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Tutorial

A Tutorial of the Effects of Sex Hormones on Laryngeal Senescence and Neuromuscular Response to Exercise

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Purpose: The purpose of this tutorial is to summarize how sex hormones affect both laryngeal senescence and neuromuscular response to exercise, highlighting the importance of considering sex differences in developing treatment for the senescent voice.
Conclusion: Men and women's voices are sexually dimorphic throughout the life span, including during the laryngeal adaptations observed during senescence. Therefore, presbyphonia (age-related dysphonia) likely clinically manifests differently for men and women due to differences in how the male and the female larynx change in response to aging. Because sexual dimorphism is evident in both laryngeal aging and response to exercise, voice therapy programs aimed at treating the typical and disordered aged voice should consider sex differences in their design.

It is well established that age differentially impacts the voices of men and women (W. S. Brown, Morris, Hollien, & Howell, 1991; Goy, Fernandes, Pichora-Fuller, & van Lieshout, 2013; Linville, 2002; Morsomme, Jamart, Boucquey, & Remade, 1997). Many physiological factors likely contribute to age-related changes in vocal function, including changes in pulmonary function, neuromuscular properties of intrinsic laryngeal muscles, and the cover of the vocal fold (Awan, 2006; Kahane, 1987; Takeda, Thomas, & Ludlow, 2000). For example, age-related changes within the vocal cover, such as decreases in mass, elastic fibers, and glandular secretions, affect the viscoelasticity properties of the vocal folds in advanced age (65+ years of age) and, thus, affect vocal fold vibration (Bloch & Behrman, 2001; Hirano, Kurita, & Nakashima, 1983; Kahane, 1983; Tomita, Nakashima, Maeda, Umeno, & Sato, 2006). Also, neuromuscular changes of the intrinsic laryngeal muscles likely partially contribute to age-related vocal changes (Connor, Suzuki, Lee, Sewall, & Heisey, 2002; McMullen & Andrade, 2006, 2009; Suzuki, Connor, Lee, Leverson, & Ford, 2002). However, little information exists on the extent of sexual dimorphism within the aging laryngeal muscles.

Both men and women's voices change as a normal process of aging; however, as with many other subsystems of the body, disorders of the voice are more likely to occur in old age (Bhattacharyya, 2014). Presbyphonia, age-related dysphonia, is generally associated with age-related anatomical changes in the larynx, similarly known as presbylaryngis (Sauder, Roy, Tanner, Houtz, & Smith, 2010). The literature has described presbyphonia as perceptually weak, hoarse, breathy, and unsteady with the hallmark characteristic of presbyphonia, vocal fold bowing (Kendall, 2007; Sauder et al., 2010; Verdonck-de Leeuw & Mahieu, 2004). Vocal fold bowing is attributed to the atrophy of the muscular portion of the thyroarytenoid (TA), observed in both aged men and women's vocal folds, and to the decreased depth of the superficial and intermediate layers of the lamina propria, primarily observed in the aged male larynx (Kersing & Jennekens, 2004; Martins et al., 2015; Mueller, Sweeney, & Baribeau, 1985; Tanaka, Hirano, & Chijiwa, 1994). Vocal fold bowing results in the incomplete closure of the membranous vocal fold edges during phonation and is one of the contributing factors to perceived hoarseness and breathiness and loss of vocal intensity in the aged voice (Linville, 1996; Lundy, Silva, Casiano, Lu, & Xue, 1998). Studies have estimated that the prevalence of presbyphonia

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is between 4% and 30% in older adults but have not identified the ratio of men to women with presbyphonia (Verdonck-de Leeuw & Mahieu, 2004; Woo, Casper, Colton, & Brewer, 1992). Because many other voice disorders are sexually dimorphic in prevalence rates, presbyphonia may occur more frequently for one sex than for the other (Bhattacharyya, 2014; Roy et al., 2004).

