

# Aging Thyroarytenoid and Limb Skeletal Muscle: Lessons in Contrast

\*Lisa B. Thomas, †Anne L. Harrison, and ‡Joseph C. Stemple

*Lexington, Kentucky*

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**Summary:** Voice production is vital throughout life because it allows for the communication of basic needs as well as the pursuit and enjoyment of social encounters. Unfortunately, for many older individuals the ability to produce voice is altered. Structural and functional declines in the neuromuscular system occur with aging and likely contribute to the modification of voice. One specific target of the aging process is the thyroarytenoid (TA) muscle, the primary muscle of voice production. The objectives of this overview article are to (1) share current findings related to the aging of limb skeletal muscle, (2) identify age-related morphological and physiological features of TA muscle, (3) compare and contrast age-related changes in TA with those in limb skeletal muscle, and (4) describe therapies for reversing sarcopenia in limb muscle and consider the applicability of these therapies for addressing vocal fold atrophy and age-related voice changes. The article shares current knowledge from the basic sciences related to skeletal muscle aging and compares/contrasts typical muscle aging to TA aging. Current evidence suggests that (1) the TA muscle undergoes notable remodeling with age, (2) aging of the TA is multifactorial, resulting from a myriad of neurologic, metabolic, and hormonal changes, many of which are distinct from the age-related processes of typical limb skeletal muscle, (3) investigation of the aging of the TA and its role in the aging of voice is in its infancy, and (4) potential behavioral and non-behavioral therapies for reversing aging of the TA must be further examined.

**Key Words:** Aging—Voice—Thyroarytenoid—Skeletal muscle—Presbyphonia.

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From the \*Rehabilitation Sciences Doctoral Program, University of Kentucky, Lexington, Kentucky; †Division of Physical Therapy, University of Kentucky, Lexington, Kentucky; and the ‡Division of Communication Disorders, University of Kentucky, Lexington, Kentucky.

Address correspondence and reprint requests to Lisa B. Thomas, MA, Rehabilitation Sciences Doctoral Program, University of Kentucky, Charles T. Wethington Building, Room 106C, 900 South Limestone, Lexington, KY 40536-0200. E-mail: [Lisa.Thomas@uky.edu](mailto:Lisa.Thomas@uky.edu)

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## INTRODUCTION

Voice production is essential for oral communication. Unfortunately, for many, vocal function deteriorates over the course of later life. Research has consistently demonstrated age-related changes in pitch, pitch range, loudness, and quality.<sup>1-7</sup> For many elders, these voice changes are of sufficient magnitude to alter quality of life and limit social interaction.<sup>8,9</sup> Although a number of factors likely contribute to functional changes associated with the aging of voice, also known as presbyphonia, one primary contributor is felt to be deterioration

of the primary muscle of voice production, the thyroarytenoid (TA).<sup>3,10,11</sup>

The TA muscle is a paired skeletal muscle that makes up the bulk of the true vocal fold body. During voice production, the TA draws the vocal folds to midline and manages tension along the vocal fold edge.<sup>12</sup> Aging often brings about atrophic changes in the TA.<sup>13–15</sup> Such changes may alter the linear nature of the vocal fold edge and limit the muscle's ability to contribute fully to voice production.

Atrophic changes, such as those observed in the TA, are also identified in other skeletal muscles throughout the body. Sarcopenia refers to a loss of muscle mass, strength, and quality that is observed with aging.<sup>16,17</sup> The loss of mass occurs gradually and has little effect on functional skills until threshold of loss is met. Once individuals progress beyond threshold levels, notable declines in functional ability surface in daily life and the risk of disability increases. The link between sarcopenia and disability has led to a surge of research into this facet of the aging process. Reviews of aging muscle literature have attributed sarcopenic changes to a combination of hormonal, neurological, environmental, and metabolic factors.<sup>16–21</sup> The identification of these etiologic factors has spurred the investigation of potential therapies to reverse the effects of sarcopenia at the cellular level and enhance the life of older adults in our society. Much of the research in aging muscle to date has been conducted on limb skeletal muscle, and its applicability to the muscles of the head and neck remains to be defined.

Although we might presume that all voices age, the exact prevalence of clinical presbyphonia is unknown. Estimates suggest that between 12%<sup>22</sup> and 29% (N. Roy, J. Stemple, R. Merrill, and L. Thomas, unpublished data, 2006) of elderly adults have some form of vocal dysfunction, and studies of treatment-seeking populations suggest that age-related vocal change is one of the most common voice-related diagnoses seen in otolaryngology practices.<sup>23–26</sup> Yet, determining the prevalence of true presbyphonia remains a challenge for several reasons. First, vocal change in this population is often associated with age-related pathology rather than the pure effects of the aging process.<sup>27</sup>

Secondly, while vocal symptoms and glottal manifestations of presbylarynx (eg, spindle-shaped glottic chink, vocal fold bowing, and prominent vocal processes) are frequently observed in clinical practice,<sup>28</sup> the underlying mechanism of age are not well understood and are, therefore, difficult to study.

At present, treatment options for those suffering from presbyphonia are limited. Behavioral therapies aimed at strengthening the voice and restoring normal quality have been suggested by some.<sup>29–31</sup> However, many of these approaches have been adapted from treatment protocols for people with pathology, and evidence supporting their effectiveness in treating presbyphonia is limited. In addition, surgical intervention involving the augmentation or medialization of atrophic vocal folds has also been suggested.<sup>32–36</sup> Studies have found such treatments effective in reducing glottal gap and improving voice and swallowing function.<sup>32,34–36</sup> However, studies have yet to examine the long-term benefits of these treatments. Although the above behavioral and surgical methods offer a degree of relief to some, they do not address fundamental changes in the TA muscle observed in some older adults even in the absence of pathology. To address these basic tissue alterations, collaborative research efforts between bench and clinical scientists must translate cellular discoveries to functional outcomes. Such efforts would provide a greater knowledge of the TA and its aging process and lead the field toward more well-defined programs of prevention and rehabilitation.

The purpose of this overview article is to share with clinicians current findings from the bench sciences regarding age-related changes in skeletal muscle in hopes of preparing clinicians for future trends in the management of the aging voice. To meet this aim, the article will (1) share current findings related to the aging of limb skeletal muscle, (2) identify age-related morphological and physiological features of TA muscle, (3) compare and contrast age-related changes in TA with those in limb skeletal muscle, and (4) describe therapies for reversing sarcopenia in limb muscle and consider the applicability of these therapies for addressing vocal fold atrophy and age-related voice changes. The article will be translational in nature, linking the science

of the bench to the intervention of the bedside in hopes of creating a bridge between basic science and clinical practice. Each section begins with an overview of scientific findings regarding limb skeletal muscle and concludes with a review of findings specific to the vocal fold musculature.

