Research Article

Ovine Vocal Fold Tissue Fatigue Response to Accumulated, Large-Amplitude Vibration Exposure at Phonatory Frequencies

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Purpose: The contribution of tissue mechanical response to vocal fatigue is poorly understood. This study investigated the fatigue response of vocal fold tissues to large-amplitude vibration exposure at phonatory frequencies, using an ex vivo ovine model.

Method: Twelve sheep vocal fold mucosal specimens were subjected to sinusoidal, simple-shear deformation for prolonged cycles, under a large but physiological shear strain (46%) in a frequency range of 100–230 Hz. The duration of shear varied from a critical vibration exposure limit of 1,040 s to 4 times the limit (4,160 s). Tissue viscoelastic response was quantified by the elastic shear modulus (G'), viscous shear modulus (G''), and damping ratio (G''/G').

Results: Distinct response patterns were observed at different frequencies. G' and G'' generally decreased with

vibration exposure at 100 Hz, whereas they generally increased with vibration exposure at 200 and 230 Hz. Statistically significant differences were found for G'' increasing with vibration exposure at 200 Hz and damping ratio decreasing with vibration exposure at 200 Hz. Significant increases with frequency were also found for all viscoelastic functions. Results suggested that the contribution of tissue viscoelastic response to vocal fatigue could be highly frequency dependent. In particular, increases in G'' with vibration exposure could lead to high phonation threshold pressures and difficulty sustaining phonation at higher frequencies following prolonged vocalization.

Conclusion: These preliminary findings may help us better understand vocal fatigue and recovery and should be corroborated by studies with human vocal fold tissues.

Vocal fatigue is frequently experienced by individuals in vocally demanding professions and by individuals with various kinds of voice disorders, and it is often related to the perception of a “tired voice,” a weak voice, an increased vocal effort or phonatory effort, laryngeal discomfort, and reduced capability of control of the vocal mechanism, as among the most common symptoms (Nanjundeswaran, Jacobson, Gartner-Schmidt, & Verdolini Abbott, 2015; Solomon, 2008; Vilkman, 2004; Welham & MacLagan, 2003). Nanjundeswaran et al. (2015) developed the Vocal Fatigue Index (VFI) as a standardized, psychometrically validated clinical self-assessment tool to objectively quantify the symptoms and characteristics of

vocal fatigue, in order to more systematically define vocal fatigue and to more reliably identify individuals with vocal fatigue. The VFI has since been applied to quantify vocal fatigue symptoms in occupational voice users at risk of developing voice disorders, such as teachers (Hunter & Banks, 2017). As identified in the VFI, one important symptom of vocal fatigue is the perception of increased vocal effort, which can be correlated to the clinical aerodynamic measure of phonation threshold pressure (PTP). PTP can be defined as the minimum subglottal pressure or lung pressure required to initiate and sustain vocal fold oscillation (Titze, 1988), and it has been repeatedly shown that vocal fatigue can be associated with an elevated PTP and the perception of an increased level of vocal effort (Chang & Karnell, 2004; Eustace, Stemple, & Lee, 1996; McCabe & Titze, 2002; Solomon & DiMattia, 2000; Solomon, Glaze, Arnold, & van Mersbergen, 2003). A closely related aerodynamic measure is phonation threshold flow, the airflow equivalent of PTP, which can be defined as the minimum airflow that can initiate stable vocal fold oscillation and could also serve as an objective

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indication of the level of vocal effort required for phonation, including in subjects with voice disorders (Zhuang et al., 2009).

Physiologically, vocal fatigue is presumably related to laryngeal tissue fatigue, which could be broadly classified into two kinds of tissue fatigue: muscle fatigue and nonmuscular tissue fatigue (Hunter & Titze, 2009; Titze, 1999). Muscle fatigue in different parts of the body in general has been extensively studied in the literature, involving the accumulation of metabolic biomarkers during muscle fatigue, including serum lactate, oxidative stress biomarkers, and inflammatory biomarkers such as tumor necrosis factor- α and interleukins (Kent-Braun, Fitts, & Christie, 2012; Wan, Qin, Wang, Sun, & Liu, 2017). Muscle fatigue in the larynx, in particular, has also been studied with electromyography, with observations of significant gains in lateral cricoarytenoid muscle activity during effortful phonation, and compensatory increases in cricothyroid and thyroarytenoid muscle activities upon decreasing electromyographic activities of fatiguing lateral cricoarytenoid muscles (Boucher, Ahmarani, & Ayad, 2006).