In general, physical exercises seek to increase strength, endurance, and power by overloading the skeletal muscles to evoke metabolic, morphologic, and neural adaptations of muscle fibers (MacInstosh, Gardiner, & McComas, 2005). Vocal training has been used to mitigate the adverse laryngeal changes that occur in advanced age (Oates, 2014). Vocal training is related to physical exercises; however, rather than taxing the vocal mechanism to achieve hypertrophy or recruitment of additional muscle fibers, the goal of vocal training is to improve overall vocal function by targeting the balance between respiratory support and laryngeal effort (Stemple, Lee, Damico, & Pickup, 1994). Common features of vocal training programs used in the treatment of presbyphonia include maximum vocal effort tasks, such as maximum sustained phonation of vowels and ascending/descending pitch glides of vowels (Gorman, Weinrich, Lee, & Stemple, 2008; Kaneko et al., 2015; Lu, Presley, & Lammers, 2013; Tay, Phyland, & Oates, 2012; Ziegler, Verdolini Abbott, Johns, Klein, & Hapner, 2014). Vocal training programs designed to treat the senescent voice currently do not consider differences between men and women in their therapeutic vocal techniques.

Despite the high prevalence of voice disorders in the elderly and the well-known negative consequences of voice disorders, the neuromuscular mechanisms underlying sexual dimorphism of the aging larynx remain relatively unexplored. The purpose of this tutorial is to demonstrate why sexual dimorphism should be considered when designing voice therapy programs for the aging voice by examining the literature on how sex hormones influence normal laryngeal aging and neuromuscular responses to exercise. Therefore, this tutorial will summarize the sex differences and hormonal influences of vocal aging, muscle aging, and response to physical exercise. Age-related laryngeal adaptations mentioned throughout this text are considered part of normal laryngeal aging, unless otherwise specified. Typical age-related sexually dimorphic laryngeal adaptations provide a physiological rationale for considering sex differences when developing and evaluating the effectiveness of diagnostic and therapeutic techniques for the aged voice. This document focuses on the literature pertaining to sexual dimorphism of human and rat TA muscles, the primary muscle of the vocal fold. The rat model was included because it is the most studied animal model of laryngeal muscle aging.

Effect of Hormones on the Aging Voice

For both men and women, hormonal changes are associated with vocal changes, which are most prominent during periods of chronic changes in the sex hormone levels (such as during menopause), and likely partially mediated by the concentration of circulating hormones (Kirgezen, Sunter, Yigit, & Huq, 2017). The vocal fold contains estrogen, progesterone, and androgen receptors with a similar distribution between sexes (Kirgezen et al., 2017). Hormone receptors allow for cell signaling to occur, and the presence of sex hormone receptors in the vocal fold tissue indicates a relationship between hormones and vocal fold functions (Kirgezen et al., 2017). Although both aged men and women experience endocrine function changes resulting in declines in sex hormone production, the dramatic and sudden decline of estrogen production following menopause greatly differs from men's progressive decline of androgens (Kaufman & Vermeulen, 2005; Lamberts, van den Beld, & van der Lely, 1997). Therefore, the senescence of the endocrine system may contribute to the sexually dimorphic pattern of vocal aging.

Several age-related vocal changes affecting the female voice have been well documented. Morphological changes of the larynx include vocal fold thickening, increased vocal fold edema, and increased prominence of the vocal process (Abitbol, Abitbol, & Abitbol, 1999; Hirano, Kurita, & Sakaguchi, 1989; Honjo & Isshiki, 1980; Kahane, 1983; Pontes, Brasolotto, & Behlau, 2005; Pontes, Yamasaki, & Behlau, 2006). Following menopause, the vocal folds thicken and become more edematous (Honjo & Isshiki, 1980; Kahane, 1983). The thickening of the vocal folds postmenopause may partially explain why older women had greater vocal fold contact (inferred by electroglottograph measures) than young women in Higgins and Saxman's (1991) study. This thickening of the vocal folds may contribute to closing the posterior glottal gap, which is commonly observed via stroboscopy in young women (Chandran, Hanna, Lurie, & Sataloff, 2011). In fact, Linville (1992) found that young women more frequently displayed incomplete glottic closure and posterior glottal gaps than older women, with older women having a higher incidence of anterior glottal gaps. The increased incidence of anterior gaps may be due to the increased incidence of the vocal prominence found in elderly women's larynges in studies by Pontes et al. (2005, 2006). Because the degree of glottic closure correlates with perceived breathiness (Sodersten & Lindestad, 1990), it is not surprising that postmenopausal women have decreased perceived breathiness when compared to young women's voices (D'Haeseleer et al., 2011). Thus, these age-related vocal changes are likely partially due to the biomechanical and morphological changes that occur following chronic dramatic changes in hormone levels.