### LIMB SKELETAL MUSCLE STRUCTURE AND FUNCTION

The basic organization of skeletal muscle is notably consistent across muscles of the human body. Whole skeletal muscles are made up of multiple individual muscle fibers (muscle cells), which are grouped into fascicles. Muscle fibers are, in turn, made up of smaller units called myofibrils, which are composed of myofilaments, the contractile proteins of muscle. Actin and myosin are the myofilaments that are linked during a muscle contraction.<sup>37</sup> Contraction of skeletal muscle is prompted by a complex series of events. Motor neurons from the spinal cord or brainstem send messages along a peripheral nerve to the target muscle. Release of acetylcholine at the neuromuscular junction (NMJ) excites the muscle membrane, resulting in the release of calcium ions from the sarcoplasmic reticulum of the muscle. These ions diffuse throughout the muscle fiber reaching the contractile proteins of the fiber. Calcium bonding with myofilaments permits the bonding of actin and myosin myofilaments. The hydrolysis of adenosine triphosphate (ATP) results in the production of energy. The energy created during this process facilitates the sliding of actin on myosin, resulting in a muscle contraction.<sup>38</sup> The energy supplied from ATP is also used to pump calcium back into storage within the sarcoplasmic reticulum of the muscle in order for relaxation of the muscle to occur. Despite the fact that all skeletal muscles share this same basic molecular mechanism of contraction, the potential of individual muscles can vary markedly in regard to total tension development, fatigue resistance, and the ability to create motion. Characteristics that are known to influence these abilities include ratios of various muscle fiber types within the muscle, muscle metabolism, motor innervation, and sensory innervation.<sup>39</sup>

### Fiber type and metabolism

Actin and myosin comprise the contractile proteins of the muscle fiber. Actin myofilaments are composed of thin chains of intertwining protein molecules, whereas myosin filaments are thicker and characterized by periodic enlargements called head groups, which provide binding sites for actin during contraction. Speed of contraction of the muscle fiber is determined by the fiber's myosin heavy chain (MyHC) isoform.<sup>40</sup> Three basic isoforms are commonly expressed in muscle<sup>a</sup>: MyHC- $\beta$  yields slow fiber contractions, whereas MyHC IIa and IIx produce rapid contractions.<sup>41</sup> The recruitment of a large proportion of fibers composed primarily of MyHC IIa and IIx will be a determinant of the muscle's ability to develop tension and force.

Sustainability of contraction, or resistance to fatigue, is determined in part by the muscle fiber's method of energy production. Energy is produced by the hydrolysis of ATP; continual stores of ATP are required for muscle contraction. The mitochondria of the muscle fiber use oxygen and nutrients delivered through the blood vessels and associated capillaries to produce ATP. Some fibers produce ATP at a high rate through the use of oxygen to synthesize ATP. These oxidative pathways of energy production are highly efficient and yield multiple ATP molecules per metabolic exchange, resulting in the ability to sustain contractions for long periods of time. During rapid and forceful contractions, ATP production through oxidative phosphorylation is limited by the time it takes to supply adequate oxygen to the cell through the circulatory system. Other fibers rely on anaerobic energy production. Anaerobic glycolysis produces ATP molecules quickly for fast-contracting fibers. These glycolytic pathways produce fewer ATP molecules per metabolic exchange, but the process is faster and can occur in the absence of oxygen. The glycolytic process

<sup>a</sup> In addition to the three primary MyHC isoforms described above, a few highly specialized isoforms have been found in some human skeletal muscles. Muscles demonstrating these unique profiles appear to be refined for specialized patterns of contraction. Superfast MyHC- $\epsilon$  and MyHC- $\alpha$  isoforms have been found in human extraocular muscles and masseter muscles, respectively.<sup>44</sup> The presence of these rapidly contracting isoforms illustrates the sophistication of movement required by these muscle systems.

requires substantial glucose to produce small amounts of ATP, and while effective in producing rapid and forceful muscle contractions, the process is not resistant to fatigue.<sup>38</sup> Our ability to function adequately in the world requires muscles that can generate substantial power, as needed in walking, and those that can endure, as needed when standing erect. To underscore the important clinical relevance of fatigue resistance or power generation, we should note that muscle fatigue or weakness manifests itself initially during function as a lack of movement coordination and control, which in turn predisposes the associated tissue to injury.<sup>42</sup>

The cross-correlation of information related to a specific muscle fiber's MyHC isoform and method of energy production permits the grouping of muscle fibers by their contractile properties. Using this model, three primary categories of human muscle fiber types have emerged<sup>41,43</sup>: type I, type IIA, and type IIX.<sup>b</sup> Type I fibers demonstrate slow rates of contraction and high levels of endurance (MyHC- $\beta$ , high oxidative profile, low- or moderate-glycolytic profile). Type I fibers are commonly found in higher ratios in postural muscles (eg, soleus, erector spinae) or other muscles requiring sustained contraction.<sup>43</sup> Conversely, type IIX fibers demonstrate faster rates of contraction and lower levels of endurance (MyHC IIX, high-glycolytic profile, low-to-moderate oxidative profile). Finally, type IIA fibers are often referred to as "intermediate" fibers. These fibers contract at a rate that is faster than type I fibers but slower than type IIX fibers. In addition, they demonstrate moderate resistance to fatigue (MyHC IIA, indicators of both oxidative and glycolytic energy production). Fibers of the type IIA and/or type IIX phenotype appear in larger ratios in fast-contracting powerful muscles such as the gastrocnemius and gluteus maximus of the lower extremities, the latissimus dorsi of the trunk, and the diaphragm.<sup>41,44</sup> All skeletal muscles in humans are comprised in varying ratios of these different fiber types,<sup>37</sup> and

they are typically arranged in a mosaic-type pattern throughout the muscle.<sup>45</sup> The muscle's overall fiber type composition is a determinant of its functional characteristics.