Nonmuscular tissue fatigue, on the other hand, has received far less attention in the literature, especially for fatigue of the vocal fold connective tissue layer, commonly called the *lamina propria* (Gray, Titze, Chan, & Hammond, 1999; Titze, 2006). Although there have been studies of nonmuscular vocal fold tissue fatigue focusing on the surface tissue layer, that is, the vocal fold epithelium, such as the electron microscopic characterization of epithelial damage in association with accumulated doses of vibration exposure (Gray & Titze, 1988; Kojima et al., 2014), virtually nothing is currently known about the effect of accumulated vibration exposure on the vocal fold lamina propria (Hunter & Titze, 2009; Titze, 1999). This gap of knowledge in the literature is significant, because the vocal fold cover, which includes the superficial layer of the lamina propria, is the major vibratory layer of the vocal fold during phonation (Titze, 1988, 2006), and the vocal ligament, which refers to the intermediate layer plus deep layer of the lamina propria, is primarily responsible for providing the tensile stress of the vocal fold critical for fundamental frequency control (Chan, Fu, Young, & Tirunagari, 2007; Titze, 2006). To better understand the effect of accumulated vibration exposure on the vocal fold lamina propria, this study focused on quantifying the tissue fatigue response of the lamina propria in terms of changes in tissue viscoelastic properties, in order to provide some preliminary data on the role of nonmuscular tissue fatigue for vocal fatigue.

The critical level of accumulated tissue acceleration for hand-transmitted vibration in industrial power tool use has been well established in the literature (Griffin, 1990), but a corresponding critical level for vocal fold vibration exposure was not readily available. Titze, Švec, and Popolo (2003) did such an analysis based on hand-transmitted vibration and estimated that a safety limit for accumulated vibration exposure prior to tissue damage in terms of the total distance traveled by vocal fold tissue particles in a periodic vibratory trajectory is 520 m. In terms of duration,

this safety limit of accumulated distance would correspond to continuous phonation for 1,040 s with no rest, at a large but physiological vibratory amplitude, corresponding to about 50% shear strain, which is typically observed in vocal fold vibration during human phonation (Titze & Hunter, 2015; Titze et al., 2003).

Based on this safety limit of 1,040 s of continuous vibration exposure corresponding to a level at risk of tissue injury, this study was designed to quantify the tissue fatigue response of the vocal fold lamina propria when subjected to large-amplitude vibration exposure accumulated over varying duration, across different frequencies in the phonatory range, using an ex vivo ovine model. The duration of continuous vibration exposure of the current study ranged from this critical level of 1,040 s to a significantly prolonged duration, namely, four times the safety limit (4,160 s), in order to investigate the relatively long-term effects of accumulated vibration exposure. The frequency of continuous vibration exposure covered the typical male and female mean speaking fundamental frequency range, at 100, 150, 200, and 230 Hz (Titze, 2006). The continuous vibration exposure was delivered with a simple-shear rheometer, capable of precise, large-amplitude nonlinear translational shear deformation of vocal fold tissues at phonatory frequencies (Chan, 2018), as well as measurements of the corresponding changes in tissue viscoelastic response (Chan & Rodriguez, 2008), as functions of the frequency and duration of vibration exposure. The ex vivo ovine data could provide some preliminary insights on how tissue fatigue response may contribute to vocal fatigue at phonatory frequencies.

Method

Laryngeal Tissue Specimens

Twelve male Dorsett sheep larynges were excised immediately postmortem from a local abattoir, which sacrificed animals specifically for meat packing purposes. No animals were sacrificed specifically for the present experiment. The animals were around 2 years old, weighing around 100 lb each. The animal euthanization protocol was approved by the USDA Meat and Poultry Inspection Program and was in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.).

Sheep has been shown to be a reasonable animal model for the human larynx in terms of its structural morphology, layered structure of the vocal fold, histochemical compositions of the intrinsic laryngeal muscles, elastic properties and failure response of the vocal fold tissues, glottal airflow resistance, and phonatory characteristics including fundamental frequency, oscillation amplitude, and PTP (Alipour & Jaiswal, 2008; Alipour, Jaiswal, & Vigmostad, 2011; Chan & Siegmund, 2004; Happak, Zrunek, Pechmann, & Streinzer, 1989; Zrunek, Happak, Hermann, & Streinzer, 1988).