Other acoustic changes of women's voices have been reported following menopause. Following menopause, women's voices decreased in speaking fundamental frequency (f_0; D'Haeseleer et al., 2011; D'Haeseleer, Depypere, & Van Lierde, 2013; Raj, Gupta, Chowdhury, & Chadha, 2010; Schneider, van Trotsenburg, Hanke, Bigenzahn, & Huber, 2004). Also, women's signal-to-noise ratio increases until the age of 50 years and then decreases with age, which may relate to the onset of menopause (Stathopoulos, Huber, & Sussman, 2011). Furthermore, Abitbol et al. (1999) described a "menopausal vocal syndrome" characterized by reduced vocal intensity, vocal fatigue, a decreased frequency range with loss of the high tones, and a loss of harmonics negatively impacting the timbre of the voice in 17% of postmenopausal women. For aging women, voice changes simultaneously occur with respiratory decline, such as reduced vital capacity and maximum phonation time (Awan, 2006). Therefore, some postmenopausal vocal changes may reflect reduced pulmonary function with aging rather than changes within the larynx itself.

Menopause does not affect all women's voices similarly. Adipose tissue is the primary source of estrogen production in postmenopausal women, which may explain why a high body mass index is correlated to a higher f_0 postmenopause (D'Haeseleer et al., 2013). Therefore, investigating the role of estrogen in vocal fold maintenance postmenopause is essential to interpret acoustic findings that occur postmenopause.

Age-related declines in testosterone, known as andropause, may contribute to acoustic changes observed in the aging male voice. Following the age of 30 years, serum testosterone levels decrease by 1% per year (Matsumoto, 2002). With aging, the vocal folds of men tend to thin, which can contribute to vocal fold bowing (Mueller et al., 1985; Tanaka et al., 1994). This thinning of the vocal fold tissue likely contributes to the decreased vocal fold contact (inferred by electroglottograph measures) and higher incidence of spindle-shaped glottal gaps of aged men's larynges compared to young men's (Higgins & Saxman, 1991; Linville, 2002). These morphological and biomechanical changes of the voice likely also contribute to the increased breathiness perceived of the aged male voice (Linville, 2002). Finally, with aging, men's voices increase in speaking f_0 (W. S. Brown et al., 1991; Gugatschka et al., 2010).

It has been speculated that the change in speaking f_0 in old age for men and women is attributed to the thinning/thickening of the vocal folds (Abitbol et al., 1999; W. S. Brown et al., 1991; Pontes et al., 2005). Particularly for women, increased vocal fold mass is cited as the contributing factor to the decline in f_0 following menopause, with androgens speculated to take a larger role in tissue mucosa maintenance in the postmenopausal woman (Abitbol et al., 1999; Linville, 1996; Pontes et al., 2005; Raj et al., 2010; Sebastian, Babu, Oommen, & Ballraj, 2012). Because few studies have utilized serum hormone testing, sex hormones and their relationship to vocal fold thinning/thickening cannot be determined. Furthermore, vocal fold mass may not be useful in understanding age-related frequency changes (Titze, 2011). Neuromuscular and vibratory vocal fold adaptations during aging are more likely to influence f_0 than mass itself. The neuromuscular parameters of the TA are discussed in subsequent sections.

Sex hormones are associated with the senescence of the human larynx. In the context of aging, men and women experience opposite directional changes in vocal fold contact during phonation, speaking f_0, and breathiness. Men's voices become breathier and higher in f_0, and women's voices become less breathy with lower f_0. Because of the distribution of hormone receptors within the vocal fold and the clinical observation of vocal changes during suspected changes in hormone levels (menopause/andropause), research is warranted in investigating how sex hormones affect the underlying laryngeal neuromuscular mechanisms.

