

Dysphagia as the primary clinical abnormality in two dogs with inflammatory myopathy

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- ▶ Dysphagia can result from functional or morphologic abnormalities of the pharyngeal or esophageal musculature. Functional dysphagias include myopathies, neuropathies, and disorders of neuromuscular transmission.
- ▶ Evaluation of serum creatine kinase activity, electromyography, esophagraphy, and histologic and immunohistochemical evaluation of muscle biopsy specimens are required to identify functional dysphagias in dogs.
- ▶ Dysphagia may be the predominant clinical sign in dogs with inflammatory myopathy.

A 6-year-old 22-kg (48.4-lb) sexually intact female Boxer (dog 1) and a 4-year-old 25-kg (55-lb) spayed female Boxer (dog 2) were evaluated at the University of Tennessee Veterinary Teaching Hospital because of progressively worsening dysphagia of 5 years' (dog 1) and 2 years' (dog 2) duration. The owner of dog 1 reported that the dog would become cyanotic and collapse when it was allowed to eat or drink from the floor or an elevated position. When the owner opened the dog's mouth, he would find food impacted in the pharynx that required manual removal. Dog 2 also had a history of oropharyngeal impaction but would not collapse after meals. No exercise intolerance or alterations in behavior or attitude were reported for either dog. Dog 1 had been treated by the referring veterinarian with prednisone (1 mg/kg [0.45 mg/lb], PO, q 12 h), enrofloxacin (3 mg/kg [1.36 mg/lb], PO, q 24 h), and levothyroxine sodium (0.2 mg/kg [0.09 mg/lb], PO, q 12 h) for 1 week, but there had not been any clinical improvement.

On initial examination at the veterinary teaching hospital, both dogs were bright, alert, and active; body condition scores were 4 and 3 on a scale from 1 to 9. Neurologic evaluation revealed the absence of a gag reflex following pharyngeal stimulation in both dogs. Oropharyngeal and laryngeal examinations performed

after the dogs were anesthetized revealed a slightly elongated soft palate in dog 1.

A CBC, serum biochemical profile (including determination of serum creatine kinase [CK] activity and free thyroxine concentration), barium esophagraphy, and endoscopy were performed on both dogs. The only hematologic and serum biochemical abnormalities were high CK activity (982 U/L in dog 1 and 1,189 U/L in dog 2; reference range, 59 to 895 U/L). Serum acetylcholine receptor antibody titers were within reference limits (0.06 nmol/L for dog 1 and 0.04 nmol/L for dog 2; reference range, < 0.6 nmol/L). For both dogs, results of an indirect immunocytochemical assay¹ for antibodies against masticatory muscle type 2M fibers were negative.

Feeding trials were performed before and after IV administration of edrophonium chloride² to help rule out focal myasthenia gravis in dog 1. Administration of edrophonium did not improve the dog's ability to swallow. Feeding the dog resulted in collapse and cyanosis of the oral mucous membranes. The dog recovered after the food was manually removed from the pharynx.

Three-phase esophagraphy was performed in both dogs. Briefly, liquid barium sulfate suspension, barium mixed with soft food, and barium mixed with kibble were administered, and swallowing was evaluated fluoroscopically. In dog 1, prehension and bolus formation were normal during all 3 phases of the study, with the bolus propelled into the pharyngeal region by the base of the tongue. However, normal pharyngeal contractions did not follow pharyngeal distention, and the bolus remained in the distended pharynx during repeated swallowing attempts with only small amounts passing through the cricopharyngeal opening. Primary waves of esophageal motility were not generated after arrival of a single bolus into the proximal portion of the esophagus, but esophageal motility was stimulated by subsequent boluses. In dog 2, once a small bolus arrived in the proximal portion of the esophagus, repeated swallowing attempts were necessary for further movement into the stomach.

In both dogs, electromyography of the muscles of the pharynx, larynx, and proximal portion of the esophagus revealed scattered fibrillation potentials, positive sharp waves, and complex repetitive discharges. Results of electromyography of the other muscles of the head and forelimbs were normal in dog 1, but in dog 2, a few fibrillation potentials were observed in selected muscles of the forelimbs and hind limbs.