For each excised larynx, the mucosa of each vocal fold was carefully dissected. Left–right symmetry in tissue mechanical properties was assumed based on previous studies on linear viscoelastic properties of the human vocal fold cover (Chan & Rodriguez, 2008; Chan & Titze, 1999), so that fatiguing experiments conducted on only one vocal fold specimen isolated from each larynx can be assumed to be also representative of the contralateral vocal fold. The vocal fold mucosa, consisting of the epithelium and the lamina propria, was isolated from the thyroarytenoid muscle underneath using sharp surgical instruments similar to that described previously for the human larynx (Chan & Rodriguez, 2008). As sheep does not have a vocal ligament (Zrunek et al., 1988), the vocal fold mucosal specimens isolated and tested may be considered as the portion of the vocal fold that is primarily responsible for vibration, corresponding to the human vocal fold cover, which is the major vibratory layer during phonation (Titze, 1988, 2006). In total, 12 sheep larynges were harvested. For each of the four testing frequencies (100, 150, 200, and 230 Hz), three vocal fold mucosal specimens dissected from three different larynges were subjected to the continuous, accumulated, large-amplitude vibration exposure experiments. While it has been quite common for tissue viscoelastic experiments based on smaller sample sizes (e.g., Alipour et al., 2011; Chan & Rodriguez, 2008; Chan & Titze, 1999), results of the current study were nonetheless limited by the small sample size and should be corroborated by further studies.

Application of Sinusoidal, Large-Amplitude Vibration Exposure

For each vocal fold mucosal specimen, accumulated, large-amplitude vibration exposure was applied in a controlled-strain, simple-shear rheometer previously validated for the nonlinear deformation and linear viscoelastic characterization of vocal fold tissues (Chan, 2018; Chan & Rodriguez, 2008), at one of four frequencies in a physiological range (100, 150, 200, and 230 Hz), covering the male and female phonatory range for typical conversational speech (Titze, 2006). The continuous vibration exposure applied to each specimen was in the form of large-amplitude, translational, sinusoidal simple-shear deformation of the specimen between the upper plate and the lower plate of the rheometer (see Figure 1). The simple-shear mode of vibration can be considered an approximation of the shear wave propagating on the vocal fold mucosa (human vocal fold cover), which is called the mucosal wave during vocal fold vibration in vivo (Titze, 2006; Titze & Hunter, 2015; Titze et al., 2003). Similar to our previous study (Chan & Rodriguez, 2008), each specimen was mounted with the epithelium completely in contact with the lower plate of the rheometer, in an orientation such that the translational simple-shear deformation applied between the upper plate and the lower plate would correspond to the direction of the mucosal wave during vocal fold vibration (Titze, 2006; Titze & Hunter, 2015). The amplitude of

shear displacement was always half of the rheometer gap size used (e.g., 0.15-mm displacement for a gap of 0.3 mm), resulting in a large but physiological amplitude of shear strain: $\arctan(0.5) = 46.36\%$ (Chan & Rodriguez, 2008). This shear strain amplitude approximately corresponded to a typical amplitude of vocal fold vibration of 1.0–1.5 mm for human phonation (Titze & Hunter, 2015; Titze et al., 2003).

The continuous vibration exposure applied to each specimen started with a duration of shear of 30 s and then progressed to 1,040 s, 2,080 s, and, finally, 4,160 s. After continuous vibration exposure for a duration of each of these time points of 30, 1,040, 2,080, and 4,160 s, measurements of the linear viscoelastic response of the specimens were conducted. The same mounting of each specimen was maintained in the rheometer throughout the process of large-amplitude shear (vibration exposure) and small-amplitude shear (viscoelastic measurements), as confirmed by video inspection of the specimen with a camera directly mounted above the transparent upper plate.