Hormones Are Associated With Sarcopenia

Differences in altered endocrine function of older men and women may contribute to the sexual dimorphism observed in the severity and rate of sarcopenia, age-related loss of muscle mass and strength (Kirchengast & Huber, 2009). In human limbs, age-related changes at the neuromuscular junction (NMJ) contribute in part to the development of sarcopenia in advanced age (Deschenes, Roby, Eason, & Harris, 2010). The C-terminal agrin fragment (CAF) is indicative of NMJ degeneration and is a biological marker of sarcopenia in men; however, the decline in muscle mass in women is not strongly CAF dependent (Drey et al., 2013). Furthermore, in limbs, NMJ degradation and remodeling occurs during muscle disuse and aging (Wilson & Deschenes, 2005). Because the number and size of NMJs within the TA muscle decrease with aging, disuse (such as vocal rest) may detrimentally impact the aged voice, particularly in men, who are more CAF dependent than women.

In limb muscles, menopause contributes to sarcopenia, which results in an accelerated loss of muscle strength and mass during perimenopausal years that may be due to several morphological changes that occur during menopause (M. Brown, 2008; Maltais, Desroches, & Dionne, 2009). Sarcopenia results in a preferential loss of fast-twitch muscle fibers, which have higher concentrations of estrogen receptors than slow-twitch muscles (M. Brown, 2008). Therefore, menopause may contribute to this preferential loss of fast-twitch muscle fibers. However, in humans, it is difficult to tease apart aging from estrogen deprivation. Several animal studies have demonstrated adverse neuromuscular adaptations and functional outcomes following menopause within the hindlimb muscles in both young and old animals (Bunratsami, Udomuksorn, Kumarnsit, Vongvatcharanon, & Vongvatcharanon, 2015; Kadi et al., 2002; Moran, Warren, & Lowe, 2006; Velders & Diel, 2013). Nevertheless, the effects of menopause on the intrinsic laryngeal muscles are unexplored.

The progressive loss of testosterone after the age of 30 years contributes to sarcopenia in men (Matsumoto, 2002). Higher androgen levels are associated with greater muscle mass development in men than in women, and the tapering of testosterone in men corresponds to declining rates of muscle mass and strength in old men (Matsumoto, 2002; Welle, Tawil, & Thornton, 2008). Because the process of andropause is gradual, sarcopenia develops gradually and accelerates in later decades of life (Kirchengast & Huber, 2009). Thus, sex hormones also likely contribute to sarcopenia in men.

Sexual Dimorphism of TA Muscle Senescence

Sarcopenia affects all skeletal muscles, including the intrinsic laryngeal muscles. The structure of the human

TA muscle is composed of Type I, IIA, and IIX muscle fibers in a mosaic-like pattern (Kersing & Jennekens, 2004). In response to aging, some studies report loss of Type I and II muscle fibers, only Type I, or only Type II; therefore, muscle fiber composition changes remain undefined for the TA muscle (Kersing, 1986; Kersing & Jennekens, 2004; Malmgren, Fisher, Bookman, & Uno, 1999; Sato & Tauchi, 1982; Thomas, Harrison, & Stemple, 2008). When sex is not considered (either sexes are grouped or one sex is excluded), studies have shown that aging generally affects the number and size of muscle fibers, muscle fiber type composition, and metabolic function in the TA muscles in both human and rat models (Kersing & Jennekens, 2004; Martins et al., 2015; McMullen & Andrade, 2006, 2009; Nishida et al., 2013).

One of the age-related muscular changes of the TA muscles is the decrease of the cross-sectional area of the muscle fibers. Atrophy has been observed in the male TA muscles with an 11% decrease in the diameter of the TA muscle fibers from adulthood (17.2 μm) to advanced age (15.3 μm), whereas the diameter of the TA muscle fibers in women decreases by 5% from adulthood (15.5 μm) to advanced age (14.7 μm; Martins et al., 2015). These atrophic changes of the TA muscle fibers likely contribute to vocal fold bowing observed of the aged larynx. Although Martins et al. (2015) suggest no sex difference in their study, it is likely due to the small sample size (five per group). A large effect size ($d = 1.05$) can be calculated between the mean muscle fiber diameters of young (30–50 years of age) men and women, but no effect size ($d < 0.2$) is found between the mean muscle fiber diameters of aged (60–70 years of age) men and women using the data from Martins et al.. These data suggest that young men have larger muscle fibers in the TA muscles than women but experience a greater rate of atrophy that eliminates the sex differences in muscle size in advanced age.