### Motor innervation

The structure and function of the muscle fiber is also dependent on its motor innervation. The motor unit comprises a motor neuron and all the muscle fibers innervated by that neuron. When the neuron fires, all associated fibers will contract. Large motor units consist of many fibers innervated by a single neuron, and are typically able to develop more force than smaller motor units. The smaller units comprise fewer fibers per neuron, facilitating coordination and control. For example, the powerful gluteus maximus muscle consists of large motor units, whereas the well-controlled postural muscle, the soleus, is made up of small units.<sup>42</sup> Interestingly, muscles with higher ratios of slow contracting type I fibers tend to have smaller motor units overall than those with higher ratios of type II fast-contracting fibers. Furthermore, motor neurons innervating type I fibers tend to be characterized by cell bodies and axons with smaller diameters, and reduced conduction velocity compared to those innervating type II fibers. The hamstring muscles that provide rapid mobility of the knee through a large range of motion are composed of a larger proportion of type II fibers, with fast conducting (large diameter) motor neurons and large motor units. Conversely, the erector spinae muscle that is important in sustaining upright posture has relatively more fatigue-resistant type I fibers with slower conducting (small diameter) motor neurons, and smaller motor units.<sup>39</sup>

### Sensory innervation

Sensation also plays a primary role in the function of limb skeletal muscle. The sensory receptors (proprioceptors) of typical skeletal muscle include the *muscle spindle*, which lies parallel to muscle fibers and is mechanically deformed by muscle stretch, and the *Golgi tendon organ* (GTO), which is deformed by the stretch of the tendon during muscle contraction. During moments of muscle stretch, the muscle spindle activates sensory neurons, which facilitate the alpha motor neuron of

<sup>b</sup> This specific fiber type has often been referred to in the literature as type IIB. However, recent work suggests that human fibers previously categorized as IIB would be more appropriately categorized as type IIX.<sup>41</sup> For the purpose of this article, the term type IIX will be used to include fibers previously discussed as type IIB.

the associated muscle. The spindle is able to adjust its length and thus maintain its sensitivity through its own motor innervation by the gamma efferent neuron. Conversely, the GTO activates sensory neurons, which ultimately inhibit the motor neuron of the associated muscle, contributing to force modulation and muscle protection. These sensory receptors play an important role in reflexive control of muscle firing, vastly improving the efficiency of muscle firing during functional activities. The integrity and integration of these and other neural elements ensure the development of appropriate force and speed of contraction, particularly during a complex function.<sup>46</sup>

Fiber type composition, metabolic pathways, and innervation are all critical determinants of structure and function in skeletal muscle (Figure 1 for a summary). Although each plays a role in the muscle's ability to develop force, resist fatigue, and provide a controlled response to commands from higher centers, it is the complex interaction among these various domains that ultimately determines functional outcomes. The above overview provides a foundation to compare and contrast the structure and function of skeletal muscles involved in voice production.

### TA STRUCTURE AND FUNCTION

The TA courses from the angle of the thyroid cartilage to the vocal process and fovea oblonga of the arytenoid cartilage.<sup>12,47,48</sup> The TA is often considered to be a two-compartment muscle made up of

the medially positioned vocalis and the more laterally positioned thyromuscularis.<sup>12,48</sup> Authors propose that the muscularis portion of the TA plays a role in rapid shortening of the true vocal cord (TVC) complex, whereas the smaller vocalis region plays a role in fine tuning tension along the TVC edge.<sup>12,48</sup> Contraction of the TA results in a thickening and stiffening of the muscle layer and a corresponding loosening of the transitional layers of the lamina propria.<sup>49</sup>

When compared to typical limb muscle, the TA muscle has been shown to stand apart along a number of fronts (Figure 2 for a summary). Fiber size, contractile protein profiles, and mitochondrial content are areas where laryngeal muscles have evidenced marked variability from limb skeletal muscle.<sup>50-59</sup> Because of the TA's notable divergence from other skeletal muscle, a brief review of the structure and function of the TA is in order.

The TA in humans consists of type I, IIA, and IIX fibers.<sup>10,57,59-63</sup> Hybrid fibers coexpressing more than one MyHC isoform have also been identified.<sup>63</sup> The various fiber types are typically arranged in the classic mosaic pattern across the breadth of the muscle.<sup>10,60</sup> Exact proportions of the various fiber types within the muscle have not been made, and variability among study methods makes comparisons difficult. Most, however, agree that the human TA type II fibers are slightly more abundant than type I fibers<sup>50,59,62,63</sup> and that within the type II category, type IIA fibers are the most common form.<sup>54,59</sup> Sanders<sup>64</sup> suggests that fast and slow fibers are arranged along a gradient,

Characteristic	Fiber Type		
	Type I	Type IIA	Type IIX
Metabolic pathway	Oxidative	Oxidative-Glycolitic	Glycolitic
Mitochondria number	High	High	Low
Capillary density	High	High	Low
Glycogen content	Low	Intermediate	High
Speed of contraction	Slow	Intermediate	Fast
MyHC-isoform	MyHC- $\beta$	MyHC IIA	MyHC IIX
Fatigue resistance	High	Intermediate	Low
Diameter motor neurons	Small	Intermediate	Large
Muscle fiber diameter	Small	Intermediate	Large

FIGURE 1. Characteristics of muscle fiber types.<sup>39,41,42</sup>

Feature	Limb Skeletal Muscle	Thyroarytenoid
<b>Fiber Types</b>	I, IIX, IIA* <sup>41,43</sup>	I, IIX, IIA <sup>10, 57, 59-63</sup> Specialized isoforms in humans <sup>54,57,59</sup> MyHC-eo in animal models <sup>52,53,58</sup>
<b>Fiber Arrangement</b>	Mosaic pattern <sup>45</sup>	Mosaic pattern <sup>10,60</sup>
<b>Contractile Features</b>	Speed variable among muscles <sup>41,43,44</sup>	Rapidly contracting; speeds in excess of limb skeletal muscle <sup>57</sup>
	Fatigue-resistance variable among muscles <sup>41,43,44</sup>	Fatigue resistant <sup>65</sup>
<b>Motor Unit (MU) Size</b>	Large and small MUs present <sup>42</sup>	Small MUs <sup>50,51,55,56</sup>
<b>Proprioception</b>	Muscle spindle <sup>46</sup>	Presence of spindle debated <sup>67,68</sup>
	Golgi Tendon Organ <sup>46</sup>	May be mediated by mucosal mechanoreceptors <sup>69</sup>

\*Ratio of these fiber types vary across limb skeletal muscle. Ratios determine the functional characteristics of the muscle.

**FIGURE 2.** Limb skeletal muscle and the TA.<sup>10,41–46,50–63,65,67–69</sup>

with the medial aspect of the TA being composed primarily of slow fibers and the lateral aspect being composed primarily of fast fibers. This particular fiber composition places the TA as a fast-contracting muscle, with contractile speeds that often meet or exceed those of limb skeletal muscle.<sup>57</sup> Recent studies have suggested the presence of additional specialized MyHC isoforms in some human TA fibers.<sup>54,57,59</sup> To date, these isoforms have not been positively identified; however, they approximate the MyHC-eo found in specialized human muscles and the laryngeal muscles of other species.<sup>52,53,58</sup> These findings highlight the uniqueness of the TA muscle relative to limb skeletal muscle.