Among the time points of 30, 1,040, 2,080, and 4,160 s, the duration of 30 s was chosen to quantify the initial tissue viscoelastic response before fatigue, but after achieving the “preconditioned” state, or stabilization of the cyclic stress–strain response. This stabilized state has been found to be achieved following around 100 cycles of large-amplitude oscillatory shear at 100 Hz for the human vocal fold lamina propria (Chan, 2018). The duration of 30 s at the lowest testing frequency (100 Hz) would correspond to 3,000 cycles of shear, well past the preconditioned state. The time point of 1,040 s (or 17 min 20 s) corresponded to the critical vibration exposure distance limit (520 m) established by Titze et al. (2003), based on tissue damage criteria for hand-transmitted vibration (Griffin, 1990). The time points of 2,080 and 4,160 s corresponded to twice and four times the accumulated distance limit to simulate significant, relatively long-term fatigue conditions. Tissue viscoelastic response (elastic shear modulus G' and viscous shear modulus G'') was measured at each of these four time points to quantify the effects of duration and frequency of accumulated vibration exposure on the tissue fatigue response.

Rheometric Measurement of Tissue Viscoelastic Response

Viscoelastic response of each vocal fold specimen was quantified by the same simple-shear rheometer for applying the continuous vibration exposure, without having to disrupt the mounting of the specimen. The rheometer was custom-built based on the Bose ELF 3200 system (Bose Corporation, ElectroForce Systems Group) and was previously validated for the linear viscoelastic characterization of vocal fold specimens at phonatory frequencies, with valid measurements of the elastic shear modulus G' and viscous shear modulus G'' obtainable at up to 250 Hz (Chan & Rodriguez, 2008).

Figure 1. The application of large-amplitude translational vibration exposure in conjunction with small-amplitude rheometry based on the controlled-strain, simple-shear rheometer reported in Chan and Rodriguez (2008). A large-amplitude sinusoidal shear (46% shear strain) is applied to a tissue specimen at 100, 150, 200, or 230 Hz. Following 30, 1,040, 2,080, and 4,160 s of vibration exposure, linear viscoelastic response of the specimen can be quantified through the principle of simple-shear rheometry (with known displacement x , harmonic shear force F , gap size d , and specimen contact area A ; Chan & Rodriguez, 2008; Chan & Titze, 1999).

As shown in Figure 1, each specimen was subjected to translational, sinusoidal simple-shear deformation between two rectangular acrylic tissue plates. A linear motor applied a translational displacement (x) to the specimen at a specified amplitude and frequency via the upper plate. The shear force (F) from the viscoelastic response of the specimen was detected by a piezoelectric transducer (PCB Piezotronics Model 209C12) via the lower plate. A normal load transducer measured the compressive force between the specimen and the two tissue plates, to ensure no slippage between the specimen and the two plates at a specified gap size ($d = 0.3$ mm). Experiments were performed in a transparent acrylic environmental chamber with temperature maintained at 37°C and relative humidity maintained at 100%, to prevent tissue dehydration. A digital camera mounted directly above the chamber allowed for scaled images of the mounted specimen to be taken, in order to determine the area of contact (A) between the specimen and the upper plate, using Image J (National Institutes of Health).

Based on the theory of linear viscoelasticity, when a specimen is subjected to small-amplitude shear deformation, at a strain amplitude of 1.0%–2.0%, stress–strain linearity can be maintained within the linear viscoelastic region. Given the amplitude of the translational displacement (x), the amplitude of the shear force (F), the phase shift between them, the gap size (d), and the area of contact (A), the elastic shear modulus (G'), viscous shear modulus (G''), and damping ratio (or damping factor; $\zeta = G'' / G'$) of the specimen can readily be determined as functions of frequency (Chan & Rodriguez, 2008; Chan & Titze, 1999). The experimental error of measurements made with the simple-shear rheometer used has been reported to be within 9% of the measured values (Chan & Rodriguez, 2008), which was similar to the magnitude of experimental errors for other similar rheometers (at 5%–10%; Chan, 2004; Chan & Titze, 1999).

Statistical Analysis

All statistical analyses were conducted with IBM SPSS Version 24.0. The level of significance (α) was .05 (two-tailed). To examine the effect of the duration of

vibration exposure, Friedman test was conducted to examine if there were any significant changes in G' , G'' , and ζ with vibration exposure duration at each of the four testing frequencies (100, 150, 200, and 230 Hz). The nonparametric Friedman test was used due to the small sample size ($n = 3$) at each frequency. To examine the effect of frequency, repeated-measures analysis of variance was conducted to examine if there were any significant differences in G' , G'' , and ζ across the four testing frequencies (the parametric test became feasible given the increase in sample size, with the data being examined across all durations of vibration exposure). Post hoc pairwise comparisons were performed using multiple t tests with Bonferroni corrections.

