Dorsal displacement of the soft palate is a common cause of respiratory noise and exercise intolerance in racehorses.\textsuperscript{1–3} Its etiology is not completely known, although most investigators believe the disease is secondary to neuromuscular dysfunction of the soft palate.\textsuperscript{3} Various surgical procedures have been developed to treat DDSP in horses.\textsuperscript{3,6–8} Such procedures include sternohyoideus and sternothyroideus myectomy, sternothyroideus tenectomy, staphylectomy, oral palato-pharyngoplasty, epiglottic augmentation, laryngeal tie forward, and laser palatoplasty.\textsuperscript{1,2,5,8–10}

Laser palatoplasty has been used to stiffen the soft palate (ie, increase its elastic modulus) through laser-induced fibrosis. Laser palatoplasty has been used alone or in combination with other surgical procedures.\textsuperscript{2,10} Two studies\textsuperscript{2,10} of laser palatoplasty in horses have been performed. In one,\textsuperscript{2} standing diode laser palatoplasty was used in combination with sternothyroideus tenectomy, resulting in 50 of 52 horses returning to racing after surgery.\textsuperscript{2} In the other,\textsuperscript{10} a carbon dioxide laser via laryngotomy was used, leading to improved racing performance in 63% of treated horses afterward.

Because surgical lasers have been used to treat snoring in people, it has been speculated that laser palatoplasty in horses results in fibrosis of the soft palate.\textsuperscript{11} It is also believed that development of soft palate fibrosis following laser palatoplasty results in increased soft palate stiffness, which may lead to a decreased likelihood of soft palate displacement during exercise.\textsuperscript{2,10} However, to the authors’ knowledge, this supposition has not been objectively evaluated. The purpose of the

**Objective**—To determine the effects of diode laser palatoplasty on the soft palate in horses.

**Animals**—6 clinically normal horses and 6 euthanized horses from another study.

**Procedures**—6 horses underwent diode laser palatoplasty (treated horses); 3 received low-dose laser treatment (1,209 to 1,224 J), and 3 received high-dose treatment (2,302 to 2,420 J). Six other horses received no treatment (control horses). The upper respiratory tracts of all treated horses were evaluated immediately following surgery (day 0) and on days 2, 7, 14, 21, 30, and 45. Horses were euthanized on day 45, and magnetic resonance imaging (MRI) of the head was performed. The soft palate was removed from treated and control horses, evaluated grossly, and scored for edema, inflammation, and scarring. Soft palates from all horses were sectioned for histologic and biomechanical evaluations.

**Results**—Endoscopic examination revealed a significant increase in soft palate scarring and decrease in edema and inflammation in treated horses by day 7. Gross postmortem findings corresponded with MRI findings. Gross and histologic examination revealed a significant increase in scarring, edema, and inflammation at day 45. Histologic evaluation of palatal tissue from high-dose–treated horses revealed full-thickness injury of skeletal muscle, with atrophy of muscle fibers; findings in low-dose–treated horses indicated superficial injury to skeletal muscle. After surgery, treated horses had a significant decrease in soft palate elastic modulus, compared with control horses.

**Conclusions and Clinical Relevance**—Laser palatoplasty resulted in soft palate fibrosis and skeletal muscle loss; however, the fibrosis did not result in an increase in soft palate elastic modulus. (Am J Vet Res 2010;71:575–582)

**Abbreviations**

<table>
<thead>
<tr>
<th>DDSP</th>
<th>Dorsal displacement of the soft palate</th>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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study reported here was to determine the effects of a diode laser on the soft palate following laser palatoplasty in horses. We hypothesized that diode laser palatoplasty would result in an increase in fibrosis and stiffening (elastic modulus) of the soft palate within 45 days after surgery.

Materials and Methods

Animals—Twelve horses were used in the study. Those intended for the treatment group (n = 6; mean age, 10.6 years) were endoscopically examined to ensure they had clinically normal upper respiratory tracts (nasal passage, soft palate, larynx, and trachea). They were then randomly allocated to 2 groups of 3: group 1, with a mean age of 7.3 years, and group 2, with a mean age of 13.6 years. Those intended for the control group (n = 6; mean age, 7 years), which was used for histologic and biomechanical evaluations only, were euthanized for another, unrelated research project at our institution. The study protocol was approved by the Institutional Animal Care and Use Committee of Purdue University.

