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Biomechanical Properties of Recurrent Laryngeal Nerve in the Piglet

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Abstract

Unilateral vocal fold paralysis (UVP) results from damage to the recurrent laryngeal nerve (RLN). The most common causes of UVP are associated with compromised RLN tissue. The purpose of this research was to investigate the biomechanical properties of piglet RLN and identify differences in these properties along its length and in between the left and right side. Quasi-static uniaxial tensile testing and isotropic constitutive modeling was performed on seven piglet RLNs. Stiffness and other biomechanical parameters were derived from these tests and compared from conducting two different statistical analysis for the between and within nerve comparisons. Results showed higher stiffness values in the left RLN segment than for the right. Descriptive data demonstrated a higher stiffness in RLN segments surrounding the aortic arch, indicating a more protective role of the extracellular matrix in these nerves. This research offers insight regarding the protective function of the RLN connective tissues and structural compromise due to its environment.

Keywords

Mechanical properties; Ortner's syndrome; Unilateral vocal fold paralysis; Stiffness; Yield stress; Epineurium

INTRODUCTION

Unilateral vocal fold paralysis (UVP) most commonly occurs subsequent to surgery, idiopathic (i.e., unknown cause), and nonupper respiratory malignancies associated with

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impaired function of the recurrent laryngeal nerve (RLN) supplying the vocal folds in the voice box, or larynx.^{28,33,39,44,58} The majority of individuals diagnosed with UVP are older than 45 years of age with an average age of 64 years at onset.^{9,23} Slightly more females present with this disorder than males, on average.^{28,39} UVP significantly impacts affected individuals as it is associated with impaired voice production and airway protection.^{1,10–12,17–21,24,25,29–32,35,36,40,41,64}

The primary etiology of impaired RLN function in humans appears to be linked to structural damage. That is, the surgical etiology likely occurs as a result of trauma to the RLN due to the nerve being cut, excessively stretched, or compressed during the procedure. In many cases, RLN impairment occurs despite great care taken during procedures to avoid contact with the nerve. In these cases, it is possible that postsurgical edema may compress the nerve resulting in damage affecting RLN function.⁴²

Interestingly, 12–42% of laryngeal paralyses are diagnosed as idiopathic or unknown etiology.⁴⁶ Recent literature has suggested that some of these cases may, in fact, be caused by diseases affecting the proximal region of the RLN.² Several such cases have been published in recent years further elucidating the link between UVP and aortic or pulmonary artery aneurysm, enlarged atrium, or neoplasm affecting the lower lobe of the left lung.^{8,16,34,65} Thus, some proportion of those previously diagnosed with idiopathic UVP may have impaired RLN function due to excessive stretch or compression of the nerve associated with changes in the aortic arch, pulmonary vein, or atrium. Evidence that chronic compression may be indicated in idiopathic onset of UVP has been addressed by research investigating its histopathology in horses.^{7,13,14} This research showed that pathologic changes in the RLN were consistent with that of chronic nerve compression. Further, increased occurrence of pathology in the distal RLN segments suggested that chronic compression occurred near the aortic arch or inferior trachea.

Given that the most common causes of UVP are associated with compromised nerve tissues, investigation of the biomechanical properties of the RLN may be helpful to characterize the environmental forces this nerve is able to withstand. Such research would test the ability of the connective tissues that compose the RLN to protect it from typical stress and strain forces in its environment.

Connective tissues of peripheral nerves play an important role in insulating and protecting peripheral nerves.^{26,37,48–51,53–56} The *endoneurium* immediately surrounds individual neurons and has two layers composed of collagen fibrils.^{37,51,60,63} Groups of nerve fibers, or nerve fascicles, are contained within several layers of flattened cells that comprise the *perineurium*. This layer acts as a “blood-nerve barrier” by preventing the transport of harmful agents into the endoneurium.^{5,37,45,51,60} The most external layer surrounding all of the fascicles within each nerve is the *epineurium*. This layer contains a loose framework of collagen fibrils, adipose tissue,^{22,37,47,60} elastin filaments (10 nm width), and elastin fibers (250–500 nm in width).^{37,59,60}

