Differential effects of targeted tongue exercise and treadmill running on aging tongue muscle structure and contractile properties

Heidi Kletzien, John A. Russell, Glen E. Leverson, and Nadine P. Connor

Department of Surgery, Otolaryngology-Head and Neck Surgery, University of Wisconsin, Madison, Wisconsin; and Department of Communicative Disorders, University of Wisconsin, Madison, Wisconsin

Submitted 13 November 2012; accepted in final form 19 December 2012

Kletzien H, Russell JA, Leverson GE, Connor NP. Differential effects of targeted tongue exercise and treadmill running on aging tongue muscle structure and contractile properties. J Appl Physiol 114: 472–481, 2013. First published December 20, 2012; doi:10.1152/japplphysiol.01370.2012.—Age-associated changes in tongue muscle structure and strength may contribute to dysphagia in elderly people. Tongue exercise is a current treatment option. We hypothesized that targeted tongue exercise and nontargeted exercise that activates tongue muscles as a consequence of increased respiratory drive, such as treadmill running, are associated with different patterns of tongue muscle contraction and genioglossus (GG) muscle biochemistry. Thirty-one young adult, 34 middle-aged, and 37 old Fischer 344/Brown Norway rats received either targeted tongue exercise, treadmill running, or no exercise (5 days/wk for 8 wk). Protrusive tongue muscle contractile properties and myosin heavy chain (MHC) composition in the GG were examined at the end of 8 wk across groups. Significant age effects were found for maximal twitch and tetanic tension (greatest in young adult rats), MHCIIb (highest proportion in young adult rats), MHCIX (highest proportion in middle-aged and old rats), and MHCI (highest proportion in old rats). The targeted tongue exercise group had the greatest maximal twitch tension and the highest proportion of MHCI. The treadmill running group had the shortest half-decay time, the lowest proportion of MHCIIa, and the highest proportion of MHCIIb. Fatigue was significantly less in the young adult treadmill running group and the old targeted tongue exercise group than in other groups. Thus, tongue muscle structure and contractile properties were affected by both targeted tongue exercise and treadmill running, but in different ways. Studies geared toward optimizing dose and manner of providing targeted and generalized tongue exercise may lead to alternative tongue exercise delivery strategies.

The tongue has an important role in swallowing and respiration. For swallowing actions, the tongue is active in bolus formation, transport, and propulsion (82). The genioglossus (GG) muscle, the primary tongue protruder, is a major force generator during the swallow and is crucial to dilation and/or narrowing of the pharynx during breathing (19, 21, 23). More specifically, the GG is involved in opening the oropharynx and reducing resistance to breathing (57, 70, 77). Due to the cross-system role of the GG muscle, age-related changes in muscle structure and physiology may result in decrements in both respiration and swallowing actions.

Age-related changes in GG muscle structure and physiology have been noted in humans and in animal models (11, 15, 28, 45, 50, 56, 67, 68, 71, 75). Exercise may serve as an effective remediation because recent studies have identified increased GG muscle contraction amplitudes with exercise, as measured with electromyography (EMG) (73, 83, 88). In addition, exercise-induced increases in GG EMG activity were correlated with increases in nasal, oral, and total ventilatory rates and neural drive to the GG (1, 3, 20, 73, 88). However, the optimal type of exercise to employ for improving tongue muscle contractile function and the manner in which different types of exercise may alter GG muscle biochemistry have not been elucidated.