Both dogs were euthanatized because of the poor quality of life and prognosis for recovery. Complete necropsies were performed, and pharyngeal, masseter,

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Supported in part by the Muscular Dystrophy Association.

The authors thank Norma Prades and David Chen for technical assistance and Dr. Eva Engvall for providing antibodies against laminin α 2, integrin α 7, and β -sarcoglycan.

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glossal, diaphragmatic, cranial, and peripheral muscle specimens of both dogs were examined grossly and histologically.

In dog 1, diameter of the rostral to middle portion of the pharyngeal lumen was narrowed to two thirds of the diameter of the caudal portion by hypertrophy of the muscles of the pharyngeal wall, primarily the hyopharyngeal and thyropharyngeal muscles. The pharyngoesophageal lumen was more prominent than normal (Figure 1). The pharyngeal musculature was

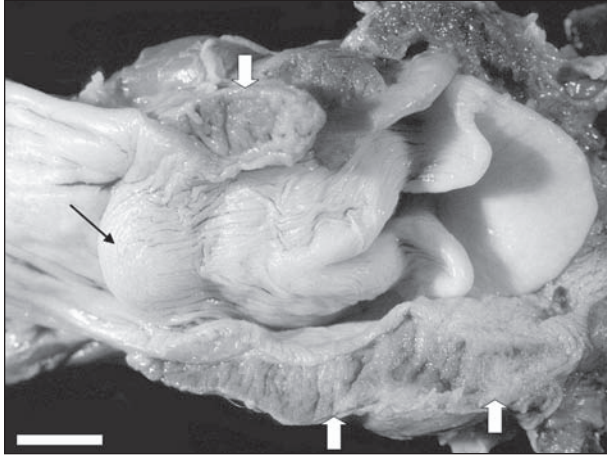


Figure 1—Postmortem appearance of the pharynx of a dog examined because of dysphagia secondary to inflammatory myopathy. The pharynx has been opened along the dorsal midline. The thyropharyngeal and cricopharyngeal muscles were thicker than normal and irregularly pallid (white arrows). The pharyngeal lumen was prominent (black arrow). Bar = 1 cm.

pale on cut section. No gross abnormalities were identified during necropsy of dog 2. Specimens from the thyropharyngeus, hyopharyngeus, gluteal, and masseter muscles and the tongue and diaphragm from both dogs were immersion fixed in neutral-buffered 10% formalin or frozen in isopentane.

Fresh-frozen muscle biopsy specimens were evaluated by use of a standard panel of histochemical stains and enzyme reactions.³ In dog 1, there was a marked variation in the diameter (20 to 300 μ m) of myofibers in the thyropharyngeus and hyopharyngeus muscles, with numerous atrophic and hypertrophic fibers and with some fibers undergoing splitting (Figure 2). Endomysial fibrosis was prominent throughout the tissue sections. Internal nuclei and central rowing were present in several muscle fibers. Multifocal areas of endomysial mononuclear cell infiltration with invasion of non-necrotic fibers were also present. Scattered necrotic fibers and basophilic regenerating fibers were observed. The tongue was similarly affected. Mild changes were detected in the diaphragm and left gluteal muscles. No abnormalities were seen in the masseter muscle.

In dog 2, the most prominent abnormalities involved the thyropharyngeal and masseter muscles. Multifocal areas of mononuclear cell infiltration, scattered degenerating and regenerating fibers, and internalized nuclei were seen in the thyropharyngeal muscle. Multifocal areas of mixed cellular infiltration having a pericapillary and endomysial distribution with invasion of muscle fibers were seen in the masseter muscle.