The amount of epineurium present in a peripheral nerve varies relative to the environment that the nerve travels through. For example, peripheral nerves exhibit increased amounts of

epineurium where they cross joints.⁶⁰ Such increases in epineurium would improve protection from nerve compression resulting from joint movement.^{48–50,52,53,55–57} This is especially true when a nerve contains large amounts of adipose tissue in the epineurium.²²

Previous work compared the epineurium of the left RLN to the flexor hallicus longus branch of the tibial nerve (NFHL) in the canine.⁴ This study demonstrated a significant difference in the amount and makeup of the epineurium in the left RLN compared to the NFHL. The RLN exhibited a significantly greater amount of adipose tissue and relative cross-sectional area consisting of epineurium than the NFHL nerve. Also, the quantity and distribution of adipose tissue in the RLN epineurial tissue was greater than seen in the comparison nerve. The comparison nerve demonstrated similar proportions and distribution of adipose tissue as was previously reported for noncranial peripheral nerves.^{27,47,50} In addition, the left RLN of a puppy was acquired during the course of the adult canine study and exhibited noteworthy differences from the adult nerves. Specifically, the puppy nerve exhibited significantly less cross-sectional epineurium that was primarily comprised of collagen with only three fat cells identified. Although an unpublished finding, the contrasting size and makeup of the puppy and adult canine left RLN epineurium raised questions about changes in epineurium associated with aging.³ This is of particular interest since spontaneous damage to the left RLN tends to occur most often beyond the age of 55 years suggesting that tissue changes may occur with aging.

Overall, the extensive amount of epineurium and large amount of adipose tissue contained within the epineurium of the adult canine's left RLN may have functional significance. That is, the observed characteristics of the RLN epineurium may serve to protect the nerve against structural damage to nerve fibers during mechanical deformation or constriction such as occurs during neck flexion/extension or laryngeal elevation. The amount and makeup of epineurium offers clues regarding the environmental forces to which the RLN is subjected. However, the relation between the individual structural components of RLN epineurium and the type of protective function they offer has not yet been elucidated. Specifically, how these structural components (e.g., adipose, collagen) contribute to the overall biomechanical response of the RLN is of interest, as this may relate to the interplay between mechanical forces and signal transduction. Identifying these structure–function relationships may be important in understanding nerve damage associated with vocal fold paralysis.

The purpose of this study was to investigate the biomechanical properties of the piglet RLN using uniaxial tensile testing before the porcine animal has experienced full growth and development. Given prior research demonstrating changes in epineurium composition and relative cross-sectional proportion along its length, the changes in mechanical response as a function of segment location of the RLN were studied. Specifically, maximum tangential modulus (MTM), yield stress, yield stretch, and constitutive model parameters were derived from uniaxial tensile tests of each RLN segment. Yield stretch and yield stress are useful to note as this is the point at which permanent deformation will begin to occur. MTM and the isotropic constitutive model parameters are measures of stiffness, which is indicative of the ability of the RLN to resist deformation (in this case stretching).

METHODS

Nerve Specimen Acquisition and Preparation

The RLN was excised bilaterally in eight 2-day-old piglets (four males and four females) within 6 h postmortem. All nerve specimens were excised in entirety from the vagus nerve to insertion into the posterior larynx and stored in physiologic saline at 4 °C. To maintain orientation of proximal and distal aspects of each nerve, a large segment of the vagus was excised with the RLN branch. Three nerve samples broke away from the vagus during excision so that this method of determining nerve orientation was not possible for these samples. In those specimens, a suture was attached to the laryngeal end of the nerve to indicate the distal portion of the nerve. One nerve lost its orientation marker during storage making it impossible to determine which end of the nerve was proximal or distal. This nerve and its companion nerve were not analyzed consequently. Thus, seven right and left RLNs were tested within 48 h of acquisition.