Results

Effects of Vibration Exposure Duration and Frequency on Tissue Viscoelastic Response

Figure 2 shows the effects of vibration exposure duration and frequency on the linear viscoelastic response (elastic shear modulus G' and viscous shear modulus G'') of the vocal fold specimens. Parts A–D depict the results at the four test frequencies of 100, 150, 200, and 230 Hz, respectively, with the effect of vibration exposure duration shown in each part, varying from 30 to 4,160 s. A general trend of G' and G'' decreasing with the duration of simple-shear deformation (duration of accumulated vibration exposure), starting at 1,040 s, can be observed at 100 Hz (Part A), whereas at 150 Hz, there were no obvious trends, but rather, the data were characterized by a much higher variability as indicated by the magnitude of the error bars, when compared to other frequencies (Part B). Another general trend of G' and G'' increasing with the vibration exposure duration, again starting at 1,040 s, can be observed at 200 Hz (Part C) and at 230 Hz (Part D), after G' and G'' initially decreased from 30 to 1,040 s.

The Friedman test was conducted to determine if any of these general trends were statistically significant. Results showed that G'' increased significantly with vibration exposure duration at 200 Hz, with the other trends

Figure 2. Elastic shear modulus (G') and viscous shear modulus (G'') of the sheep vocal fold mucosa as functions of vibration exposure duration at the frequencies of (A) 100 Hz, (B) 150 Hz, (C) 200 Hz, and (D) 230 Hz. Means and standard deviations (error bars) of three specimens are shown for each frequency.

not reaching statistical significance (see Table 1). Next, repeated-measures analysis of variance was conducted to determine if there were any significant changes of G' and G'' with frequency. Results showed that both G' and G'' increased significantly with frequency. Post hoc pairwise comparisons showed that, for both G' and G'' , the data at 230 Hz were significantly higher than those at other frequencies (see Table 1).

Figure 3 shows the effects of vibration exposure duration and frequency on the damping ratio or damping factor ($\zeta = G'' / G'$) of the vocal fold specimens. It can be seen that there was a general trend of ζ decreasing with vibration exposure, especially at the higher frequencies (200 and 230 Hz), where ζ decreased from around 0.5 to around 0.31–0.36 from 30 to 4,160 s. On the other hand, there was another general trend of ζ increasing with frequency, particularly for the lower frequencies, where ζ increased from around 0.21 to around 0.45 from 100 to 200 Hz (see Figure 3). The Friedman test was conducted to determine if the observed changes with vibration exposure duration were statistically significant. Results showed

that ζ decreased significantly with vibration exposure duration at 200 Hz, with the changes at other frequencies not reaching statistical significance (see Table 1). Next, repeated-measures analysis of variance was conducted to determine if there were any significant changes of ζ with frequency. Results showed that ζ increased significantly with frequency. Post hoc pairwise comparisons showed that the values of ζ at 230 and 200 Hz were significantly higher than those at 100 Hz (see Table 1).

Parametric Curve-Fitting

To better understand the time course of the tissue fatigue response, that is, the change in tissue viscoelastic response with vibration exposure, two parametric models were used to curve-fit the empirical data, namely, (a) a sigmoidal model and (b) an exponential model, in order to examine if the time course of the tissue viscoelastic response may mirror one of these two distinct tissue recovery trajectories (Hunter & Titze, 2009). It has been quite well established in the wound healing literature that the time course

Table 1. Results of statistical analysis of the effects of vibration exposure duration (t) and frequency (f) on the elastic shear modulus (G'), viscous shear modulus (G''), and damping ratio (ζ) of sheep vocal fold mucosa specimens ($n = 3$ for each frequency).