Laser palatoplasty—All 6 horses in the treatment group underwent standing transendoscopic diode laser palatoplasty. However, group 1 was assigned to receive low-dose laser treatment (1,209 to 1,224 J), and group 2 was assigned to receive high-dose laser treatment (2,302 to 2,420 J). Each horse was placed in metal stocks and sedated with detomidine hydrochloride (0.01 to 0.02 mg/kg, IV) and butorphanol tartrate (0.01 to 0.02 mg/kg, IV). The rostral 5 cm of the mucosa of the right nasal passage was topically anesthetized. A videoendoscope was inserted into the right nasal passage, and the caudal margin of the soft palate was made visible. The surgical procedure was video recorded, and digital images were obtained. An endoscopic spraying device was inserted into the biopsy channel of the endoscope, and 10 to 20 mL of 2% lidocaine hydrochloride solution was sprayed topically onto the nasopharyngeal mucosa of the soft palate and epiglottis. To access the caudal aspect of the soft palate, DDS was initiated by passing the video endoscope into the trachea and stimulating a cough reflex. If persistent DDS could not be induced, the epiglottis was retracted with bronchoesophageal grasping forceps to facilitate the surgical procedure. The diode laser was set to 15 W with 1 second of activation followed by 1 second of interval activation. A 600-μm contact-sculpted fiber was passed through the biopsy channel of the endoscope to contact the caudal free edge of the soft palate, where it was activated for 1 second. The power density of the laser beam was calculated at 10,417 W/cm². The fiber was then moved 2 to 4 mm and the procedure repeated. The laser was brought into contact with the soft palate, starting at the free edge and extending 2 to 3 cm rostral from the free edge (not exceeding the rostral aspect of the epiglottis). The laser-treated area did not extend more than 2 to 3 cm abaxially from midline. Once the desired amount of energy (in joules, as automatically calculated by the laser unit) was applied to the soft palate, the surgery was terminated. No other surgical procedures were performed.

Postoperative treatment—Following surgery, all treated horses received anti-inflammatory medications, including phenylbutazone (4.4 mg/kg, PO, q 24 h for 7 days), dexamethasone (0.04 mg/kg, IV, q 24 h for 2 days), and pharyngeal spray (dimethyl sulfoxide, glyc erin, prednisolone, and sterile water; 10 mL, administered topically via transnasal catheter, q 12 h for 7 days).

Postoperative antemortem evaluation—Horses in the treatment group were monitored for clinical signs of coughing or dysphagia. These horses were endoscopically evaluated immediately after surgery (day 0) and on days 2, 7, 14, 21, 30, and 45 afterward. Each horse was made to stand in metal stocks, and a nose twitch was applied. When needed, horses were sedated with detomidine hydrochloride and butorphanol tartrate, as previously described. Video recorded and digital endoscopic images were obtained at each follow-up examination and were reviewed by 3 clinicians. Each clinician independently evaluated each endoscopic examination by use of a 4-point scoring system designed to subjectively measure 3 types of tissue reaction: edema, fibrosis, and inflammation. A grade of 0 was assigned when no edema, scarring, or inflammation was evident. Grades 1, 2, and 3 were assigned upon detection of mild, moderate, or severe edema, scarring, or inflammation, respectively. Endoscopic evidence of scarring was defined as white mucosal surface discoloration of the soft palate or evidence of mucousal contracture (e.g., stellate appearance). On day 45 after surgery, all horses were euthanized by IV injection of 100 mL of a solution of phenytoin and pentobarbital.