Nerves were segmented using a cross-sectional cut such that all nerve segments were measured to have an average gauge length of 11.86 (± 1.63) mm and a diameter of 0.42 (± 0.095) mm. The average values of gauge length and diameter for each nerve segment are reported in Table 1. Thus, two segments were tested from the right RLN (segment 1 and 2) and four segments from the left RLN (segments 1, 2, 3, and 4). Segment 1 corresponded to the proximal end, or the segment at the vagus nerve location for both the right and left RLN. Segment 2 corresponded to the portion of the left RLN that wraps around the aortic arch. Segments 3 and 4 of the left RLN were associated with the distal portion within the neck where the nerve inserts into the larynx. In contrast, segment 2 of the right RLN corresponded to the distal segment where the nerve inserts into the larynx. The difference in the number of segments between the left and right RLN was due to the longer length of the left RLN (71.5 ± 8.70 mm) compared to the right (48.25 ± 8.88 mm). In addition, the right nerve resides almost entirely within the neck region whereas the left RLN transitions from the thoracic environment into the neck region. Figure 1 illustrates the environment and segmentation of the right and left RLNs.

Uniaxial Testing

Uniaxial testing was completed using a commercially available Perkin–Elmer Diamond Dynamic Mechanical Analyzer (DMA). The DMA had a load and spatial resolution of 0.5 μ N and 1 μ m, respectively. All nerve specimens were tested in 37 °C PBS at a strain rate of 5%/min up to 25% strain. Cauchy stress, T_{11} , was calculated at each time point as

$$T_{11} = \left(\frac{P_{11}}{A_0} \right) \lambda_{11}, \quad (1)$$

where P_{11} is the instantaneous load, A_0 is the initial cross-sectional area (assumed to be circular), and λ_{11} is the stretch.

Constitutive Modeling

Following the work of Raghavan and Vorp,³⁸ we modeled the nonlinear mechanical behavior of the RLN as a homogeneous, incompressible, isotropic, and hyperelastic material using a strain energy density function of the following form

$$W=W[I_{\mathbf{B}}, II_{\mathbf{B}}], \quad (2)$$

where W is the strain energy density, $I_{\mathbf{B}} = \text{tr} \mathbf{B}$, $II_{\mathbf{B}} = 1/2[(\text{tr} \mathbf{B})^2 - \text{tr}(\mathbf{B}^2)]$ (representing the first and second invariants of $\mathbf{B}$), $\mathbf{B} = \mathbf{F}\mathbf{F}^T$ ($\mathbf{B}$ is the left Cauchy-Green tensor and $\mathbf{F}$ is the deformation gradient tensor).

For this type of material, the constitutive equation is

$$\mathbf{T} = -p\mathbf{I} + 2\frac{dW}{dI_{\mathbf{B}}}\mathbf{B} + 2\frac{dW}{dII_{\mathbf{B}}}\mathbf{B}^{-1}, \quad (3)$$

where $\mathbf{T}$ is the Cauchy stress tensor, $\mathbf{I}$ is the identity tensor, and p is a Lagrange multiplier.^{38,62}

Figure 2 shows a representative plot of the first and second invariants of the stretch tensor. This plot demonstrates a nearly linear relation between the $\mathbf{B}$ invariants associated with Cauchy stress. This result was true for all specimens tested. For this reason, it was assumed that $W = W(I_{\mathbf{B}})$ only. If we consider an incompressible ($\lambda_1\lambda_2\lambda_3 = 1$) and isotropic ($\lambda_2 = \lambda_3$) specimen extended uniaxially and subjected to a traction of magnitude T_{11} and a stretch ratio of λ_{11} , which is stress free in the other two directions ($\mathbf{T} = \text{diag}[T_{11}, 0, 0]$), then from Eq. (3) we find

$$\frac{dW}{dI_{\mathbf{B}}} = \frac{T_{11}}{2(\lambda_{11}^2 - \lambda_{11}^{-1})}. \quad (4)$$