Variable	Effect of vibration exposure duration ^a				Effect of frequency ^b
	At 100 Hz	At 150 Hz	At 200 Hz	At 230 Hz	
G'	Nonsignificant	Nonsignificant	Nonsignificant	Nonsignificant	$F(3, 7) = 18.668$ ($p < .01$)
G''	Nonsignificant	Nonsignificant	$p < .05$	Nonsignificant	100 Hz = 150 Hz = 200 Hz < 230 Hz ^c $F(3, 7) = 42.441$ ($p < .001$)
ζ	Nonsignificant	Nonsignificant	$p < .05$	Nonsignificant	100 Hz = 150 Hz = 200 Hz < 230 Hz ^c $F(3, 8) = 11.951$ ($p < .01$) 100 Hz = 150 Hz; 100 Hz < 200 Hz = 230 Hz ^c

^aFriedman's repeated-measures analysis of variance by ranks. ^bRepeated-measures analysis of variance. ^cAdjustments for multiple pairwise comparisons with Bonferroni corrections.

of tissue recovery, or wound healing trajectories, can be characterized either by an exponential model, which would correspond to chronic wound healing, or by a sigmoidal model, which would correspond to acute wound healing (Franz, Kuhn, Wright, Wachtel, & Robson, 2000; Steed, 2003). These models have been applied for the description of vocal fatigue recovery as well (Hunter & Titze, 2009). With these models, the dependence of the viscoelastic functions G' , G'' , and ζ on vibration exposure duration t can be described by:

$$G' = e^{(a+b/t)} \quad (1a)$$

$$G'' = e^{(a+b/t)} \quad (1b)$$

$$\zeta = e^{(a+b/t)} \quad (1c)$$

Figure 3. Damping ratio (ζ) of the sheep vocal fold mucosa as a function of vibration exposure duration and frequency. Means and standard deviations (error bars) of three specimens are shown for each frequency. Only the upper error bars are shown to avoid visual confusion.

based on the sigmoidal model, and by:

$$G' = a e^{bt} \quad (2a)$$

$$G'' = a e^{bt} \quad (2b)$$

$$\zeta = a e^{bt} \quad (2c)$$

based on the exponential model, where for both models, a and b are the coefficients of parametric curve-fitting. Previously, studies on the linear viscoelastic properties of vocal fold tissues used the power law ($G' = a f^b$) and the logarithmic equation ($G' = a \log f + b$) to describe the dependence of G' and G'' on frequency f (Chan & Rodriguez, 2008; Chan & Titze, 1999). For the current study, however, the point of interest was rather on the dependence of the viscoelastic functions on the vibration exposure duration t ; hence, the exponential model and the sigmoidal model were used for quantifying the time course of the fatigue response. Average data for the three vocal fold specimens at each frequency were fitted to the models with least-squares regressions. Results of the curve-fitting are summarized in Table 2, showing the values of the coefficients a and b and the coefficient of determination R^2 , as an estimate of the goodness of fit.

Results of the parametric curve-fitting showed that, in general, the viscoelastic functions G' , G'' , and ζ can be better described by the exponential model (Equation 2) as compared to the sigmoidal model (Equation 1), with higher values of R^2 at 100, 200, and 230 Hz, except for G'' at 230 Hz. In fact, the exponential model was capable of describing G' and G'' reasonably well at these frequencies, with $R^2 \geq .445$ except for G'' at 230 Hz, whereas neither model was able to curve-fit the data at 150 Hz with reasonable R^2 values (see Table 2).

Discussion

Vocal fatigue has often been characterized by a state of progressive increase in phonatory effort and loss of

Table 2. Results of least-squares nonlinear regressions for the parametric description of elastic shear modulus (G'), viscous shear modulus (G''), and damping ratio (ζ) of sheep vocal fold mucosa specimens according to Equations 1 and 2 at the frequency of (a) 100 Hz, (b) 150 Hz, (c) 200 Hz, and (d) 230 Hz ($n = 3$ for each frequency).