Sexual dimorphism is also present within the recurrent laryngeal nerve of aged men and women (de Campos et al., 2013). The overall parameters of the recurrent laryngeal nerve, including total number of fibers, intraperineural area, axonal area, axonal diameter, and the area of the nerve occupied by myelinated fibers, are larger in aged men than age-matched women (de Campos et al., 2013). This finding suggests that aged men can present a higher conduction velocity than aged women, which may partially contribute to divergent f_0 changes in advanced age (de Campos et al., 2013).

In the rat literature, the muscle fiber compositions of adult rats are similar in both the medial and lateral divisions of the TA muscles between sexes; however, male rats have a larger muscle fiber size in the lateral (muscularis) portion of the TA (Lenell & Johnson, 2017). Whether this muscle size difference is eliminated in old age (analogous to the human study by Martins et al., 2015) is unknown. Age-related changes at the cellular level (NMJs and metabolic features) and loss of muscle fibers likely contribute to the contractile dysfunction of aged laryngeal muscles (Connor et al., 2002; McMullen & Andrade, 2006).

NMJ adaptations likely precede these atrophic changes (Deschenes et al., 2010). Several studies have demonstrated age-related NMJ remodeling within the TA muscles of aged male rats (Connor et al., 2002; Johnson, Ciucci, & Connor, 2013; McMullen & Andrade, 2009). In the male rat model, aging results not only in decreased total number and size of the NMJs within the TA muscles but also in NMJ morphological changes of increased motor endplate dispersion and increased axonal sprouting (Connor et al., 2002; Johnson et al., 2013; McMullen & Andrade, 2009; Yamamoto, Tanaka, Tsubone, Atoji, & Suzuki, 2003). These age-related changes of the NMJ in the TA muscles are part of the larger process of motor unit remodeling that occurs in advanced age (Johnson et al., 2013; Santo Neto & Marques, 2008; Takeda et al., 2000).

In TA muscles, there are more NMJ cluster fragments in young female rats than in age-matched male rats, which correlates with higher synaptic strength; however, this observation is not present in the other intrinsic laryngeal muscles (Feng, Zhang, Ralston, & Ludlow, 2012). The hormonal influence of estrogen may improve synaptic strength in female rats' TA muscles (Feng et al., 2012). Therefore, NMJs in the TA muscles may be less affected in the aged female rats than in the aged male rats, because unlike aged women, female rats continue to secrete steady estrogen levels into old age (Huang, Steger, Bruni, & Meites, 1978; Naftolin et al., 2007). Thus, further investigation of the effect of estrogen on NMJ remodeling and TA muscle fibers in aged female rats is warranted.

Mitochondrial abnormalities likely contribute to the contractile dysfunction of the TA muscles (McMullen & Andrade, 2006). In humans, significant mitochondrial abnormalities develop in the TA with age, which may play a role in the functional deficits of the larynx in old age (Kersing & Jennekens, 2004). In male rats, abnormal mitochondrial accumulations (ragged red fibers) are present in aged rat TA muscles but are absent in young and middle-aged rat TA muscles; however, these observations have not been investigated in aged female rats (McMullen & Andrade, 2006). Abnormal mitochondrial accumulations affect the contraction of the muscles because these noncontracting materials occupy portions of the muscle's cross-sectional area (McMullen & Andrade, 2006). Also, in male rats, the number of mitochondria decreases with age, which is associated with the metabolic profile shift from an oxidative (high percentage of fatigue-resistant fibers) to a glycolytic (low percentage of fatigue-resistant fibers) profile (McMullen & Andrade, 2006). These changes occurring in the mitochondria contribute to a loss of endurance of this muscle (McMullen & Andrade, 2006). Thus, changes in muscle fiber size and type, NMJs, and mitochondria comprise some of the neuromuscular underpinnings of the senescent larynx.

Whether sexual dimorphism exists in the aging TA muscles remains unexplored in the rat model. In aging female rats, the number of muscle fibers, but not diameter, decreases in the TA; however, this finding must be considered independent of the previously mentioned neuromuscular changes of the aging TA due to differences in the sex and strain of the rats (Nishida et al., 2013). Furthermore, studies have not investigated sexual dimorphism in muscle fiber composition in the TA muscles of men and women. In human studies, men and women were grouped together due to small sample sizes (Kersing & Jennekens, 2004). In animal models, most studies only used one sex (McMullen & Andrade, 2006, 2009; Nishida et al., 2013). However, the studies that compared male and female TA muscles revealed sexual dimorphism of the NMJ and fiber sizes (Feng et al., 2012; Lenell & Johnson, 2017). Therefore, the questions of whether the sexually dimorphic features of the muscle fiber compositions of the TA muscles exist and whether they are differentially impacted by age remain unexplored.