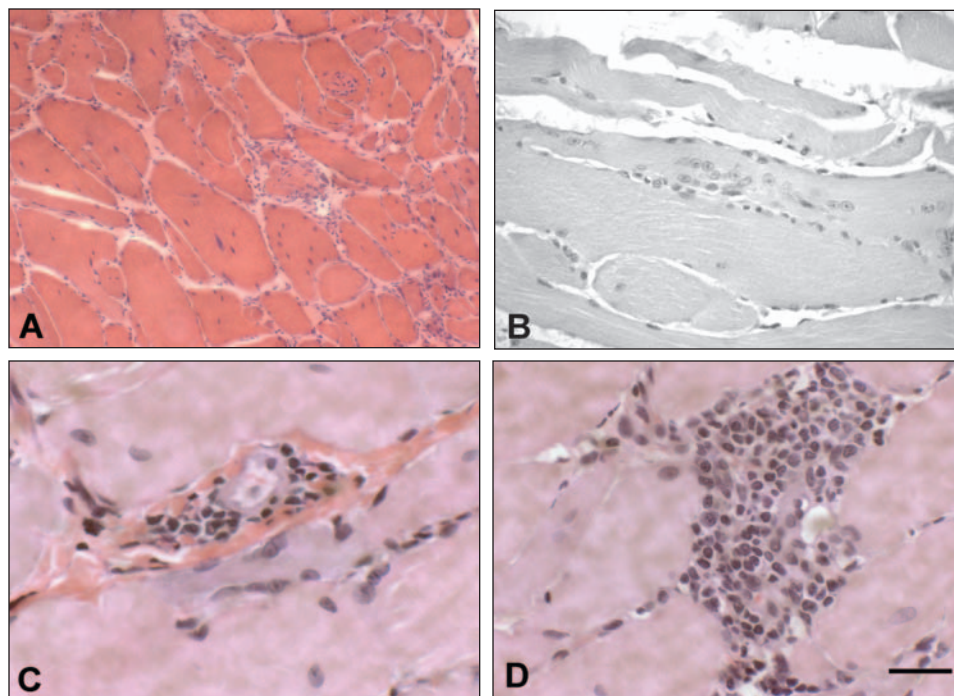


Figure 2—Photomicrographs of sections of the thyropharyngeus and masseter muscles from 2 dogs with dysphagia secondary to inflammatory myopathy. Notice the variability of myofiber size, fiber splitting, endomysial fibrosis, numerous internal nuclei, and mononuclear cell infiltrates with an endomysial distribution (A) and the rowing of internal nuclei (B) in one dog and the multifocal areas of mixed mononuclear cell infiltration with a perivascular (C) or endomysial (D) distribution in the other. H&E stain; bar = 100 μ m for A and 25 μ m for B, C, and D.

For both dogs, lineages of inflammatory cells in fresh-frozen sections of the thyropharyngeus muscle were identified through the use of a panel of monoclonal antibodies against canine leukocyte cell-surface proteins.⁴ A mixed population of cell types was present in dog 1 (Figure 3); T lymphocytes were identified with monoclonal antibodies against CD3, CD4, and CD8 antigens; dendritic cells were identified with antibodies against CD1c and CD11c antigens; and macrophages were identified with antibodies against CD11b and CD11c antigens. Neither B lymphocytes (CD21 positive) nor eosinophils (direct peroxidase reaction) were identified. In dog 2, a mixed population of CD4 and CD8 lymphocytes, scattered macrophages, and dendritic cells was seen.

Histologic abnormalities in both dogs were consistent with chronic inflammatory myopathy or dystrophic myopathy. To distinguish chronic inflammatory

myopathy with fibrosis from dystrophic myopathy, indirect immunofluorescence staining of fresh-frozen muscle biopsy specimens was performed to determine whether proteins associated with dystrophy were present. Monoclonal antibodies^a against the rod domain (1:20, NCL-DYS1) and carboxy terminus (1:20, NCL-DYS2) of dystrophin, utrophin (1:5, NCL-DRP2), spectrin (1:100, NCL-SPEC2), β -sarcoglycan (1:50, NCL-b-SARC), γ -sarcoglycans (1:50, NCL-g-SARC), and β -dystroglycan (1:100, NCL-b-DG) were used. Antibodies against α -sarcoglycan (1:200, rabbit polyclonal),⁵ laminin α 2,⁶ and integrin α 7⁷ were also used. Compared with control muscle specimens, staining for integrin α 7 was reduced in both dogs (Figure 4). Staining for other dystrophy-associated proteins was similar to staining in control muscle specimens. Thus, a diagnosis of inflammatory myopathy was made for both dogs.