Current clinical treatments for oropharyngeal dysphagia have targeted the tongue with the goal of strengthening associated musculature (5, 38, 58, 63, 89). Targeted tongue exercises, such as progressive resistance tongue exercise, have been shown to increase tongue forces and have been associated with improved swallowing function (29, 58). Animal models have shown that 8 wk of progressive resistance tongue exercise is associated with changes in GG muscle fiber structure and tongue force, even in old animals (11, 67). However, targeted tongue exercise may not be the only approach for positively affecting tongue muscle structure and physiology. It may be possible to systematically increase tongue muscle activity not only through targeted tongue exercise, but also with the use of other, nonspecific exercises that serve to increase respiratory drive, such as treadmill running. The rationale for this concept is the previously discussed upregulation of tongue muscle activity and neural drive to the GG that is associated with increased respiratory activity during exercise (1, 3, 20, 73, 83, 88). Thus, treadmill running may strengthen the tongue muscle and improve overall function by inducing changes in tongue muscle contractile properties and structure. However, targeted tongue exercise and treadmill running may not yield the same types of changes in muscle structure and function. Investigations geared toward discovery of potential differential effects are necessary, and results may have clinical implications.
The purpose of this study was to determine the differential effects of targeted tongue exercise and treadmill running across the adult age range using a rat model. We hypothesized that both exercise methods would alter tongue muscle contractile properties and lead to changes in GG muscle biochemistry. We also hypothesized that GG alteration associated with targeted tongue exercise and treadmill running would be distinct because of the highly dissimilar nature of these exercise methods and greater specificity of targeted tongue exercise.

METHODS

Animals. This study was performed in compliance with the NIH Guide for Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of the University of Wisconsin School of Medicine and Public Health. Male Fischer 344/Brown Norway rats (n = 102) were obtained from the National Institute on Aging colony (Harlan Laboratories, Indianapolis, IN) at the ages of 6, 21, and 29 mo old. Upon the completion of the study, rats were 9 mo old (young adult, n = 31), 24 mo old (middle aged, n = 34), and 32 mo old (old, n = 37). The median life expectancy of the Fischer 344/Brown Norway rat is ~36 mo (81).

The rats were housed in pairs in standard polycarbonate cages on a 12:12-h light-dark reversed light cycle. Animals were obtained 2–3 wk before the start of the experiment to allow for acclimation to the vivarium, light cycle reversal, water restriction, and familiarization to the experimental equipment (tongue force operandum or treadmill). All animals were water restricted to 3 h/day (78). Body weights were recorded weekly at the same time of day to monitor the effects of the water restriction and training paradigms. Rats within all three age groups were randomly assigned to either: 1) a targeted tongue exercise group that received 8 wk of progressive resistance exercise (n = 36), 2) a group that received 8 wk of treadmill running (n = 35), and 3) a no exercise group, representing the control condition (n = 31).

Targeted tongue exercise. Experimental methods for animals in the targeted tongue exercise group have been detailed previously (6, 11). Briefly, thirsty rats were trained to press the tongue against an instrumented disc to receive a water reward. Targeted tongue exercise was performed 5 days/wk for a total of 8 wk. Each exercise session lasted 10 min. Before and midway through the 8 wk of exercise, an estimated maximum pressure (EMP, mN) was determined for each rat. Throughout the 8 wk of exercise, force increments for obtaining a water reward were increased to mimic a progressive resistance training program. Rats were trained at 50% EMP (weeks 1 and 2), 60% EMP (weeks 3 and 4), 70% EMP (weeks 5 and 6), and 80% EMP (weeks 7 and 8). After the 8 wk of targeted tongue exercise, posttreatment maximum voluntary tongue force (mN) values were obtained.

Treadmill running. One week after arrival, rats were trained over the course of 2 wk to run on an adapted motor-driven treadmill designed for rats (model LE8710R; Panlab, Harvard Apparatus, Holliston, MA). During the 1st wk of training, rats were individually placed into a treadmill lane at a 20° incline for a total of 10 min. The treadmill was stationary, and the rats explored and became familiar with the apparatus. In the 2nd wk of training, rats ran at 10 cm/s and by the end of the 3rd wk progressed to 20 cm/s at a 20° incline for 10 min. If an animal did not run on the treadmill by the end of the training period, the rat was classified as a “nonrunner” and removed from the study (34).