The laryngeal muscles demonstrate notable variability from limb skeletal muscle in relation to mitochondrial density. Work by Hinrichsen and Dulhunty identified that the posterior cricoarytenoid and cricothyroid muscles of the larynx possessed unusually high densities of mitochondria per fiber volume when compared to limb skeletal

muscle.<sup>65</sup> The authors proposed that the high mitochondrial counts were reflective of the muscles' continuous action in the process of respiration. Although the TA muscle was not among the laryngeal muscles under study by Hinrichsen and Dulhunty, recent work by Andrade and McMullen (unpublished data, 2006) has identified high mitochondrial densities in the TA also. The elevated mitochondrial presence of the TA suggests the importance of fatigue resistance in the muscle's functioning.

Thus, the TA appears distinctive in terms of its contractile properties. The muscle combines the feature of rapid contraction with the feature of fatigue resistance, an atypical finding among skeletal muscle. The contractile profile may be reflective of the TA's complex role, as a muscle of respiration, airway protection, and voice production.

In addition to its unique contractile properties, the TA has long been recognized for its rich neural support from the vagus nerve.<sup>12,50,51,55,56,66</sup>

In most cases, motor innervation is received solely from the vagus nerve's recurrent laryngeal branch (RLN); however, there is new evidence that, in a small number of cases, motor innervation may also be received through the internal or external branches of the superior laryngeal nerve (SLN).<sup>66</sup> The TA is highly innervated with motor units characterized by only a few muscle fibers innervated by a single motor neuron.<sup>50,51,55,56</sup> Sensory information within the larynx is identified by mechanoreceptors, chemoreceptors, taste buds, and free nerve endings located at various points in the laryngeal complex. Interestingly, the presence of proprioceptive organs, such as the muscle spindle, within the human TA continues to be a matter of debate and discussion.<sup>67,68</sup> Work by Andreatta et al<sup>69</sup> suggests that the proprioceptive sense within the larynx may be mediated outside of the muscle layer through mucosal mechanoreceptors located in the posterior region of the larynx, near the TA's attachment to the arytenoid cartilage. Sensory information from the laryngeal receptors is transmitted *via* the internal branch of the SLN (points down to the level of the vocal folds) and the RLN (points below the level of the vocal folds).<sup>64</sup>

### AGE-RELATED CHANGE

Roubenoff and Hughes<sup>17</sup> note that sarcopenia is, at its core, a disorder of muscle *cells*, rather than a disorder of muscle *mass*, suggesting that changes at the cellular level are primary and lay the foundation for subsequent alterations in muscle mass. These authors, along with others,<sup>16,20,21</sup> offer that aging brings about a cascade of morphologic, neurologic, metabolic (mitochondrial and vascular), hormonal, and environmental changes that affect the muscle cell and ultimately the muscle structure and function as a whole. The question remains as to whether the sarcopenic features of limb skeletal muscle can be generalized to other muscles throughout the body. Some have argued that muscles of the head and neck are unique in their morphology as well as in their response to aging<sup>70,71</sup> and that these muscles should, therefore, be considered separately during studies of age-related decline. Below is a review of the morphological

changes observed with age and the primary influences or domains associated with these changes. Similarities and differences between limb skeletal muscle and the TA are discussed and are summarized in Figure 3.

### Morphology

#### *Limb skeletal muscle*

Muscle morphology changes in a number of ways in later life. One of the most marked features of sarcopenic muscle is a reduction in overall muscle mass.<sup>72,73</sup> Authors have estimated the loss of mass in some muscle groups to be as great as 20–40%.<sup>74–79</sup> In limb skeletal muscle, the loss of mass comes about secondary to reduced numbers of type I and II fibers<sup>79,80</sup> along with the preferential atrophy of type II fibers.<sup>79,81,82</sup> Areas within the muscle left void by fiber loss and atrophy are partially infiltrated by connective tissue and fat.<sup>79,80</sup> As a result, estimates of muscle cross-sectional area may not fully express the degree of muscle loss that has been experienced.<sup>45</sup> It is reasonable to assume that as the ratio of connective tissue to muscle increases, the work of remaining muscle must also increase to achieve the same function. Finally, some studies have demonstrated that older muscles lose the mosaic-like distribution of fiber types and take on a pattern whereby fibers of a similar type group together.<sup>72,83</sup> This phenomenon, known as *fiber type grouping*, is felt to be an indicator of ongoing denervation-reinnervation within muscle. Under this assumption, the denervated fibers of failing motor neurons are “adopted” by nearby motor neurons. The reinnervated muscle fibers, then, take on the profile of the adoptive neurons, resulting in clustering of similar fiber types.<sup>21</sup>

The morphological changes of skeletal muscle noted above have a marked effect on muscle function, including the parameters of speed, force, strength, and endurance.<sup>84</sup> During aging, the speed of muscle contraction declines. Although the exact mechanism of the decline is unknown, it has been speculated that the slowing may be secondary to a reduction in the number and size of fast-contracting fibers or an alteration in the fundamental contractile properties of fibers.<sup>84</sup> Further, aged muscle produces less force than younger muscle. This