	Sigmoidal model (Equation 1)			Exponential model (Equation 2)		
	a	b (s)	R^2	a (Pa)	b (s ⁻¹)	R^2
(a) Viscoelastic function at 100 Hz						
G'	7.839	-0.734	.005	2822.54	-0.00006154	.445
G''	6.269	0.312	.001	617.33	-0.00008392	.537
ζ	-1.556	0.086	.001	0.2155	-0.00001106	.154
	Sigmoidal model (Equation 1)			Exponential model (Equation 2)		
	a	b (s)	R^2	a (Pa)	b (s ⁻¹)	R^2
(b) Viscoelastic function at 150 Hz						
G'	7.565	-0.228	.001	1895.34	0.000008487	.008
G''	6.453	-0.961	.008	627.05	0.000001726	.001
ζ	-1.124	-2.493	.241	0.3237	-0.000009784	.043
	Sigmoidal model (Equation 1)			Exponential model (Equation 2)		
	a	b (s)	R^2	a (Pa)	b (s ⁻¹)	R^2
(c) Viscoelastic function at 200 Hz						
G'	7.768	-4.624	.041	1639.93	0.0002	.701
G''	6.907	-1.304	.007	801.25	0.0001	.598
ζ	-0.834	4.269	.226	0.5215	-0.00007957	.911
	Sigmoidal model (Equation 1)			Exponential model (Equation 2)		
	a	b (s)	R^2	a (Pa)	b (s ⁻¹)	R^2
(d) Viscoelastic function at 230 Hz						
G'	8.673	-2.086	.039	4967.94	0.00007893	.649
G''	7.664	5.095	.221	2231.3	-0.000001085	.0001
ζ	-0.916	6.87	.262	0.5281	-0.0001	.913

Note. R^2 is the coefficient of determination indicating the goodness of curve-fitting.

phonatory abilities, after a period of vocalization or voice usage. An elevated level of phonatory effort or vocal effort, which can be correlated to an increase in PTP, has been reported to be one of the most common signs of vocal fatigue (Chang & Karnell, 2004; Eustace et al., 1996; McCabe & Titze, 2002; Solomon & DiMattia, 2000; Solomon et al., 2003). Prior to the recent development of the psychometrically validated self-assessment tool, the VFI (Nanjundeswaran et al., 2015), vocal fatigue has been described by a variety of subjective experience related to physiological and functional voice changes, including the perception of increased stress in the neck and shoulder region, increased laryngeal discomfort, and increased throat pain, as well as reduced vocal control, difficulty speaking, and voice loss (Nanjundeswaran et al., 2015; Solomon, 2008; Vilkman, 2004; Welham & MacLagan, 2003). As suggested by the findings of the VFI, individuals with vocal fatigue tend to become aware of fatigue-related changes by initially a perception of the sense of vocal “tiredness,” followed by an avoidance of voice use, and eventually physical discomfort during vocalization (Nanjundeswaran et al., 2015).

Nonmuscular tissue fatigue of the vocal folds could involve two distinct types of tissue injury and damage as the vocal folds are subjected to repeated, accumulated large-amplitude vibration exposure during phonation. The

first type of injury and tissue damage may involve the epithelial cells and the basement membrane, including significant reduction in the depth of the epithelial cell layer and separation of structural proteins and anchoring fibers (collagen and elastin fibers) from the lamina propria (Gray & Titze, 1988; Hunter & Titze, 2009; Kojima et al., 2014). The second type of injury and tissue damage may involve a redistribution of extracellular fluids partly due to rises in capillary pressures, which would likely adversely impact tissue viscoelasticity (Czerwonka, Jiang, & Tao, 2008; Hunter & Titze, 2009). Results of the current study could provide insights on how exactly tissue viscoelasticity might vary with vibration exposure and how such tissue viscoelastic response could contribute to vocal fatigue at different frequencies.

The distinct response patterns of changes in G' , G'' , and ζ with vibration exposure observed at different frequencies suggested that phonation could be differentially affected by the tissue fatigue response across different frequencies. At 100 Hz, the gradual decrease in G'' with vibration exposure (see Figure 2A) suggested there could be a gradual decrease in PTP and a correspondingly lower level of vocal effort during phonation, since PTP depends critically on the viscous properties of vocal fold tissues (G'' ; Chan & Titze, 1999, 2006; Titze, 1988). On the other hand, at the higher frequencies of 200 and 230 Hz, the gradual

increases in G'' with vibration exposure (see Figures 2C and 2D) suggested there could be corresponding increases in PTP and higher levels of vocal effort during phonation. Indeed, a significant increase in G'' with vibration exposure duration was observed at 200 Hz (see Table 1 and Figure 2C). Since PTP is also determined by frequency alone, with PTP increasing significantly with the fundamental frequency of phonation (Titze, 1988, 2006), the significant increase in G'' with vibration exposure suggested that it would become even more difficult (or effortful) to sustain phonation at higher frequencies beyond the effect of frequency alone, following prolonged periods of accumulated, large-amplitude vibration exposure.