As seen in Fig. 3, $dW/dI_{\mathbf{B}}$ varies linearly with $I_{\mathbf{B}} - 3$, so that we can write

$$\frac{dW}{dI_{\mathbf{B}}} = \alpha + 2\beta(I_{\mathbf{B}} - 3), \quad (5)$$

where α and β are the model parameters. This relation also held for all specimens tested. Equation (5) suggests that at most a quadratic term is necessary so that the strain energy can be written

$$W = \alpha(I_{\mathbf{B}} - 3) + \beta(I_{\mathbf{B}} - 3)^2. \quad (6)$$

From Eqs. (5) and (3), we obtain

$$\mathbf{T} = -p\mathbf{I} + 2[\alpha + 2\beta(I_{\mathbf{B}} - 3)]\mathbf{B}. \quad (7)$$

The constitutive model proposed above for the RLN can be reduced by combining Eqs. (4) and (5) and utilizing the traction free boundary conditions to obtain

$$T_{11} = [2\alpha + 4\beta(\lambda_{11}^2 + 2\lambda_{11}^{-1} - 3)][\lambda_{11}^2 - \lambda_{11}^{-1}]. \quad (8)$$

Equation (8) was used in this study to model each nerve segment's behavior during uniaxial extension.

Data Acquisition and Analysis

The parameters for quantification of the RLN tissue biomechanical behavior were obtained using two programs. A custom written Matlab program was used to generate individual specimen $T_{11} - \lambda_{11}$ plots. The program also calculated the yield stress and yield stretch as the point at which the incremental modulus first decreased. The incremental modulus at yield, herein termed the MTM, was also quantified using the Matlab program.

The constitutive model described above (8) was fit to each nerve segment's $T_{11} - \lambda_{11}$ data using a Marquardt–Levenberg least-squares algorithm in the commercially available statistical software SigmaStat. In order to prevent violating fundamental thermodynamic principles, α and β were required to be greater than or equal to zero. Figure 4 shows the Cauchy stress–stretch ratio data from a representative nerve specimen and corresponding fit by Eq. (8).

Statistical analyses were conducted to compare the measures of yield stress, yield stretch, MTM, α and β between and within the right and left RLN segments. Two different statistical tests were conducted for the between and within nerve comparisons. The first test used the generalized estimating equations approach to determine differences in MTM, yield stress, yield strain, α and β between the right and left RLN and along their lengths. Due to the differing lengths and associated numbers of segments tested between the right and left RLNs, values for each dependent variable for the left RLN were collapsed for segments 1 and 2 (i.e., most proximal segments within the thorax), and segments 3 and 4 (i.e., most distal segments within the neck) to create two nerve segments for comparison to the right RLN segments 1 (i.e., proximal) and 2 (i.e., distal). That is, data associated with left RLN segments 1 and 2 were analyzed together as segment 1 and left RLN segments 3 and 4 were analyzed together as segment 2. Collapsing adjacent segments along the length of the left RLN allowed for statistical comparison of analogous proximal and distal segments of the left and right RLN to determine differences between the left and right nerves. This comparison is of interest as the entire right side resides within the neck region compared to the left RLN that resides within both the thoracic and neck regions. Thus, the independent variables tested included left vs. right RLN and a comparison between nerve segments from proximal to distal.

The second statistical test conducted was a generalized estimating equations analysis to determine differences between nerve segments along the length of the right and left RLNs across the dependent variables of interest. Thus, all four nerve segments were compared to each other within the left RLN to determine differences within the thorax compared to the

neck region of this nerve and both segments of the right RLN were compared to each other during this analysis.

RESULTS

Results for Comparisons Between the Right and Left RLN

Maximum Tangential Modulus—A significant main effect was identified for nerve segment (Wald χ^2 (1, $N = 38$) = 15.1, $p < 0.0001$), with the average MTM of the left nerve (16.8 (± 7.62) MPa) significantly less than the right nerve (18.5 (± 5.65) MPa). In addition, a significant interaction was found for nerve segment $\times$ nerve (Wald χ^2 (1, $N = 38$) = 6.875, $p = 0.009$). *Post hoc* pairwise comparisons using a Bonferroni correction for comparison of nerve and for nerve segment ($\alpha = 0.05/2 = 0.025$) were completed to determine which segments differed between nerves. The results demonstrated that the MTM of the right RLN segment 2 was significantly higher than left RLN segments 1 and 2. Figure 5 shows the average MTM differences between the left and right nerve specimens.