Postmortem evaluation—Immediately after treated horses were euthanized, the head was harvested from each and stored on ice until an MRI examination was performed (within 1 hour after euthanasia). A 3.0-T magnetic resonance scanner was used with the following settings: slice thickness, 3 to 5 mm, and interslice gap, 1 to 3 mm. The field of view was variable and chosen to maximize the area of soft palate imaged. The following pulse sequences were obtained for each head: T1-weighted sagittal plane, T1-weighted transverse plane, T2-weighted dorsal plane, T2-weighted fat-suppressed sagittal plane, and T2-weighted fat-suppressed transverse plane. T2-weighted fat-suppressed dorsal plane, T2-weighted dorsal plane, and T1-weighted fast spoiled gradient echo axial plane. Pulse sequences in the dorsal plane were in an oblique plane relative to the head but parallel to the long axis of the soft palate and epiglottis. Transverse plane images were obtained in a standard fashion, perpendicular to the sagittal plane of the head. In addition, 2 heads from horses belonging to neither the treated nor control group and euthanized for reasons unrelated to upper respiratory tract disease were harvested, underwent MRI, and served as control specimens.

All MRI sequences were evaluated by 1 board-certified veterinary (WW) and 1 board-certified human (DD) radiologist. Sagittal T1- and T2-weighted images were evaluated initially for lesions. When pathological changes were detected, imaging software was used to cross-reference lesion locations on transverse and dorsal plane images of the same weighting.
Gross postmortem examination—Soft palates of the 6 cadaveric control horses were harvested and prepared, along with the soft palates of all 6 treatment horses, for further evaluation. The soft palate of each horse was removed from the head in bloc by first dividing the horizontal rami of the mandible with a handsaw. Next, each larynx and soft palate were removed by first searing the stylohyoid bone, sharply dividing the palatopharyngeal arches from the pharyngeal wall, and cutting the soft palate from the hard palate with a scalpel blade. The soft palate and larynx were photographed, and then the soft palate was removed from the larynx by dividing the palatopharyngeal arch on the dorsal midline. The soft palate was spread out on a wax board and tacked down with 20-gauge needles for digital photography of the dorsal (nasopharyngeal) surface.

Photographs were reviewed and scored independently for edema, scarring, and gross evidence of inflammation by 3 examiners who used the previously described scoring system. Examiners were not blinded to treatment group. The soft palate was examined for gross abnormalities, including local hemorrhage, surface disruptions, and palpable thickening or increased firmness, indicative of fibrosis.

The section for histologic evaluation was tacked to wooden tongue depressors to prevent soft palate muscular contraction and immersed in neutral-buffered 10% formalin. The opposite half of the soft palate was sharply divided into 3 segments (rostral, middle, and caudal), each approximately 6 × 6 cm, for biomechanical testing. Each section was wrapped in gauze soaked in saline (0.9% NaCl) solution and placed on ice until frozen with liquid nitrogen. For rapid freezing, the sections were immersed in liquid nitrogen for 3 minutes, placed in plastic containers, and stored at −70°C.12 (Figure 1).

The formalin-fixed soft palate was divided and embedded in 3 paraffin blocks, designated block 1 (medial sagittal sections, including the caudal margin), block 2 (paramedian sagittal sections), and block 3 (cross section of soft palate rostral to the sagittal sections). Serial sections were stained with H&E and Masson trichrome and Verhoeff Van Gieson stains. Histologic examination consisted of an evaluation of each tissue block for edema, fibrosis, and inflammation by 1 veterinary pathologist (MAM). The blocks were then scored in accordance with the previously described scoring system. Photomicrographs of the sagittal sections were merged into a panoramic image by use of image-processing software. The panoramic images were evaluated for area of skeletal muscle (mm²), area of skeletal muscle injury (mm²), depth (superficial vs full thickness) of laser injury, rostral extension of laser-induced injury from the caudal border of the soft palate, and extent of soft palate glandular damage present.

Prior to biomechanical testing, soft palate sections were placed in a water bath set to 37°C and thawed for approximately 30 to 40 minutes. Section thickness was measured in millimeters, and each section was pinned over a hole in a wax board. The square holes in the wax board ranged from 1.0 to 1.3 cm to 3.0 to 3.3 cm in size. Each tissue section was fitted over a hole approximately to its size. Care was taken to ensure each sample was tacked down so that no tension over the hole existed. Tissue sections were moistened with lactated Ringer's solution and placed under a deformation rod (indentor). The rod was connected to a 100-g load cell. Each sample was compressed to a total displacement of 24.8 mm at a loading rate of 3 mm/s, and the extent of tissue deformation was obtained by use of computer software.1 Because tissue deformation after a compression test causes each section to be unusable for further testing, only 1 compression test was performed. The elastic modulus of the tissue deformation for the caudal, mid, and rostral section of each soft palate was calculated as the force (force applied to achieve compression [N]) divided by area (per square meter of tissue). To control for the age differences among the treated horses (2 to 19 years) versus the control horses (all 7 years), the ratio of the caudal to rostral elastic modulus was calculated.