For the damping ratio or damping factor ζ , it was found that ζ generally decreased with vibration exposure, particularly at 200 and 230 Hz, where on average, ζ decreased from around 0.5 to between 0.31 and 0.36 from 30 to 4,160 s (see Figure 3). Indeed, a significant decrease in ζ with vibration exposure duration was observed at 200 Hz (see Table 1 and Figure 3). Since $\zeta = G'' / G'$, this finding suggested that the increases in G' with vibration exposure were even higher in magnitude than the increases in G'' with vibration exposure (147% increase in G' vs. 89% increase in G'' at 200 Hz, 51% increase in G' vs. 28% increase in G'' at 230 Hz).

On the other hand, ζ increased significantly with the frequency of vibration exposure, increasing from an average of 0.21 at 100 Hz to an average of 0.43 at 230 Hz, with the data at 230 and 200 Hz significantly higher than those at 100 Hz (see Table 1 and Figure 3). At first glance, this finding may seem to be at odds with the results of previous studies showing ζ decreasing with frequency (Chan & Rodriguez, 2008; Chan & Titze, 1999). However, in the current study, it was actually the effect of frequency of large-amplitude vibration exposure, whereas in the previous studies, it was the effect of frequency of small-amplitude shear deformation during rheometric testing (Chan & Rodriguez, 2008; Chan & Titze, 1999). In fact, implications of the current findings in terms of the dependence of ζ on frequency were consistent with those of G'' as discussed above, in that phonation would become more difficult at higher frequencies as the vocal fold mucosa is subjected to accumulated, large-amplitude vibration exposure.

The duration of large-amplitude vibration exposure in the current study started at 1,040 s, which was based on the critical, acceptable level of accumulated tissue acceleration for vocal fold vibration exposure prior to tissue injury as derived from the hand-transmitted vibration literature (Griffin, 1990; Titze et al., 2003). However, it can be expected that the vocal fold lamina propria is likely better adapted for vibration than hand tissues and that active metabolic tissue recovery could raise the acceptable safety limit to beyond 1,040 s of nonstop vibration, as tested by the longer periods of vibration exposure in the current study, up to 4,160 s. In addition, the importance of pauses and vocal rest cannot be underestimated, as a voicing ratio or duty ratio of approximately 50% has been found for teachers during their daily vocalizations, meaning that

the total duration of vocal rest was about the same as the total duration of nonstop, continuous phonation (Titze & Hunter, 2015; Titze et al., 2003).

Results of the parametric curve-fitting showed that the time course of the tissue fatigue response could be better described by the exponential model, rather than by the sigmoidal model, except for the frequency of 150 Hz, which could not be described by either model (see Table 2). This finding was consistent with those of Hunter and Titze (2009), who found that the time course of vocal fatigue recovery followed a pattern of chronic dermal wound healing trajectory, that is, an exponential curve, rather than a pattern of acute dermal wound healing trajectory, that is, a sigmoidal curve (Franz et al., 2000; Hunter & Titze, 2009; Steed, 2003). This recovery pattern could reflect a state of ongoing metabolic tissue repair concurrent with continuous, repeated tissue injury secondary to vibration exposure. Hunter and Titze also argued that the chronic wound healing trajectory could, at some point, shift to an acute wound healing trajectory, if vocalization is continued beyond a certain threshold of tissue injury, as indicated by signs of vocal fatigue. Our findings indicated that such a threshold of wound healing pattern shift could be beyond 4,160 s of nonstop, continuous large-amplitude vibration exposure of the vocal fold mucosa, considering only the passive component of tissue viscoelastic response without active tissue recovery, and would warrant further studies.

Sheep vocal fold tissues were used for this study, with a small sample size; hence, the current findings should be considered preliminary. Although sheep is a viable model of the larynx, the tissue viscoelastic response of the human vocal fold lamina propria could be different from that of the sheep under accumulated, long-term, large-amplitude vibration exposure. Further studies should involve a larger sample size and should target the fatigue response of human vocal fold tissues.

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