After 3 wk of training, each rat completed an endurance test (ET) and a progressive running test (PRT). For the ET, rats were given a 1-min warm-up running period at 20 cm/s. Treadmill speed was then increased to 30 cm/s on a 20° incline, and rats ran until exhaustion, which was defined as the inability of the rat to return to and maintain the pace of the treadmill (13, 40, 41). The test began at 20 cm/s, and speed was increased 5 cm/s every 1 min. The maximal speed (cm/s) reached during the PRT was recorded for each rat.

Following baseline testing, the rats were matched into treadmill running groups based on their performance in the PRT. For consistency with the targeted tongue exercise group, each running session lasted 10 min a day, 5 days/wk for a total of 8 wk, and treadmill speeds were increased throughout the experimental period as rats achieved performance goals. Rats were trained at 50% of their PRT speed (weeks 1 and 2), 60% PRT (weeks 3 and 4), 70% PRT (weeks 5 and 6), and 80% PRT (weeks 7 and 8). After 8 wk of treadmill running animals completed a posttreatment ET and PRT. Times for the ET were recorded for each rat, as were maximal speeds from the PRT.

No exercise. Rats were handled in an identical manner to the targeted tongue exercise and treadmill running groups but did not participate in any exercise treatment for the 8-wk duration of the experiment.

Tongue muscle contractile properties. Muscle contractile properties were determined following 8 wk of targeted tongue exercise, treadmill running, or no exercise treatment conditions. Rats were anesthetized with isoflurane (3–4%) followed by pentobarbital sodium (70 mg/kg, ip injection). The hypoglossal nerves were exposed bilaterally under microscopy, and the bifurcation of the nerve into the medial and lateral branches was identified. Nerve cuff stimulation electrodes were placed around the hypoglossal nerve inferior to the bifurcation. The lateral branch of the hypoglossal nerve was sectioned bilaterally using microscissors, leaving an intact medial branch. A suture was placed through the tongue and connected to a force transducer. The transducer was oriented inferiorly to the rat to maximize the force vector during evoked protrusive tongue actions. Following a 45-min stabilization period, the medial branch of the hypoglossal nerve was supramaximally stimulated at 1.5 times the maximum twitch response (1 Hz rectangular-wave pulses, pulse width 0.1 ms), and provocative muscle contractile properties were measured as described previously (10, 45). Stimuli were delivered at 1 Hz to elicit a twitch contraction. Following twitch contraction data collection, tetanic tension was induced with stimulation at 80 and 100 Hz with 200-ms trains; the maximum force value at 80 or 100 Hz was used in statistical analyses. Fatigueability was determined by repetitive stimulation at either 80 or 100 Hz (based on maximum force obtained in tetanic tension trials) for 2 min. Twitch contraction time, half-decay time, maximal twitch tension, maximal tetanic tension, and fatigue index (ratio of force at the end of the 2 min of stimulation relative to initial force) were measured for each rat. Thus, a high fatigue index reflected resistance to fatigue. The stimulation signal and tongue force signal were acquired digitally on a dedicated laboratory computer with an analog-to-digital converter (Data Translation, Marlboro, MA) using data acquisition software written and customized for our use (Acquire version 1.3.0).

Following data collection, the force transducer was removed from the tongue, and the rat was euthanized by an overdose of Beuthanasia (0.3 mL, ip injection). The GG muscle from the left or right side of the tongue of each rat was extracted, suspended in 0.9% saline solution, and frozen. The specimens were frozen, individually, in 2-methylbutane cooled by liquid nitrogen and stored in a −80° freezer.