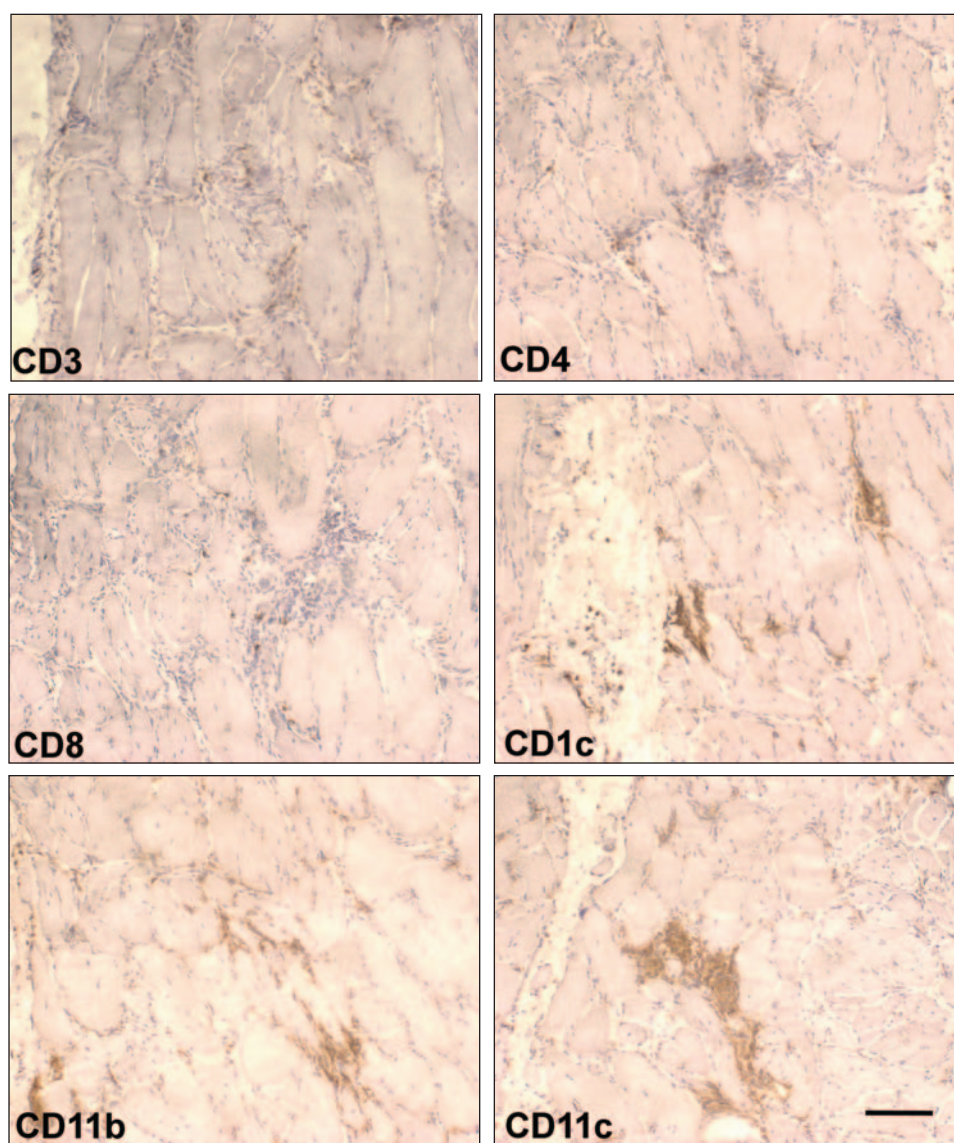


Figure 3—Photomicrographs of sections of the thyropharyngeus muscle of a dog with dysphagia secondary to inflammatory myopathy. Sections were stained immunohistochemically with antibodies against CD3, CD4, and CD8 to identify T lymphocytes; antibodies against CD1c and CD11c to identify dendritic cells; and antibodies against CD11b and CD11c to identify macrophages. Avidin-biotin immunoperoxidase stain; bar = 100 μ m for all images.