In the human and rat literature, two sexually dimorphic neuromuscular laryngeal differences have been discovered. The TA muscle fibers are larger for males (both humans and rats) than for females, and female rats have larger NMJ cluster fragmentation in the TA muscles. However, the sexually dimorphic feature of men's TA muscles experiencing greater rates of atrophy in advanced age than women's observed by Martins et al. (2015) warrants further investigation and validation. An obvious dearth of information exists regarding the underlying neuromuscular laryngeal differences between sexes and how sex hormones may influence these parameters of the voice.

Hormones and Response to Exercise

Hormones are associated with sexually dimorphic responses to physical exercise. Physical exercise is a well-established treatment to offset sarcopenia (Johnston, De Lisio, & Parise, 2008). In general, there are two primary types of exercise: endurance training and strength training. Aged men and women's limb muscles respond differently to each of these types of exercise (Bamman et al., 2003; Ivey et al., 2000; Kosek, Kim, Petrella, Cross, & Bamman, 2006; Melnyk, Rogers, & Hurley, 2009). Nevertheless, how exercise may differentially influence neuromuscular adaptations in the TA muscles remains unknown.

In response to endurance training, aged men and women experience similar increases in maximum oxygen uptake, muscle enzyme activities, muscle bed capillarization, and muscle transformations from Type IIb to Type IIa (Coggan et al., 1992; Kohrt et al., 1991; Rogers & Evans, 1993). Type IIb muscle fibers, which are undifferentiated fibers, are currently referred to as Type IIx fibers in humans (Powers & Howley, 2014; Sandage & Smith, 2017). Overall, endurance training similarly affects the skeletal muscle properties and maximum oxygen uptake in men and women (Coggan et al., 1992). Sexually dimorphic muscle properties that are present pretraining, however, persist posttraining, such as overall smaller muscle fibers, fewer capillaries per muscle fiber, and lower enzyme activities of succinate dehydrogenase and citrate synthase in women (Coggan et al., 1992). These two metabolic enzymes participate in the citric acid cycle to generate mitochondrial energy via adenosine triphosphate. Thus, both aged men and women benefit from endurance training with similar neuromuscular adaptations (Rogers & Evans, 1993).

In response to strength training, studies have found that aged men experience greater gains in hypertrophy of trained muscles than do aged women; women's strength gains come primarily from neural adaptations, such as enhanced motor unit recruitment (Bamman et al., 2003; Ivey et al., 2000; Kosek et al., 2006; Melnyk et al., 2009). This greater hypertrophic response observed in males may be due to higher levels of both chronic circulations of free testosterone and activity-induced testosterone (Kraemer & Ratamess, 2005). With increased age, however, the mechanisms for gaining muscle strength in men increasingly rely on neural adaptations and less so on muscle fiber hypertrophy (MacInstosh et al., 2005).

Metabolic adaptations of muscle fibers secondary to behavioral vocal training both in general and for sex differences specifically have not been explored. In young adult male rats, chronic electrical stimulation (a model of vocal training) increased NMJ density, mitochondrial content, and glycogen-positive fibers in the TA muscles (McMullen et al., 2011). Additionally, maximal chronic electrical stimulation induced hypertrophy of the TA muscle fibers in both young and old male rats (Stemple et al., 2015). These results demonstrated that the TA muscles of adult male rats can adapt in response to maximal stimulation in ways that are associated with enhanced muscle performance (McMullen et al., 2011).