Myosin heavy chain. The protein concentration of each GG muscle was optimized following tissue homogenization using either a dilution series or a standardized protein concentration assay (Bradford Protein Assay) such that each concentrate contained 0.4 µg of protein. SDS-PAGE was performed on each concentrate with a 0.75-mm-thick 6% acrylamide-30% glycerol separating gel (18 × 16 cm) and a 4% acrylamide-30% glycerol stacking gel. A silver staining kit was used to stain the gel for visualization of protein bands. Each silver-stained gel was digitally imaged, and the optical density of each band was determined by investigators masked to rat age and treatment group using computer-assisted image analysis and densitometry (UN-
Fig. 1. Maximal voluntary tongue force was significantly greater following 8 wk of targeted tongue exercise for each age group \( (P < 0.0001) \).

Scan-IT gel version 6.1; Silk Scientific, Orem, UT. The ratio of the density of an individual myosin heavy chain (MHC) band to the total density within a column was used to determine the percentage of each MHC isoform per lane (14, 25, 27, 32, 68, 76, 84). Previous studies have demonstrated good to excellent reliability using these procedures (68).

**Statistical analysis.** Within each age group, paired \( t \)-tests were used to compare maximal voluntary tongue force \( (\text{mN}) \), ET time \( (\text{s}) \), and progressive running test speed \( (\text{cm/s}) \) before and following targeted tongue or treadmill running treatments.

ANOVA was used to compare age groups on the following measures: mean number of tongue presses above threshold, percent change in maximal voluntary tongue force, ET time, and PRT speed.

Two-way ANOVA was used to examine age effects, treatment effects, and interactions for muscle contractile property variables and the MHC isoform composition of the GG. Body weight was used as a covariate for analysis of maximum twitch tension and tetanic tension because body weight was found to correlate highly with these variables in prior studies (11, 45). Pairwise comparisons were made between groups using Fisher’s protected least significant difference tests. SAS statistical software was used for all analyses (SAS Institute, Cary, NC). The critical value for obtaining statistical significance was set at \( \alpha = 0.05 \).

For some measures, missing data resulted in a smaller sample size and thus smaller degrees of freedom. Computer error accounted for missing behavioral data \( (n = 3) \). Unexpected animal expiration or computer error resulted in missing muscle contractile property data \( (n = 16) \). In some cases, use of the GG muscle tissue in other experiments accounted for missing MHC composition data \( (n = 4) \). All other available data were used.

**RESULTS**

**Targeted tongue exercise.** All rats in the targeted tongue exercise group completed the 8 wk of progressive resistance tongue exercise. The average number of tongue presses per session for the last 4 wk of targeted tongue exercise were not significantly different among age groups \( F(2,32) = 0.71, P = 0.5 \). The young adult group averaged 95.4, the middle-aged group averaged 73.5, and the old group averaged 100.6 presses/session. Thus, no differences were found in level of participation during targeted tongue exercise training among the three age groups throughout training.

When pre- and posttargeted tongue exercise maximal voluntary force values were compared across age groups, a significant increase in maximum force was observed using paired \( t \)-tests, \( t(33) = 9.003, P < 0.0001 \) (Fig. 1). On the average, maximal voluntary tongue force increased 152.9% over the 8-wk exercise program. Paired \( t \)-tests within each age group showed statistically significant maximal voluntary tongue force increases following the 8 wk of targeted tongue exercise within young adult \( t(10) = 9.308, P < 0.0001 \), middle-aged \( t(11) = 6.602, P < 0.0001 \), and old rats \( t(12) = 4.277, P = 0.001 \). The largest gain was seen in the young adult group with an average increase of 169.9% compared with the middle-aged (142.4%) and old (148.2%) groups. The percent change in maximum voluntary tongue force among age groups was not significantly different \( F(2,32) = 0.19, P = 0.83 \), suggesting that the young adult, middle-aged, and old groups increased maximal voluntary force values to a similar extent following targeted tongue exercise.