Statistical analysis—Data are reported as mean ± SD. Assumptions of normal data distribution and constant variance were evaluated by examining the histogram, quantile-quantile plot, and residual plot for each variable assessed. In addition, more objective assess-
ment of assumptions was performed by use of the Shapiro-Wilk test for normality and modified Levene test for homogeneity of variance. An ANOVA for repeated measures was used to compare the mean scores for ordinal response variables (ie, degrees of edema, fibrosis, and inflammation) by treatment group and age. A multiple pairwise comparison of mean scores was performed by computing 95% confidence intervals and applying a Bonferroni adjustment only when the overall model was significant. A 1-way ANOVA was used to analyze the difference in mean effect for quantitative response variables (eg, elastic modulus, area of skeletal muscle, and area of skeletal muscle injury) between high-dose and low-dose laser treatments. To ensure that the ANOVA model for the ordinal variables was reliable, some of the ordinal variables, which did not have repeated measures, were tested by use of the equivalent non-parametric Kruskal-Wallis test. A value of $P \leq 0.05$ was considered significant, and all statistical analyses were performed with commercial software.

Results

Surgical outcome—Duration of transendoscopic diode laser palatoplasty ranged from 15 to 33 minutes in the 6 treated horses. Two horses developed clinical signs of mild dysphagia and coughing, beginning 2 days after surgery (day 2) and resolving by day 7. Both affected horses were difficult to treat postoperatively with anti-inflammatory spray and therefore only received pharyngeal medication and therefore only received pharyngeal medication.

Postoperative endoscopic evaluation—Endoscopic examinations revealed edema in all treated horses on day 2. This edema decreased with time, whereas scarring (white focal spotting or star-like mucosal contraction) became more evident (Figures 2 and 3). The mean scarring scores for group 1 (low-dose laser treatment) and group 2 (high-dose laser treatment) increased from 0 and 0 on day 2 to mean ± SD values of 2.2 ± 0.7 and 2.3 ± 0.1 on day 45, respectively. On the other hand, the mean edema score decreased from 1.8 ± 0.7 in group 1 and 1.8 ± 0.4 in group 2 on day 2 to 0.1 ± 0.3 and 0.3 ± 0.5 on day 45, respectively. For inflammation, the mean score decreased from 1.6 ± 0.5 in group 1 and 1.7 ± 0.5 in group 2 on day 2 to 0 for both groups on day 45. When horse age was controlled for, overall significant differences were detected between groups 1 and 2 with respect to scarring ($P = 0.002$), edema ($P = 0.029$), and inflammation ($P = 0.002$). These differences did not exist when age was not included in the analysis. Endoscopically, speed of mucosal healing ranged from 14 days in horses that underwent low-dose laser treatment (group 1) to 30 days in horses that underwent high-dose (group 2) treatment.

The mean scores for scarring, edema, and inflammation were significantly ($P < 0.001$) associated with the number of days elapsed after surgery. Whereas the mean score for scarring appeared to increase as the number of days after surgery elapsed, the opposite was evident for edema and inflammation; however, these patterns were not significant. There was no significant difference in the day-specific mean score for scarring or edema between groups 1 and 2. However, group 2 had a significantly higher mean score for inflammation on day 7 (2.0 ± 0.9), compared with the mean score for group 1 on the same day (1.2 ± 0.7).

Gross postmortem evaluation—All horses had moderate (group 1 horses) to severe (group 2 horses) scarring at the caudal free margin of the soft palate. The 3 horses in group 2 had stellate mucosal scarring. These horses all developed an area of redundant tissue at the caudal free margin of the soft palate and had subjectively greater palpable thickening (compatible with fibrosis) than did group 1 horses. Three of 6 horses (2 horses from group 1 and 1 horse from group 2) had mild to moderate hemorrhagic areas on the soft palate; 1 developed a 1 × 0.5-cm mass of granulation tissue at one of the laser sites.