Yield Stress and Yield Strain—No significant differences were identified between nerves and segments 1 and 2 for either of these dependent variables. Average yield stress and strain data for the collapsed data can be found in Table 2.

α : A significant main effect for nerve segment was identified (Wald χ^2 (1, $N = 38$) = 14.116, $p < 0.0001$). Using a Quasi Likelihood test, nerve segment 1 was identified as significantly different between nerves (Wald χ^2 (1, $N = 38$) = 7.226, $p = 0.007$). The average α value for each specimen of the collapsed data group can be found in Table 2. As can be seen in Table 2, the right RLN exhibits higher average values of α in segment 1 compared to the same segment in the left RLN.

β : A significant main effect for nerve segment was identified (Wald χ^2 (1, $N = 38$) = 7.304, $p = 0.007$). Using a Quasi Likelihood test, nerve segment 1 was identified as significantly different between nerves (Wald χ^2 (1, $N = 38$) = 10.183, $p = 0.001$). The average β values for the left and right RLN segments can also be found in Table 2. As can be seen in Table 2, the left RLN segment 1 shows higher average values for β than does the right RLN segment 1.

Results for Comparisons Within the Left and Right RLN

Maximum Tangential Modulus—A significant difference for the main effect of nerve segment was found for the left RLN (Wald χ^2 (3, $N = 24$) = 8.994, $p < 0.05$), but not for the right RLN. After several pairwise comparisons were completed during the *post hoc* analysis, no statistical differences were found between left RLN segments. Figure 6 shows the average MTM within left and right nerve specimens.

Yield Stress and Yield Strain—No significant differences were identified within the right or left RLNs for yield stress or yield strain variables. Average yield stress and strain data for the within nerve segment comparison data can be found in Table 3.

α : A significant difference for nerve segment was found for the right RLN (Wald χ^2 (1, $N = 14$) = 4.569, $p < 0.05$), but not for the left RLN. However, *post hoc* pairwise comparisons did

not show statistical differences within the right RLN segments. The average α values calculated for each specimen for within nerve comparisons can be found in Table 3.

β : A significant difference for nerve segment was found for the left RLN (Wald χ^2 (3, $N=24$) = 17.703, $p=0.001$), but not for the right RLN. *Post hoc* pairwise comparisons were completed using a Bonferroni adjustment. Segments 2 and 4 (95% Wald CI for difference = 3.36 to 11.3), and segments 3 and 4 (95% Wald CI for difference = 1.5–4.6) were significantly different at the $p < 0.0001$ level. The average β values calculated for each specimen for within nerve comparisons can also be found in Table 3. As seen in this Table 3, β values decreased from segments 2 to 4 within the left RLN. In contrast, β values appeared to remain relatively consistent along the length of the right RLN.

DISCUSSION

The responses of the left and right RLNs to longitudinal tensile stress were investigated in piglets using a uniaxial tension setup. Significant differences were demonstrated between the left and right RLNs for MTM, α and β . However, the differences showed that the MTM of the distal portion of the right RLN differed from both proximal and distal segments of the left RLN while only the proximal segments of both nerves were different for α and β measures. The right RLN demonstrated greater average MTM in the proximal and distal values compared to values found in the same segments within the left nerve. In contrast, higher β values were found in the proximal left RLN compared to both segments in the right RLN. These findings may relate to the very different anatomical locations of the proximal sections of each nerve. The left RLN resides deep within the thorax at the level of the aortic arch, a prominent pulsating vessel. In comparison, the right RLN descends to the level of the subclavian artery before it loops around this vessel and ascends toward the larynx. The latter is a smaller pulsating vessel located at the junction between the thorax and base of the neck. The increased stiffness of the left vs. right RLN (as indicated by higher β values) demonstrated there may be a protective measure to counter the complex mechanical environment the left RLN experiences as it loops around the pulsing aorta.

