submaximal velocity during HST. Although

the maximum speed (14 m/s) used in this study would not be considered a top speed for many racehorses, this can be overcome to some extent by placing the treadmill on an incline. This means that increased effort is required from the horse, without the need to increase speed. We also considered the possibility that the horses might demonstrate mild resistance or resentment associated with the mask; however, this was not appreciated and was likely minimized through appropriate acclimatization during training. The main limitation of this study is the mask used, which has been shown to cause some rebreathing of carbon dioxide. This style of respiratory mask has now been replaced by newer generation masks, which have been shown to provide more specific and more precise data acquisition.¹³ While the precise values obtained using the older mask in this study may not be highly accurate, the trends in data are valid. Additional research using a newer generation of respiratory masks is needed to obtain more accurate numerical values; however, the findings of this study can be used to guide future research surrounding dynamic ACC.

In conclusion, the findings indicate that LRLN performed in standing sedated horses effectively induces exercising Rakestraw grade C or D ACC and may be useful in future LRLN research efforts. Further investigation with larger populations, using a newer generation respiratory mask is required to eliminate the possible effect of rebreathing CO₂.

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Disclosures

The authors have nothing to disclose, and no AI-assisted technologies were used in the generation of this manuscript.

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