	Skeletal Muscle	Thyroarytenoid
<b>Morphology</b>		
<b>Muscle Fiber</b>	Reduced muscle mass <sup>72,73</sup> Loss of type I and II fibers <sup>79,80</sup> Atrophy type II fibers <sup>80-82</sup> Fiber type grouping <sup>72,83</sup>	Reduced muscle mass <sup>13,14, 15, 86</sup> Fiber loss pattern not clearly defined Loss type I, II <sup>14</sup> Loss type I only <sup>60,87</sup> Loss type II only <sup>10</sup> Maintenance of type II fiber size <sup>62,87</sup>
<b>Connective Tissue</b>	Infiltration of connective tissue <sup>79</sup>	Connective tissue patterns not clearly defined. Increase <sup>10,15,60,62</sup> No change <sup>87</sup>
<b>Function</b>	Reduced speed of contraction <sup>84</sup> Reduced force of contraction <sup>84</sup> Reduced strength <sup>75-77, 85</sup> Reduced endurance <sup>18</sup>	Reduced speed of contraction* <sup>71</sup> Reduced force of contraction* <sup>71</sup> Reduced endurance* <sup>71</sup>
<b>Mechanisms</b>		
<b>Neurologic</b>		
<b>Motor</b>	Decline in number and size of ventral horn alpha motor neurons <sup>82,84,88-90</sup> Preferential loss of neurons innervating type II fibers <sup>82</sup> Reduced number nerve fibers <sup>91-93</sup> Alterations in myelin sheath <sup>91,92</sup> Changes of neuromuscular junction <sup>84,95-100</sup>	No net loss of RLN fibers <sup>110</sup> Alterations in myelin sheath <sup>110</sup> Changes of neuro muscular junction <sup>111,112</sup>
<b>Sensory</b>	Morphological changes in muscspindle <sup>104-108</sup> Reduced proprioception <sup>104-108</sup>	Reduced number and size of SLN nerve fibers <sup>113</sup> Alterations in myelin sheath <sup>114</sup> Reduced density of sensory nerve endings <sup>112</sup> No information on proprioceptive changes
<b>Metabolic</b>		
<b>Mitochondria</b>	Increased number of mitochondrial abnormalities with associated loss of fiber number, size, and integrity <sup>124-126</sup>	Increased rate of mitochondrial mutations <sup>132</sup> Abnormal accumulations of mitochondria <sup>10,71</sup>
<b>Vascular</b>	Altered vasodilation <sup>129,130</sup>	Reduced blood flow Reduced capillary surface area <sup>111</sup>
<b>Hormonal</b>		
	Proposed relationship between hormonal changes and muscle loss <sup>17,19,20,142</sup>	Decline in number of sex hormone receptors in larynx with age <sup>153,154</sup> No studies examining shifts in hormone levels with morphological changes in TA
<b>Physical Activity</b>		
	Resistance exercise increases mass and strength <sup>74,76,77,160-162</sup> Endurance training increases mitochondrial volume density <sup>160,162, 163-166</sup> Disuse associated with atrophy <sup>172</sup>	Direct relationship between exercise and mass unable to be examined No data on disuse and atrophy

**FIGURE 3.** Morphology and mechanisms of age-related change.<sup>10,13-15,17-20, 60,62,71-77,79-93,95-100,104-108,110-114,124-126,129,130,132,142,153,154,160-166,172</sup>

limitation likely comes about due to changes in the size and makeup of motor units as well as the loss and atrophy of muscle fibers.<sup>84</sup> Strength reductions in aged limb skeletal muscle are also well documented.<sup>75–77,85</sup> In some muscle groups, the age-related reduction in strength may be as great as 30–35%.<sup>76,85</sup> These alterations in strength have been linked primarily to declines in muscle mass<sup>75</sup> and reductions in the amount of force produced per unit of cross-sectional area in aged muscle.<sup>77</sup> Decreased muscular endurance is also associated with the aging process, and is presumably related in part to the overall decline in muscle mass.<sup>18</sup>

#### *The TA*

Morphologic and physiologic changes occur in the TA muscle with aging. Bach et al first identified a reduction in human TA muscle mass in 1941.<sup>13</sup> Since the time of their work, additional authors have confirmed this age-related TA atrophy in humans.<sup>14,15,86</sup> Attempts to define more specific morphological changes within the TA have, however, resulted in little agreement across studies.

Although limb skeletal muscle demonstrates a loss of both type I and II fibers with age, patterns of fiber loss within the TA have yet to be clearly defined. Some have reported a loss of both fast and slow fibers,<sup>14</sup> whereas others have reported preferential loss of slow<sup>60,87</sup> or fast<sup>10</sup> types only. In addition, the classic reduction in type II fiber diameter seen in limb muscle has not been observed in the TA by some authors.<sup>62,87</sup> Furthermore, research is mixed in regard to the infiltration of connective and fatty tissues into the aging TA. Several authors have observed an increase in noncontractile tissues with age,<sup>10,15,60,62</sup> whereas others have not evidenced this phenomenon.<sup>87</sup> As a result, researchers continue to investigate the morphological features of the aging TA in hopes of better defining patterns of change.

Coinciding with the aforementioned changes in morphology are alterations in the TA's contractile profile. Work by McMullen and Andrade<sup>71</sup> has identified a reduction in force, speed, and endurance in the TA muscle of aged rats. Interestingly, the physiologic changes noted by McMullen and Andrade were accompanied by a shifting of the TA's metabolic properties toward a more glycolytic,

less oxidative, profile. Generalization of these findings to the human TA must be made with caution, however, as the typical rat TA has a greatly increased ratio of type II fibers compared to the human TA. At present, no studies have documented age-related changes in the contractile profile of the TA in humans.

### **Mechanisms**

#### *Neurologic*

*Limb skeletal muscle.* Skeletal muscles and the motor neurons that supply them enjoy a complex and interdependent relationship. The survival of the nervous system is critical to the survival of muscle tissue, and as the nervous system declines with age, so does its ability to sustain skeletal muscle. In fact, neurological decline is felt to be one of the primary contributors to the age-related remodeling of muscles described above. The denervation of muscle may well be the primary contributor to fiber loss, atrophy, and fiber type grouping. Therefore, it is important that an examination of age-related remodeling of skeletal muscles consider age-related modifications in the nervous system.

Aging alters the nervous system at a variety of levels, including the spinal cord and brainstem, the peripheral nerve, and the NMJ. Changes at each of these levels coalesce to produce an altered form of neural innervation for aged muscle. Within the ventral horn of the spinal cord, authors have identified a decline in both the number and size of alpha motor neurons.<sup>82,84,88–90</sup> This loss of motor neurons may be preferential to those motor neurons innervating the fast type II muscle fibers,<sup>82</sup> an interesting finding considering the preferential atrophy of type II fibers in limb muscle noted earlier.