Mean scores for soft palate scarring were significantly ($P < 0.001$) greater in laser-treated horses than in control horses; however, there was no significant difference in mean score between groups 1 and 2 (Table 1). Gross edema of the soft palate in group 2 horses was significantly ($P = 0.023$) more severe than that in group 1 horses. Horse age was not significantly associated with the severity of edema detected during gross examination, but the examiner was significantly associated with the mean score for edema ($P = 0.041$), as was the interaction effect between the examiner and study group ($P = 0.006$). Mean scores for gross inflammation were significantly ($P = 0.01$) different among treatment groups. Group 1 horses had a significantly higher mean score than did control horses (Table 1). However, differences between mean inflammation scores for group 2 and

Figure 2—Videoendoscopic images of the soft palate and larynx of a horse with a clinically normal upper respiratory tract immediately after (left), 7 days after (middle), and 45 days after transendoscopic diode laser palatoplasty at a low-energy dose (1,209 to 1,224 J). Notice the marked difference in tissue reaction in comparison with that in horses that received high-energy laser treatment (Figure 3).

Figure 3—Videoendoscopic images of the soft palate and larynx of a horse with a clinically normal upper respiratory tract immediately after (left), 7 days after (middle), and 45 days after transendoscopic diode laser palatoplasty at a high-energy dose (2,302 to 2,420 J). Notice the marked difference in tissue reaction in comparison with that in horses that received low-energy laser treatment (Figure 2).
by loose fibrous tissue from mucous glands and skeletal muscle bundles. Skeletal muscle extended caudally to within 1 mm of the free margin of the soft palate, from which it was separated by dense fibrous tissue.

In treated horses, inflammation consisted mainly of mucosal and submucosal infiltration by lymphocytes and plasma cells. Inflammation in group 1 was mild, whereas that in group 2 was moderate. In all horses in group 2, edema extended into the submucosa; such edema was not detected in group 1 horses. Group 2 horses had fibrotic changes in the nasopharyngeal submucosa that ranged from multifocal, with increased density of fibroblasts and collagen fibers, to a more widespread increase in fibrovascular (granulation) tissue, with variable collagen content. The soft palate of 1 horse in group 2 had a few irregular, pale gray plaques or bands, arranged parallel to the caudal margin of the soft palate, suggestive of fibrosis as well as an increase in fibrous tissue throughout the lamina propria and submucosa. In the same horse, necrosis of skeletal muscle with loss of myocytes and interstitial fibrosis was a prominent change and extended nearly throughout the almost 2-cm length of the section. In contrast, horses in group 1 had only mild fibroblastic proliferation and a patchy increase in fibrous tissue with minimal involvement of skeletal muscle. Only 1 horse in group 1 had prominent fibroblastic changes. That horse had multiple fibrotic bands just beneath the nasopharyngeal mucosa, and the fibrosis extended locally between bundles in the skeletal muscle at the caudal aspect of the soft palate.

Histologic scores were summarized (Table 1). Statistical analysis revealed significant associations between treatment groups with respect to mean scores for scarring (fibrosis; $P = 0.003$), edema ($P = 0.001$), and inflammation ($P < 0.001$). The mean scarring, edema, and inflammation scores of groups 1 and 2 were significantly greater than those of the control group, but there was no significant difference in mean scores between groups 1 and 2. Age was not significantly associated with the scores for any of these outcomes.

In panoramic sections of the soft palates of group 2 horses, the full thickness of the skeletal muscle was damaged, with marked atrophy of myocytes and expansion of the interstitium by collagen fibers; overlying submucosal glands were lacking (Figure 4). One horse had severe muscle atrophy in the caudal aspect of the soft palate and mild to moderate muscle atrophy in the rostral portion; however, overlying submucosal glands appeared normal, even in areas of skeletal muscle injury. In group 1 horses, skeletal muscle injury was generally superficial. In injured muscle, myocytes were rounded with intense cosinophilia or shrunkened. Submucosal glands were dense and histologically normal. One horse had severe and full-thickness muscle atrophy and fibrosis; however, submucosal glands were present in a healthy density.