The results obtained from within nerve testing allow a closer inspection of changes in biomechanical properties within the left and right RLN from proximal to distal segments. No significant differences were found for the right RLN. This finding is not surprising given that the right RLN resides almost entirely within the neck region offering a more consistent environment than for the left RLN. β was found to significantly differ between segments 2 and 4 and segments 3 and 4 within the left RLN, with higher values of β in the second and third segments. As seen in Fig. 8, β appears to be a stronger indicator of the stiffness of each nerve segment than the parameter α . At values of stretch greater than 1.10 (more than 10% strain), the slope of the model curve (representative of stiffness) varies much greater with changes in β vs. changes in α . Although no significant differences were found within nerve segments for MTM or yield stress, the descriptive data show a higher stiffness in the second and third segments of the nerve (see Figs. 6 and 7), similar to the results found for β . Given that there was a significant difference in the main effect for the left RLN within ANOVA, the insignificant results for the *post hoc* analysis on MTM are likely due to the multiple pairwise comparisons.

Previous studies have been done on the mechanical properties of peripheral nerves, including but not limited to, the tibial and sciatic nerves.^{6,15,61} However, to the author's knowledge there has been no investigation of the tensile behavior of the RLN. Comparing the stiffness of adult heterogeneous stock mice sciatic nerves elongated in an axial-loading device following crush injury revealed an average stiffness of 7.0 MPa and an average stress of 3.2 MPa at 43% strain.¹⁵ An average stiffness of fresh whole rat sciatic nerves elongated in a tensile-testing device was reported to be 0.580 ± 0.150 MPa and values of ultimate stress and strain were reported to be 2.720 ± 0.970 MPa and 0.810 ± 0.114 , respectively.⁶ These values are smaller on average than the values of the MTM and yield stress of the piglet RLN reported in this paper. These differences are most likely related to the species and size differences utilized in these previous studies.

A study of the biomechanical properties of rabbit peripheral nerves mounted on a material testing machine and stretched to failure at a rate of 1 cm/min, yielding ultimate strain and tensile strength values of $38.5 \pm 2.0\%$ and 11.7 ± 0.7 MPa, respectively.⁴³ The reported value of average ultimate tensile strength is larger than the reported value of average yield strength of the RLN in this study, but on the same order of magnitude. This is expected as our tests were not run to failure. Right and left RLN nerve segments were found to yield at strain values ranging from 11% to 20%. These findings support previous studies which demonstrate that strains of 6% to 8% for a short time can cause physiological changes within the peripheral nerve tissue tolerance and strains of 11% or greater can cause long-term damage and are considered to be states of extreme stress.⁶¹

Based on the results of this study, the RLN appears built to withstand greater amounts of stretch in the vicinity of the aortic arch in the left RLN than in more distal segments as indicated by the statistical findings for β . This suggests that the left RLN in piglets may be more prone to injury when stretched within the distal (neck) region, such as may occur during surgical procedures in the distal segments located near the larynx. Interestingly, the statistical findings for MTM were not found to be statistically different between proximal and distal segments within the left RLN as was found for β . These two measures both offer a biomechanical measure reflective of stiffness in the tissues and would be expected to have similar outcomes. Observation of the descriptive data for MTM exhibit an average increase in value at the left RLN segment associated with the aortic arch (i.e., segment 2) similar to that shown for β . MTM is a measure of the tangential slope of the Cauchy stress-stretch curve at a point at which the tangential slope no longer increases. This point varies considerably from sample-to-sample which could be the reason it was not significant while β was. It should be noted that β is a constitutive parameter which governs the shape of the curve at higher regions of stretch (greater than 10% strain). Note that, assuming material linearity, the 43% decrease in MTM from segments 2–4 of the left RLN would result in a corresponding 7% increase in stretch. These results may have important physiological relevance.