Vocal training may improve the contractile function of the intrinsic laryngeal muscles by positively affecting NMJ synaptic strength and metabolic physiology, which subsequently yields favorable behavioral outcomes. In the rat model, vocal training has yielded positive effects on age-related changes in the acoustic intensity of ultrasonic vocalizations and NMJ morphology in aged male rats; however, the impact of vocal training on vocalizations and NMJs has not been investigated in aged female rats (Johnson et al., 2013). NMJs are one of many peripheral nervous system mechanisms affected by age (Johnson et al., 2013). Estrogen may protect NMJs in the TA muscles from age-related changes. Aged female rats may not experience the same NMJ remodeling within the TA due to their preserved estrogen production in advanced age (Huang et al., 1978). The aged female rat is a useful model for understanding the role of estrogen in the preservation of the TA's NMJs. If aged female rats experience NMJ remodeling in the TA muscles to a lesser extent than male rats, estrogen (particularly estradiol) levels may be critical to the protection of the TA muscles within the female rat larynx. However, if aged female rats experience similar NMJ remodeling in the TA muscles as male rats, the influence of estradiol may not be the primary contributor of greater synaptic strength in the TA muscles of adult rats. In this case, other hormonal influences should also be considered. Therefore, the rat model allows for a unique evaluation of intrinsic laryngeal muscles that can be studied in a variety of hormonal and aging profiles: estrogen-deprived laryngeal muscles (ovariectomized young female rats), aged laryngeal muscles with ovarian hormones present (aged female rats), or aged laryngeal muscles without ovarian hormones present (ovariectomized aged female rats). Understanding the neuromuscular changes associated with aging and hormones will facilitate knowledge of the influence of hormonal senescence of the aged female voice.

Implications of Exercise for the Senescent Voice

Oates (2014) critically reviewed the 10 studies since 2008 that used behavioral vocal training programs to treat the senescent voice (typical and disordered). Some surprising highlighted findings are that, although auditory-perceptual and aerodynamic measures often improved following vocal training, acoustic measures did not commonly change (Oates, 2014). Sauder et al. (2010) hypothesized that some acoustic measures may not be sensitive enough to account for the improvements in the voice. However, that study included only seven male and two female participants, and the average of the acoustic measures for all the participants was compared pretraining and posttraining. Perhaps no changes were found because the female and male participants experienced opposite directional changes, thereby masking statistical significance. Sexual dimorphism in response to vocal training was not investigated in any of the treatment studies reviewed by Oates (2014). Studies that investigated the behavioral outcomes of vocal training have either focused on one sex or grouped sexes together due to small sample sizes. Furthermore, no information exists regarding how these exercises may differentially impact the underlying neuromuscular mechanisms of voice production.

Another interesting finding from Oates' (2014) review was that stroboscopic images have not supported the reduction of vocal fold bowing as a result of vocal training (Kaneko et al., 2015; Sauder et al., 2010). It appears that vocal training techniques that focus on maximum vocal function exercises, such as maximum sustained phonation and increasing pitch, do not tax the laryngeal muscles to the extent that strength training taxes the limb muscle. Therefore, hypertrophy of laryngeal muscles may not be a functional outcome of vocal training. Improved functional outcomes following vocal training may be due to neuromuscular changes (NMJ remodeling or more efficient entrainment of motor units) or nonlaryngeal changes (improved respiratory–vocal coordination) rather than structural changes (hypertrophy).

Conclusion

Age-related vocal changes are sexually dimorphic. Women's vocal folds become thicker and more edematous with age with a lowering f_0, increased vocal fold contact, and reduced breathiness. Men's vocal folds thin with a rising f_0, reduced vocal fold contact, and increased breathiness.

Because hormones affect normal vocal aging, it follows that presbyphonia also clinically presents differently for sexes. Referring to all age-related voice disorders as presbyphonia erroneously generalizes patterns of laryngeal aging across sexes. Differences between sexes exist in the typically aged voice and, therefore, should be considered when describing age-related voice disorders. Vocal training should target the underlying deficits contributing to age-related dysphonia. Therefore, vocal training programs should be tailored to individual patients' clinical deficits and include consideration of the underlying laryngeal mechanistic differences between sexes.

Future basic and clinical research studies should consider sexual dimorphism by investigating the impact of vocal training on the behavioral outcomes and neuromuscular properties of the intrinsic laryngeal muscles in animal models using both sexes and by examining the behavioral outcomes of aged men and women's voices separately in clinical trials. By determining the patterns of laryngeal aging in both sexes and their differential behavioral and neuromuscular responses to vocal training, we can begin to discover potential underlying mechanisms responsible for the sexual dimorphism of the aged larynx and better prevent and treat voice disorders in older adults.

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