The mean area of skeletal muscle in histologic sections obtained from block 1 of the soft palate was 29.15 and 26.36 mm$^2$ for groups 1 and 2, respectively. The mean area of skeletal muscle in block 2 for groups 1 and 2 was 20.46 and 29.46 mm$^2$, respectively. These values did not differ significantly between groups. The mean area of muscle injury in group 2 horses (6.5 ± 6.4 mm$^2$ in block 1 and 13.7 ± 15.8 mm$^2$ in block 2) was not

Table 1—Mean ± SD gross and histologic scores for degrees of gross scarring, edema, and inflammation in the soft palates from untreated control horses ($n = 6$) and horses that underwent low-energy (1,209 to 1,224 J; 3) or high-energy (2,302 to 2,420 J; 3) transendoscopic diode laser palatoplasty.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Low-energy laser</th>
<th>High-energy laser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scarring</td>
<td>0</td>
<td>2.22 ± 0.67*</td>
<td>2.67 ± 0.5*</td>
</tr>
<tr>
<td>Edema</td>
<td>0</td>
<td>0.6 ± 1.0*</td>
<td>0.7 ± 1.0*</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>1.3 ± 1.1*</td>
<td>0.7 ± 0.7</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Scarring (fibrosis)</td>
<td>0</td>
<td>2.0 ± 1.0*</td>
<td>2.7 ± 0.6*</td>
</tr>
<tr>
<td>Edema</td>
<td>0</td>
<td>1.0 ± 0.0*</td>
<td>1.7 ± 0.6*</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>2.7 ± 0.6*</td>
<td>2.0 ± 0.8*</td>
</tr>
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</table>

A 4-point subjective scoring system was used (0 = none, 1 = mild, 2 = moderate, and 3 = severe). *Value is significantly ($P < 0.05$) different from the control value for the same characteristic.

Figure 4—Panoramic photomicrographs of soft palate tissue sections from a control horse and horses that underwent low-energy (1,209 to 1,224 J; group 1) or high-energy (2,302 to 2,420 J; group 2) transendoscopic diode laser palatoplasty. Crater-like fibrotic (f) lesions secondary to diode laser fiber contact are outlined with solid lines. Muscular damage (md) and atrophy (a) are apparent in sections from group 1 and 2 horses. SPC = Caudal free margin of the soft palate. m = Nasal mucosa. Masson trichome stain; bar = 2,000 µm.

control horses or group 1 and 2 horses were not significant. Although there was no significant effect of the examiner on the mean score, there was a significant interaction effect between the examiner and study group ($P = 0.03$).

Histologic evaluation—In control horses, the oral surface and the caudal margin of the soft palate were covered by keratinizing stratified squamous epithelium with prominent broad rete ridges. Dense fibrous connective tissue with low cellularity separated the stratified squamous epithelium from the underlying mucous glands and skeletal muscle bundles. The stratified squamous epithelium changed through a transitional zone to pseudostratified columnar (respiratory-type) epithelium of the nasopharynx. The dense fibrous connective tissue changed abruptly to loose fibrous tissue at the transition from squamous to respiratory-type epithelium, and the respiratory-type epithelium was separated by loose fibrous tissue from mucous glands and skeletal muscle bundles. Skeletal muscle extended caudally to within 1 mm of the free margin of the soft palate, from which it was separated by dense fibrous tissue.

In treated horses, inflammation consisted mainly of mucosal and submucosal infiltration by lymphocytes and plasma cells. Inflammation in group 1 was mild, whereas that in group 2 was moderate. In all horses in group 2, edema extended into the submucosa; such edema was not detected in group 1 horses. Group 2 horses had fibrotic changes in the nasopharyngeal submucosa that ranged from multifocal, with increased density of fibroblasts and collagen fibers, to a more widespread increase in fibrovascular (granulation) tissue, with variable collagen content. The soft palate of 1 horse in group 2 had a few irregular, pale gray plaques or bands, arranged parallel to the caudal margin of the soft palate, suggestive of fibrosis as well as an increase in fibrous tissue throughout the lamina propria and submucosa. In the same horse, necrosis of skeletal muscle with loss of myocytes and interstitial fibrosis was a prominent change and extended nearly throughout the almost 2-cm length of the section. In contrast, horses in group 1 had only mild fibroblastic proliferation and a patchy increase in fibrous tissue with minimal involvement of skeletal muscle. Only 1 horse in group 1 had prominent fibroblastic changes. That horse had multiple fibrotic bands just beneath the nasopharyngeal mucosa, and the fibrosis extended locally between bundles in the skeletal muscle at the caudal aspect of the soft palate.