It is not expected that the RLN of piglets would experience significant tensile loading after only 2 days of growth and therefore the high stiffness found in this segment should not be attributed to growth and elongation. The portion of the left RLN adjacent to the aortic arch (i.e., segment 2) may require greater stiffness to withstand the pulsating stretching forces

from that vessel. It is possible that changes in the elasticity of the aortic arch associated with increased thoracic aortic compliance or aneurysm might impose stretching forces on the RLN beyond its capacity to withstand resulting in structural damage to the connective tissues within. Should this be the case, it would be of interest to determine how the stiffness of this segment of the RLN might change with aging, particularly in age groups in which spontaneous impairment of this nerve is known to occur. Future research is necessary to test this hypothesis directly.

The right nerve was not found to differ significantly between the proximal and distal segments for any of the dependent variables tested within this nerve. This was most likely because it resides almost entirely within the neck which offers a fairly consistent environment. Interestingly, the average MTM in the right RLN appeared to increase where the nerve inserts into the larynx. This may occur related to stretch imposed on the right RLN associated with laryngeal elevation and depression during swallowing, even during gestation.

Future research will investigate the anatomical composition of these nerves to determine how histological makeup relates to the biomechanical properties of the piglet RLN. In addition, the RLN will be studied in older pigs for comparison to the piglet research to determine changes in epineurium anatomy and biomechanical properties across the lifespan that may be linked to spontaneous impairment of the RLN with aging. Further studies will also include a finite element study of the left RLN and its surrounding anatomical environment, specifically the area corresponding to the aortic arch. RLN behavior will be modeled according to the same structure–function relationship, with values of α , β , and MTM, determined from this investigation. In addition, future work will include quantifying structure–function relationships by comparing the mechanical properties of each nerve component with the corresponding histology.

There were some limitations to the procedures of this study. Due to the size of the tensile apparatus of the DMA a maximum elongation of 25 mm was enforced. This prevented testing of the specimens to failure, which is why no values are reported here for ultimate tensile strength. These disadvantages were deemed an acceptable tradeoff for the high load and displacement resolution offered by the DMA. An additional limitation is the comparison of the data from piglet nerves with the data of mature animals. Ideally this data should be compared to those of a more similar investigation, one that studies the tensile behavior of the RLN or another peripheral nerve of piglets. However, to the author's knowledge, such information is not currently available in the literature.

In conclusion, this study provides new quantitative data regarding the biomechanical properties of the RLN in piglets. It is likely that the connective tissues comprising the left and right RLN may differ in their ability to withstand stretch as indicated by the findings of this study. The anatomic relation of the left RLN to the aortic arch could possibly render the nerve vulnerable to compression and stretch either by a thoracic aortic aneurysm or increased aortic arch compliance. In addition, the findings of this study suggest differences in stiffness along the length of the left RLN, particularly in segments 2 and 3. This offers insight regarding the protective function of RLN connective tissues and may contribute to

understanding structural compromise in this nerve due to surgical damage or thoracic aorta changes in compliance or aneurysm.

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FIGURE 1.
Illustration of the segmentation right and left RLNs.

FIGURE 2.

The experimentally determined relationship between the first and second invariants of the left Cauchy stretch tensor. Linear regression resulted in a highly correlated fit ($R^2 = 0.99$).

FIGURE 3.

The experimentally determined relationship between the first derivative of the strain energy density function and the first invariant of the stretch tensor for a typical nerve specimen. By observing a highly correlated fit ($R^2 = 0.98$), we can anticipate a linear relationship between the two.

FIGURE 4.

Cauchy stress–stretch ratio data from a representative specimen and corresponding fit by Eq. (8) ($R^2 = 0.99$).

* $p < 0.0001$ (95% Wald CI for difference = -14.35 to - 8.59)

° $p = .01$ (95% Wald CI for difference = -14.38 to -1.96)

FIGURE 5.

Data collected for segments 1 and 2, and segments 3 and 4 were collapsed to create two nerve segments for comparison to the right RLN segments 1 and 2. MTM was averaged ($\pm$ SEM) for each group of segments. The MTM of the right RLN segment 2 was significantly higher than left RLN segments 1 and 2.

FIGURE 6.