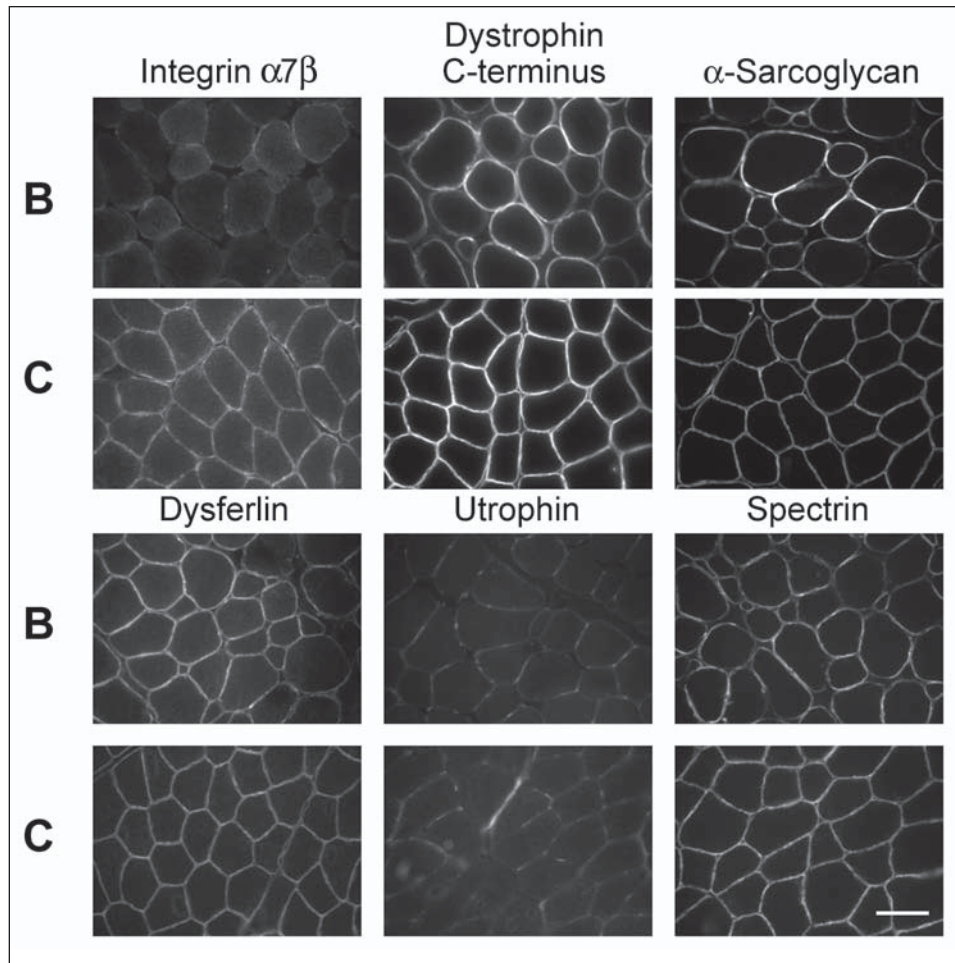


Figure 4—Photomicrographs of sections of the thyropharyngeus muscle from a dog with dysphagia secondary to inflammatory myopathy (B) and a healthy control dog (C). Sections were stained immunohistochemically for proteins associated with dystrophic myopathy. Staining for integrin $\alpha 7\beta$ was reduced in the affected dog, compared with the healthy dog, but staining for other proteins in the affected dog was similar to staining in the healthy dog. Bar = 60 μm for all images.

The 2 dogs described in the present report were initially evaluated because of chronic dysphagia of several years' duration. Dysphagia can result from an abnormality of any of the multiple components of the swallowing mechanism. These components include the musculature of the tongue, soft palate, pharynx, and esophagus.⁸ Neurologic control of the swallowing mechanism is mediated by cranial nerves VII (facial), IX (glossopharyngeal), and X (vagus), which contribute both sensory and motor function, and XII (hypoglossal), which contributes motor function only. Those involved most intimately in the pharyngeal phase of swallowing are the glossopharyngeal and vagus nerves, which form the pharyngeal plexus and innervate the pharyngeal muscles. Experimental bilateral transection of these nerves can result in severe disturbance of the swallowing mechanism.⁹ Because of the numerous components of the swallowing mechanism, a methodical approach to diagnosis was undertaken, beginning with the identification of the stage of swallowing affected in these dogs.

Swallowing can be divided into oral, pharyngeal, and cricopharyngeal phases. The oral phase involves formation of a bolus. The pharyngeal phase involves move-

ment of the bolus from the base of the tongue to the cricopharyngeal sphincter. The cricopharyngeal phase includes relaxation of the cricopharyngeal sphincter and passage of the bolus into and through the esophagus.