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significantly different from that in group 1 horses (4.53 ± 6.8 mm² in block 1 and 4.7 ± 7.5 mm² in block 2; P = 0.73 and P = 0.42, respectively).

MRI evaluation—The soft palates of all treated horses had a grossly normal relationship with the epiglottis; no horse had DDSP. Images of the head of the control horse were considered normal. On T2-weighted images of heads of treated horses, the hypointense band of the palatine muscle and the hyperintense mucosa of the nasal margin distinguished the soft palate from the overlying epiglottis. The overall signal intensity of the soft palate and epiglottis were similar for all sequences. The T2-weighted images in the sagittal plane were also the most useful for detection of pathological changes. Because of the curvature of the soft palate and epiglottis, the transverse and dorsal plane images were ineffective when used alone for interpretation. When the T2-weighted fat-suppressed sagittal images of the soft palates of treated horses were compared with those of the control horse, blooming artifacts (magnetic susceptibility artifact that is usually blood) were seen in 2 horses, a decreased signal was detected in 2 horses, and an increased signal was detected in 2 horses. On the T2-weighted fat-suppressed axial images, an increased signal was detected in 5 horses and a blooming artifact was seen in 1 horse. On the T1-weighted axial images, an increased signal was identified in 3 horses, blooming artifacts were seen in 2 horses, and a decreased signal was seen in 1 horse.

When MRI images were compared with the photographs of soft palate specimens, most lesions seen on the gross images could be identified via 1 or more MRI sequences. The features visible on MRIs corresponded to those evident in soft palate specimens from all 6 treated horses. For example, 1 horse developed a 1 × 0.5-cm mass of granulation tissue at one of the laser application sites, which could be easily recognized on the T2-weighted coronal images.

Biomechanical testing—Mean elastic modulus values for the 3 sections of the soft palate (caudal, mid, and rostral) were summarized (Table 2). Mean elastic modulus values for the caudal and mid sections of both groups of treated horses were significantly lower than those in the control group. The mean value for the rostral sections of groups 1 and 2 was higher than that in the control group; however, the difference in the mean elastic modulus between groups was not significant (P = 0.376). The mean caudal-to-rostral elastic modulus ratio differed significantly (P = 0.05) between groups 1 and 2. The mean ratio for group 2 also differed significantly (P = 0.04) from that of the control group. No significant difference in ratios was detected between the control group and group 1 or between groups 1 and 2.

### Discussion

Horses affected with DDSP can be treated with laser palatoplasty to resolve the clinical signs associated with the disease. In the study reported here, endoscopic examination of clinically normal horses treated with low- and high-dose transendoscopic diode laser palatoplasty revealed that edema and inflammation predominated in the immediate postoperative period, whereas fibrosis or scarring increased with time.

For the horses tested in this study, we choose to use the term elastic modulus instead of stiffness when referring to the biomechanical change in soft palate tissue believed to be attributable to fibrosis. In biomechanical terms, elastic modulus differs from stiffness. Elastic modulus is a property of a constituent material, and the term stiffness is better used to define properties related to a solid body. For example, a material with a high elastic modulus will undergo less deformation than a material with a lower elastic modulus. Results of histologic examination revealed significantly more fibrosis in sections of soft palate from treated horses, compared with fibrosis in control horses. In addition, a loss of soft palate skeletal muscle was evident in treated horses. Interestingly, soft palate fibrosis did not result in an increase in biomechanical elastic modulus as expected. The soft palates from treated horses were more compliant than were those from control horses, even when taking into account the differences in horse ages between the groups through calculation of the caudal-to-rostral modulus ratio. Indeed, elastic modulus ratios suggested that increasing laser intensity decreased tissue elastic modulus in clinically normal horses.