Peripheral nerves responsible for transmitting the neural impulse to the target muscle have also been shown to undergo marked changes in structure and function with age. Aging brings a reduction in the number of myelinated and unmyelinated fibers within peripheral nerves.<sup>91–93</sup> Surviving myelinated fibers demonstrate alterations in sheath thickness and regularity.<sup>91,92</sup> Finally, older fibers exhibit regions of myelin retraction and an associated lengthening of the Nodes of Ranvier, the regularly distributed gaps in the myelin sheath known to be

important in the transmission of the neural signal.<sup>92</sup> Consistent with these findings is the knowledge that peripheral nerve conduction velocity of both sensory and motor axons declines with age.<sup>82,94</sup> Furthermore, this slowing seems preferential to large diameter myelinated fibers, of the type providing motor innervation to the fast-contracting, fatigue-resistant muscle fibers.<sup>82</sup>

Finally, alterations in the morphology and physiology of the NMJ are worthy of note. Primary morphometric changes include a reduction in the number and associated increase in size of nerve terminal areas, a widening of the synaptic cleft, and an enlargement of the motor end-plate area.<sup>84,95</sup> Further, postsynaptic clefts become wider and more shallow with age.<sup>95</sup> Coinciding with these morphological changes are changes in the dynamic features of the NMJ. Authors have documented an age-related reduction in the number of synaptic vesicles.<sup>96</sup> As synaptic vesicles store neurotransmitters that are released during calcium-regulated interneuronal signaling, such a decline would appear to have marked implications for neural transmission. However, according to Robbins,<sup>97</sup> the NMJ compensates well for this loss. Remaining vesicles release increased amounts of neurotransmitter per impulse and, thereby, maintain the status of transmission. Finally, dynamic changes are also evidenced by an increase in terminal axon branching and sprouting.<sup>45,96,98–100</sup> McComas<sup>45</sup> suggests that this increased complexity of the NMJ may be the result of ongoing processes of denervation and reinnervation within aged muscles and the attempt to maintain muscle function in the presence of neurological decline.

As a result of the above changes at the spinal cord, peripheral nerve, and NMJ, the number of functional motor units declines with age<sup>101,102</sup> and surviving motor units increase in size as the orphaned muscle fibers are innervated by remaining neurons.<sup>103</sup> This compensation suggests an increase in innervation ratio (ie, more muscle fibers per neuron) with perhaps a corresponding reduction in muscular control.

The decline in limb skeletal muscle function may also be linked to the changes associated with aging in sensory innervation. The evidence strongly suggests that morphological and functional age-related changes occur in the muscle

spindle. An associated decline in proprioceptive abilities has also been demonstrated in older adults, particularly in the lower extremity muscles and joints.<sup>104–108</sup> Furthermore, as previously discussed, a decline in the function of large myelinated peripheral nerve fibers and an associated decline in myelin thickness is often associated with the age-related slowing of nerve conduction velocity in both sensory and motor neurons.<sup>109</sup>

*The TA.* Despite the importance of the TA in a number of life-sustaining functions, relatively few studies have considered age-related changes in its neural control. Only one large-scale study has investigated morphological changes of the RLN in later life.<sup>110</sup> Results of the study suggested an increase in the number of myelin-abnormal and myelin-thinning fibers with age along with an increase in myelinated fiber diameter. However, no net loss of myelinated or unmyelinated fibers was noted with age. The authors interpreted their findings as evidence for an increase in the process of degeneration—regeneration within the RLN with age. In addition to the above RLN alterations, the NMJ shows a decline in axonal terminal areas and a reduction in the size and density of motor end plates with age.<sup>111,112</sup>

Laryngeal sensory mechanisms also undergo age-related change. The primary sensory nerve of the larynx, the SLN, evidences a reduction in myelinated fiber number and size<sup>113</sup>; areas of segmental demyelination and axonal degeneration are also present.<sup>114</sup> Furthermore, the density of sensory nerve endings in the larynx declines with age.<sup>112</sup> Functionally, these changes may contribute to the documented reduction of laryngeal sensitivity with age.<sup>115,116</sup> Study of laryngeal proprioception is still in its infancy. As a result, little information has emerged pertaining to age-related change in this component of laryngeal sensation.

#### *Metabolic*

*Limb skeletal muscle.* Mitochondria are small organelles within the muscle cell that play a significant role in the metabolic determinants of muscle

contraction. It is within the mitochondria that ATP, the muscle cell's primary energy source, is produced.<sup>38</sup> One process through which mitochondrial production of ATP occurs is oxidative phosphorylation. Oxygen is taken in by the mitochondria and metabolized to form ATP; the ATP is eventually broken down to form energy for contraction. During normal mitochondrial function, by-products of the oxidative process are released in small amounts.<sup>117</sup> These by-products include the highly reactive and potentially harmful reactive oxygen species, also known as free radicals. Throughout much of life, the presence of specific enzymes and antioxidants in the body counteracts the injurious effects of these agents and prevents significant cellular damage.<sup>118</sup>

Harman<sup>119</sup> was the first to propose a relationship between the oxidative process and normal aging. He suggested that the accumulation of oxidative damage within cells during the course of the lifespan could lead to the progressive decline recognized as normal aging. Since its initial presentation, Harman's theory has been refined and further specified.<sup>120,121</sup> Authors proposed that mitochondrial DNA naturally mutate over time and demonstrate an increased rate of mutation in later life. These mitochondrial DNA mutations alter the oxidative phosphorylation process and result in the increased production of damaging free radicals within the cell. Free radicals impose further damage on the mitochondrial DNA, establishing a vicious cycle of deterioration.<sup>120,121</sup> In the years just following the theory's revision, scientists were, in fact, able to identify a mitochondrial DNA mutation common in the tissues of aging humans.<sup>122</sup> Interestingly, the common mitochondrial DNA mutation (4977-base pair deletion) was found to be more prevalent in organ systems with high oxidative demands, leading researchers to propose that strongly oxidative tissues may be more susceptible to the deleterious effects of oxidative damage than other organ systems.<sup>122</sup> Such a finding holds significant implications for those skeletal muscles that demonstrate strongly oxidative profiles as they would theoretically be more prone to oxidative damage over the lifespan. Finally, recent research has suggested that an age-related decline in antioxidant defense systems may reduce the body's ability to neutralize free radicals, and thereby, reduce its

ability to combat the deleterious effects of oxidative damage.<sup>118,123</sup>

The emergence of the mitochondrial theory of aging has brought a surge of interest into the potential relationship between mitochondrial changes and skeletal muscle aging. Studies have confirmed that aged skeletal muscles contain higher levels of mitochondrial abnormalities than younger muscles.<sup>124-126</sup> Furthermore, muscles expressing these abnormalities evidence higher rates of fiber loss, fiber atrophy, and fiber splitting than do muscles without such abnormalities. This finding has led some to suggest a direct relationship between these mitochondrial alterations and the age-related loss of skeletal muscle mass.<sup>124-126</sup> In addition to the abnormalities in mitochondrial structure, Conley et al<sup>127</sup> identified reductions in both the density and productivity of mitochondria with age. It has been suggested that such declines may alter the capacity of skeletal muscle to perform oxidative processes.<sup>127,128</sup> As oxidative phosphorylation is a primary source of energy production for many skeletal muscles, reductions in the oxidative capacity have direct implications for a muscle's energy production and susceptibility to fatigue.