Once the stage of swallowing that is abnormal has been identified, the dysphagia should be classified as either functional or morphologic. Functional dysphagia results from incoordination of muscle contractility during swallowing and is a result of neurologic abnormalities or myopathies. Focal myasthenia gravis¹⁰ and hypoadrenocorticism,¹¹ for instance, have been associated with functional dysphagia, and most diseases that result in damage to striated muscle may cause functional dysphagia, including inflammatory myopathies¹² and muscular dystrophies.^{13,14} Morphologic dysphagia, on the other hand, results from a structural abnormality such as a stricture; foreign body; trauma; or inflammatory or neoplastic lesion of the tongue, soft palate, pharynx, tonsils, larynx, or esophagus.⁸ In both dogs, endoscopy, esophagography, and histologic results ruled out morphologic dysphagia.

To our knowledge, only a few reports of dogs with neuromuscular disease in which dysphagia was the ini-

tial clinical sign have been published. A case series^{15,16} involving 24 Bouvier des Flandres with dysphagia reported that affected dogs had histologic evidence of progressive degeneration of the pharyngeal muscles, and the histologic and electromyographic findings in these Bouviers had some similarities to findings for dogs in the present report. The Bouviers were described as having muscular dystrophy, although results of immunohistochemical staining for muscular dystrophy proteins or lineages of infiltrating cells were not reported.

In the dogs described in the present report, clues to the underlying cause of the dysphagia included high serum CK activities, normal acetylcholine receptor and masticatory muscle type 2M fiber antibody titers, and electromyographic abnormalities. Complex repetitive discharges, positive sharp waves, and fibrillation potentials are highly correlated with primary myopathies and neuropathies.¹⁷ On the basis of histologic, histochemical, and immunohistochemical findings and the identification of infiltrating cell types with monoclonal antibodies against canine leukocyte cell-surface antigens, both of the dogs in the present report were confirmed to have functional dysphagia resulting from chronic inflammatory myopathy.

Immunohistochemical identification of muscular dystrophy proteins and infiltrating cells is required to differentiate between muscular dystrophies and inflammatory myopathies because pathologic abnormalities for both disease groups commonly include inflammation, degeneration, and fibrosis. Inflammation has been well described in the muscular dystrophies associated with dystrophin¹⁸ and dysferlin¹⁹ deficiencies. With the exception of staining for integrin $\alpha 7$, abnormal staining for the most common muscular dystrophy proteins was not identified in the 2 dogs described in the present report. The clinical importance of the decreased integrin $\alpha 7$ staining in these 2 dogs is not known, as this protein may be secondarily decreased in people with myopathic diseases.^{6,20}

Laminin $\alpha 2$ deficiency is a well-known cause of secondary integrin $\alpha 7$ deficiency.²⁰⁻²² However, immunofluorescence staining for laminin $\alpha 2$ in both dogs was normal. Other possible causes of a secondary integrin $\alpha 7$ deficiency include protein instability and a lack of integrin $\alpha 7$ gene expression or RNA processing. On the basis of findings in human patients with myopathies of undetermined origin,⁶ the most likely explanation is that the protein is properly produced but is mislocated or unstable at the sarcolemmal level in the absence of some as yet unknown ligand. Further studies are warranted to evaluate integrin $\alpha 7$ expression in other dogs with inflammatory myopathy. The pattern and type of infiltrating cells in both dogs were similar to those recently described for several other dogs with generalized inflammatory myopathy.³ A recent study¹² evaluating the clinicopathologic features of 200 dogs with inflammatory myopathy showed that, compared with American Kennel Club breed statistics, Boxers were overrepresented.

Chronic inflammatory myopathy can result in substantial fibrosis and myofiber loss, which, if severe enough, can lead to irreversible dysfunction²³ and muscle contracture. Treatment of patients in the chronic stage of the disease is usually ineffective, and early diagnosis and treatment are critical for a good clinical outcome. Because Boxers have been identified as having an increased rela-

tive risk for inflammatory myopathy,¹² a finding of a persistently high serum CK activity in dogs of this breed should raise the index of suspicion for this disorder.

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