Histologically, the fibrous connective tissue in the soft palates from treated horses was less organized than that in control horses. The presence of immature fibrous connective tissue as well as the loss in skeletal muscle may result in tissue that is biomechanically more compliant than is control tissue. In our experience, a decrease in tissue elastic modulus is not unexpected when healthy, untreated soft palate tissue is compared with laser-treated tissue.

The loss of soft palate skeletal muscle in the treated horses in our study, particularly those that underwent high-dose laser treatment, was most likely secondary to laser-induced thermal injury. Because the duration of this study was 45 days, it is unknown whether laser-induced muscle necrosis and, ultimately, muscle atro-
phy or loss altered the likelihood of soft palate displacement during exercise.

The hypothesis that soft palate elastic modulus would be higher in treated versus control horses 45 days after palatoplasty was not supported by our findings. However, results of clinical, gross, and histologic examinations indicated that laser palatoplasty causes scarring or fibrosis. To determine whether soft palate elastic modulus would increase beyond the 45-day study period as fibrous connective tissue matures, additional studies are required.

To the authors’ knowledge, techniques for imaging the soft palates of horses with MRI have not been described. Soft palate structures were readily identified with the MRI sequences used for the present study. Sagittal sequences were most useful because most laser application sites were axial in location. Changes in signal intensity observed with T2-weighted imaging are typically seen with fat and fluids, including edema and hemorrhage. However, when T2 weighting with fat suppression was used, the fat signal was eliminated from the image, revealing only fluid. A 3-T magnetic field yields a strong fat signal with any standard sequence. Thus, the small areas of increased signal seen on T2-weighted fat-suppressed images were consistent with inflammation and likely corresponded to laser treatment of the nasal surface of the soft palate. These changes were not seen in the control horse specimen. Hypointense areas on T1-weighted images were consistent with fluid accumulation. The gradient echo sequences were not particularly useful but were added to the sequence to help identify hemorrhage.

Overall, MRI results corresponded to gross necropsy findings. We were unable to detect MRI evidence of soft palate fibrosis following laser palatoplasty. This may have been because fibrosis generally does not result in a major change in signal intensity in soft tissues. In humans, fibrotic lesions may be isoointense on T1-weighted images and mildly hypointense on T2-weighted images. The appearance of fibrotic lesions on MRIs appears to depend on the number of macrophages, relative to numbers of other WBCs, other cellular components, and fluid and lipid infiltrates that may accompany a fibrotic reaction. Our results suggested that, although not routinely performed, MRI can be used as an alternative means of imaging the equine soft palate and adjacent structures.

One limitation of the present study is that the endoscopic and gross postmortem examiners were not blinded to the treatment horses had received. Two of the authors (KCA and JFH) performed the surgical procedures and harvested the soft palates following euthanasia. Consequently, it is possible that subjective bias could have existed when soft palates were scored for pathological change. However, there was good correspondence between results of the subjective (endoscopic, gross scoring, and MRI evaluations) and objective (histologic and biomechanical evaluations) methods of soft palate evaluation following the surgical procedure.

Our primary goal in establishing 2 laser treatment groups was to determine whether a particular dose of laser energy should be used when performing laser palatoplasty. Most likely because of the limited sample size, this goal was not achieved. Horses that underwent high-dose laser treatment consistently developed increased amounts of inflammation, edema and fibrosis, and soft palate skeletal muscle damage as evaluated endoscopically and histologically. However, mean subjective scores for these 3 variables did not differ between the 2 laser treatment groups for any type of evaluation. During the 45-day study period, no clinically important adverse effects of laser treatment (range, 1,200 to 2,400 J) were detected.

In the study reported here, transendoscopic diode laser palatoplasty effectively induced fibrosis of the soft palate. Recommended convalescence time for laser palatoplasty ranges from 3 days to 2 weeks. For the horses endoscopically examined in our study, soft palate mucosal healing ranged from 14 days in horses that underwent low-dose laser treatment to 30 days in horses that underwent high-dose treatment. This information may be useful for estimating when similarly treated horses should be returned to exercise; however, it should also be considered that the horses used in the study did not have DDSP. At our institution, we recommend that horses with clinical DDSP that have undergone laser palatoplasty receive at least 30 days of convalescent time, particularly when treated with 2,400 J of laser energy.

References