All four nerve segments were compared to each other within the left RLN and both segments of the right RLN were compared to each other during this analysis. MTM was calculated and averaged ($\pm$ SEM) for each group of segments.

FIGURE 7.

All four nerve segments were compared to each other within the left RLN and both segments of the right RLN were compared to each other during this analysis. Yield stress was calculated and averaged ($\pm$ SEM) for each group of segments. No significant differences were identified within the right or left RLNs for yield stress.

FIGURE 8.

Representative plot of Eq. (8) substituting in average values of α and $\beta \pm 1$ SD.

TABLE 1

Average measures of length and diameter and their standard deviations of each nerve segment.

Segment	Average diameter, mm ($\pm$ SD)	Average length, mm ($\pm$ SD)
PLs1	$0.41 \pm (0.050)$	$11.67 \pm (1.66)$
PLs2	$0.39 \pm (0.074)$	$11.67 \pm (1.66)$
PLs3	$0.40 \pm (0.062)$	$11.67 \pm (1.66)$
PLs4	$0.48 \pm (0.195)$	$11.67 \pm (1.66)$
PRs1	$0.45 \pm (0.096)$	$13.00 \pm (1.00)$
PRs2	$0.48 \pm (0.179)$	$13.00 \pm (1.00)$

Average diameter and length ($\pm$ SD) of each group of nerve segments measured before uniaxial tensile testing.

TABLE 2

Average measures and their standard deviation for comparison between the right and left RLN.

Sample	α (MPa)	β (MPa)	Yield Cauchy stress (MPa)	Yield stretch
LS1	$0.4 \pm 0.22^*$	$9.7 \pm 4.4^{**}$	1.93 ± 0.94	1.21 ± 0.14
LS2	0.25 ± 0.35	8.94 ± 4.57	1.30 ± 0.95	1.17 ± 0.05
RS1	$0.51 \pm 0.54^*$	$5.44 \pm 2.26^{**}$	2.07 ± 1.25	1.20 ± 0.05
RS2	0.46 ± 0.48	6.87 ± 1.86	2.13 ± 1.01	1.18 ± 0.08

Collapsed data collected for segments 1 and 2, and segments 3 and 4 were collapsed to create two nerve segments for comparison to the right RLN segments 1 and 2. α , β , yield stress, and yield stretch were calculated and averaged ($\pm$ SEM) for each group of segments. Nerve segment 1 was identified as significantly different between nerves for α and β .

* Wald $\chi^2(1, N=38) = 7.226, p = 0.007$.

** Wald $\chi^2(1, N=38) = 10.183, p = 0.001$.

TABLE 3

Average measures and their standard deviation for comparison within the right and left RLN.

Sample	α (MPa)	β (MPa)	Yield Cauchy stress (MPa)	Yield stretch
LS1	0.32 ± 0.38	8.42 ± 5.38	1.64 ± 1.31	1.11 ± 0.14
LS2	0.5 ± 0.32	$10.91 \pm 4.53^*$	1.86 ± 1.38	1.30 ± 0.27
LS3	0.58 ± 0.48	$7.1 \pm 1.72^{**}$	2.52 ± 1.25	1.19 ± 0.06
LS4	0.37 ± 0.49	$4.19 \pm 2.38^{*,**}$	1.61 ± 1.24	1.20 ± 1.08
RS1	0.25 ± 0.35	8.94 ± 4.51	1.30 ± 0.05	1.17 ± 0.05
RS2	0.46 ± 0.48	8.87 ± 1.86	2.13 ± 1.01	1.18 ± 0.08

Uncollapsed data collected for all four nerve segments were compared to each other within the left RLN and both segments of the right RLN were compared to each other. α , β , yield stress, and yield stretch were calculated and averaged ($\pm$ SEM) for each group of segments. Nerve segments 2 and 3 were found to be significantly different from segment 4 of the left RLN for β .

* 95% Wald CI for difference = 3.36–11.3, $p < 0.0001$.

** 95% Wald CI for difference = 1.5–4.6, $p < 0.0001$.