Muscle tissue relies upon the vascular system for the delivery of oxygen and other nutrients for energy production. It follows, then, that any restriction in the vascular system's ability to deliver blood to muscle tissue has the potential to alter the muscle's metabolic processes.<sup>19</sup> Normal aging may bring changes in vasodilation ability,<sup>129,130</sup> capillary size, and capillary density.<sup>131</sup> If present, such changes may limit the delivery of adequate amounts of oxygen for proper muscle function. In addition, the increased prevalence of certain vascular disease processes with age (eg, atherosclerosis, hypertension, congestive heart failure) may hinder the blood supply to muscles and, therefore, interfere with energy production within the mitochondria.<sup>19</sup>

*The TA.* The emergence of mitochondrial theories of aging has led to the investigation of cellular changes in the aged larynx. Manaligod et al<sup>132</sup> have confirmed the presence of the common 4977-base pair deletion in the lamina propria, the TA, and the cricoarytenoid joint of aged human larynges. In addition, the authors found that the

expression of the mitochondrial mutation appeared to increase with increasing age. These early findings led the group to conclude that mitochondrial alterations do play a role in laryngeal aging.

Manaligod et al's work has prompted others to examine the potential relationship between mitochondrial dysfunction and laryngeal aging. At least two studies have identified abnormal accumulations of mitochondria within aged TA muscle fibers, a finding suggestive of age-related mitochondrial deficits.<sup>10,71</sup> Interestingly, McMullen and Andrade<sup>71</sup> found that aged rats demonstrating the increased mitochondrial content also displayed a *reduction* in fatigue resistance and an *increase* in glycogen content. The authors proposed that the age-related mitochondrial dysfunction within the TA may actually alter contractile properties of the muscle by prompting a shift toward a more glycolytic profile.

Relatively few studies have examined potential vascular contributions to TA deterioration. In fact, only two studies considering age-related changes in blood flow to the TA muscle could be identified. In the first of these investigations, Malmgren and Lyon (as cited in Lyon and Barkmeier-Kraemer, 2004) identified a 42–60% reduction in laryngeal blood flow in older rats. The greatest degree of vascular decline was noted in the TA muscle. Connor<sup>133</sup> reported a reduction in the capillary surface area within the TA of aged rats when compared with younger rats. The author proposed that the age-related vascular changes may alter the supply of oxygen and other nutrients to the muscle and reduce the elimination of cellular waste products, factors which could lead to alterations in muscle function. Certainly investigations in this area are few and additional investigation will be needed to elucidate vascular alterations with age and their potential influence on laryngeal aging.

### *Hormonal*

*Limb skeletal muscle.* Hormones exert considerable influence on skeletal muscle. Anabolic hormones, such as testosterone, insulin, growth hormone (GH), and insulin-like growth factor (IGF-1) enhance muscle mass by facilitating protein synthesis, limiting protein degradation, increasing the presence of muscle-producing

satellite cells, and/or controlling the expression of agents that inhibit muscle growth.<sup>134–139</sup> Conversely, catabolic hormones, such as tumor necrosis factor and interleukin-6, exert a negative influence on muscle structure.<sup>140–142</sup> These agents likely act by signaling the death of muscle cells, inhibiting protein synthesis, or increasing the body's resistance to insulin.<sup>143–145</sup>

Interestingly, many of the above hormone levels change in later life. Levels of testosterone, GH, and IGF-1 decrease in the elderly,<sup>146–148</sup> whereas levels of some catabolic forces rise.<sup>142</sup> Several notable reviews of muscle aging literature have proposed that this age-related shift in the relative levels of anabolic and catabolic hormones may contribute to the muscle loss observed in aging.<sup>17,19,20,142</sup> These authors have proposed that the age-related decrease in anabolic forces, paired with the corresponding increase in catabolic factors, may yield a protein degradation rate that outweighs the protein synthesis rate, resulting in an overall loss of muscle mass.

*The TA.* The influence of hormones on the voice has been appreciated for some time. Hormones play a significant role in the development of the laryngeal complex and the maturation of voice.<sup>149–152</sup> Secretions of the thyroid and pituitary glands exert a degree of influence over vocal function<sup>11,15</sup>; however, the gonadal hormones appear to be the most influential in determining voice changes over the lifespan.<sup>15</sup> Interestingly, the mechanism of hormonal influence over the larynx is still disputed, as studies searching for the presence of hormone receptors within the laryngeal structure have yielded conflicting results.<sup>153–155</sup> Researchers identifying sex hormone receptors within the laryngeal tissues suggest a direct relationship between hormones and voice function.<sup>153,154</sup> However, authors failing to identify such receptors propose that hormonal influence over the voice may be mediated indirectly through hormone-related alterations in motor and sensory function.<sup>155</sup> The mechanism of hormonal influence on phonation, while still poorly defined, becomes critical as researchers attempt to link hormonal changes with presbyphonia.

According to Segre,<sup>15</sup> age-related hormonal changes are one of the primary causes of voice

changes associated with age. However, the literature base supporting Segre's assertion is limited. Most of the research linking hormonal changes with alterations in the acoustic and perceptual features of voice has emerged from studies on postmenopausal women.<sup>156–159</sup> Segre<sup>15</sup> argues vocal change at menopause should not be equated with vocal aging, a later occurring and vocally distinct phenomenon. As a result, much of the available literature discussing the hormonal influence on voice does not apply directly to the senescent voice. Further, while a number of authors have alluded to the effect of hormones on the aging of the laryngeal structure, only two studies could be identified in the literature directly linking hormones and the aging larynx. Holt et al<sup>153</sup> and Newman et al<sup>154</sup> identified a decline in the expression of sex hormone receptors in the larynx with advanced age. Whether this reduced expression was secondary to a lowering of serum hormone levels with age or to changes at the DNA level has yet to be determined. However, these findings do suggest a reduction in laryngeal sensitivity to hormonal influences in later life. Finally, no studies have linked age-related shifts in hormone levels to specific morphological modifications of the vocal folds. Although such a relationship continues to be touted as a cause of laryngeal remodeling, scientific documentation of the relationship has not emerged.

### Physical activities

#### *Limb skeletal muscle*

Muscle tissue is highly plastic and responsive to patterns of use. Thus, many have considered the effects of physical activity on muscle structure and function in later life. It has been well established that programs of exercise yield positive changes in the structure and function of muscle. Resistance exercise has been consistently linked with increases in muscle mass and strength,<sup>74,76,77,160–162</sup> whereas endurance training has been associated with increases in mitochondrial volume density and a possible shifting of fiber types toward a more oxidative profile.<sup>160,162–166</sup> Exercise mediates these changes at the cellular level by shifting levels of anabolic and GHs, modifying neuronal input, heightening enzymatic activity, and enhancing antioxidant defenses.<sup>19,74,167–170</sup> Apart from structured programs

of exercise, there is also evidence to suggest that general levels of physical activity may be predictive of muscle mass in the elderly population.<sup>161,171</sup> Congruent with the above is the knowledge that periods of muscle disuse are linked with muscle atrophy.<sup>172</sup> Disuse yields the atrophic changes by reducing protein synthesis, increasing the rate of protein breakdown, and increasing the oxidative damage within muscle cells. Older animals appear to be more negatively impacted and less able to recover from disuse than younger animals.<sup>172</sup> The reality that physical activity and exercise participation decline in the aging population suggests a clear role for use/disuse in the decline of skeletal muscle function associated with aging.<sup>173</sup> The extent of the effect is difficult to ascertain and discern from physiological changes associated with aging.

#### *The TA*

Demonstrating the relationship between vocal exercise/activity and TA structure and function has proven challenging to researchers. Physiologic voice exercise programs such as Lee Silverman Voice Treatment,<sup>174</sup> Vocal Function Exercises,<sup>175</sup> and Resonant Voice Therapy<sup>176–178</sup> have been shown to bring about positive changes in laryngeal function<sup>175,179–182</sup>; however, their effect on muscle structure has not been documented due to the delicate nature of the folds and the invasive nature of muscle biopsy. As a result, scientific evidence for the direct effect of such programs on muscle tissue is lacking. Further, the complexity of tracking general voice use patterns over time has made it difficult to establish a relationship between daily voice use patterns and TA muscle structure in later life.

Finally, periods of prolonged vocal rest (disuse) have interested voice researchers for years. However, much of the literature in this area has focused on the effect of voice nonuse on laryngeal pathology, vocal fold mucosal health, and laryngeal function. To date, no studies of the TA have linked periods of voice disuse with alterations at the muscle cell level.

Thus, there remains a void in the literature related to the effect of voice use patterns and vocal exercise on TA muscle structure. This is a critical area for study and one with significant implications for vocal training and rehabilitation. Until less

invasive methods of muscle study can be established, the field must rely upon indirect markers of vocal fold structure (eg, laryngeal videostroboscopy) to document the effectiveness of exercise interventions on the vocal fold musculature.

## CONCLUSIONS

The above review has demonstrated that the TA muscle undergoes significant remodeling and sarcopenic change with age. Most notable among these changes are muscle atrophy, fiber loss, increased glycolytic metabolism, and increased mitochondrial abnormalities<sup>10,13–15,60,86,87</sup> (Andrade and McMullen, unpublished data, 2006). In animal models, these modifications are of sufficient magnitude to alter the muscle's contractile properties toward a slower, weaker, and less fatigue-resistant profile.<sup>71</sup> Likely contributors to the structural and functional changes include myelination alterations within the RLN and SLN, reduced number of axonal terminals, decreased vascular support, systemic hormonal changes, and stochastic damage.<sup>110–114,133,142,146–148,183,184</sup>

The above discussion has also highlighted the distinctive nature of the TA muscle. Findings point to the human TA as a rapidly contracting, fatigue-resistant muscle, an atypical form among human muscles. In addition, the TA diverges from “typical” limb skeletal muscle in its architecture, innervation, contractile protein profile, mitochondrial content, and aging patterns. Because of these differences, literature pertaining to typical skeletal muscle cannot be easily generalized to the laryngeal musculature. There exists, therefore, a need for researchers to engage in systematic study of the laryngeal muscles for the purpose of clearly defining the muscle's cellular features and processes associated with aging.

It should be noted that the above discussion has focused solely on aging of the TA muscle. It is well known that the TA muscle does not act alone in voice production, but rather in harmony with other intrinsic laryngeal muscles and with the vocal folds' mucosal layer. Age-related changes have been consistently observed in these associated laryngeal structures.<sup>62,185–189</sup> In the future, it will be important to blend knowledge related to the

aging of these various laryngeal structures for the purpose of determining the relative contribution of each in the aging of voice. Defining the primary determinants of age-related change will lead to more targeted models of prevention and treatment.

Finally, the above review suggests that some treatments (eg, strengthening exercises, hormonal therapies) are beneficial in reversing sarcopenia in limb muscle. However, the appropriateness of these treatments for age-related change in laryngeal muscle has yet to be documented. Investigation of the applicability of various treatments to laryngeal muscle will likely be an area of future development.

## Future directions

Several pathways for research have emerged from the above discussion. Age-related changes in the nervous system appear to underlie a number of the morphological changes observed in the TA. However, the neural aspects of laryngeal aging, peripheral, and central, have received minimal research attention. Certainly, the door is open for the investigation of these elements and their contribution to vocal aging. Further, the review has identified notable areas of divergence between human and animal models of the larynx (eg, fiber type composition, general morphology, patterns of vocal fold use). Researchers must ascertain which areas of study are enhanced by animal models and which areas would be better served by use of the human model. Furthermore, pathways of research linking the basic and clinical sciences will be of great importance. As knowledge of the cellular aspects of vocal fold aging emerges, so does the challenge to apply that knowledge to programs of prevention and rehabilitation. Treatment models of the future will likely encompass both conventional models of behavioral treatment and innovative models of tissue engineering. Studies have consistently demonstrated the positive effect of physical exercise on the plasticity of limb skeletal muscle.<sup>74,76,77,190</sup> However, limitations inherent in TA biopsy and in the use of animal models of vocal exercise make the study of vocal exercise challenging. Future research efforts must focus on the development of methods for investigating vocal exercise models in human and/or animal models. Finally, while hormonal therapies have been documented as effective in enhancing limb muscle mass and

strength,<sup>136,191–194</sup> their effect on aging TA muscle is unknown. Studies examining the use of growth factors on denervation-related TA atrophy have shown promising results and have highlighted the importance of studying these factors in relation to age-related atrophy.<sup>195–198</sup>

According to the World Health Organization, those over 60 years of age are making up an ever-increasing segment of the world population.<sup>199</sup> Although this realization clearly touts the societal and medical advances of recent years, it also offers considerable challenges to those providing care for this population. In the coming decades, clinicians working in the area of voice can expect to see an increasing number of individuals with age-related vocal concerns. At present, the field is not equipped with a comprehensive mechanism for treating presbyphonia. Current models treat the consequences of change without offering adequate attention to the underlying mechanisms of change. New treatment models that appreciate and address the cellular foundations of vocal decline are needed. Their development may lead to better models of treatment and improved functional outcomes for patients.

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