**Treadmill running.** All but one old rat in the treadmill running group completed the 8 wk of exercise. The old rat removed from the study was classified as a nonrunner based on predetermined criteria. When the pre- and postendurance test times \( (s) \) were examined among age groups, a significant increase in the duration of time spent on the treadmill was observed using paired \( t \)-tests, \( t(34) = 2.443, P = 0.02 \). There was a 39.2% average increase in time after the 8-wk exercise program, indicating improved endurance for running after treadmill exercise. Paired \( t \)-tests within each age group showed a significant increase in time spent on the treadmill following the 8 wk of treadmill running within young adult rats \( t(12) = 2.236, P = 0.047 \). There were not significant time increases following the exercise period in the middle-aged \( t(11) = 2.166, P = 0.056 \) and old \( t(11) = -1.237, P = 0.24 \) groups (Fig. 2). The greatest percent gain in performance on the ET was seen in the middle-aged group, with an average increase of 67.24% in time compared with young adult (63.82%) and old (15.7%) groups. The percent change in ET time among age groups was not significantly different \( F(2,33) = 2.84, P = 0.07 \).

When the pre- and postprogressive running test speeds \( (\text{cm/s}) \) were examined among age groups, a significant increase in speed was observed using paired \( t \)-tests, \( t(34) = 7.979, P < 0.0001 \). There was an average overall 47.7% increase in speed after the 8-wk exercise program, demonstrating that treadmill
running increased the pace the rats were capable of running. Paired t-tests within each age group showed a significant speed increase following the 8 wk of treadmill running within young adult \((t(12) = 6.093, P < 0.0001)\), middle-aged \((t(11) = 5.398, P = 0.0003)\), and old \((t(11) = 4.944, P = 0.0006)\) groups (Fig. 3). Additionally, significant age effects were found for the PRT \([F(2,33) = 5.02, P = 0.01]\, with the greatest percent gain in speed within the young adult and middle-aged groups vs. the old group. The greatest percent gain in performance on the PRT overall was observed in the middle-aged group.

**Muscle contractile properties.** Descriptive data for each measure by age and experimental group are shown in Table 1. On two-way ANOVAs, significant main effects for age were found for maximal twitch tension \([F(2,87) = 5.93, P = 0.004]\) and maximal tetanic tension \([F(2,87) = 4.09, P = 0.02]\), in the absence of significant age by experimental group interactions, with greater tensions in young adult vs. middle-aged or old groups (Figs. 4 and 5). There were no significant age group effects for contraction time or half-decay time \([F(2,87) = 1.78, P = 0.17; F(2,87) = 1.40, P = 0.25]\).

Significant main effects for experimental group were found for half-decay time and maximal twitch tension on two-way ANOVAs in the absence of a significant interaction effect. For half-decay time, the treadmill running group had a significantly shorter decay time than the no exercise and targeted tongue exercise groups \([F(2,87) = 5.84, P = 0.004]\) (Fig. 6). Significantly greater twitch tension was found in the no exercise and targeted tongue exercise groups than in the treadmill running group \([F(2,87) = 10.74, P < 0.0001]\) (Fig. 7). There were no significant experimental group effects for contraction time and maximal tetanic tension \([F(2,87) = 0.53, P = 0.59; F(2,87) = 1.06, P = 0.35]\).

A significant interaction effect was found for fatigue index \([F(4,84) = 3.63, P = 0.009]\). Post hoc testing revealed that there was significantly less fatigue in the young adult rats within the treadmill running group than in the young adult no exercise group \((P = 0.01)\). Post hoc testing also revealed that there was significantly less fatigue in the old rats within the targeted tongue exercise and treadmill running groups than in the old no exercise group \((P < 0.0001; P = 0.0003\), respectively). The old targeted tongue exercise group manifested the least fatigue overall (Fig. 8). No other interaction effects were found.

**MHC isoform composition.** Descriptive data for each measure by age and experimental group are shown in Table 2. Representative silver-stained SDS-PAGE gels from the GG muscles of a young adult, middle-aged, and old rat in the targeted tongue exercise, treadmill running, and no exercise groups are presented in Fig. 9, A–C. On two-way ANOVAs, significant main effects for age were found for MHCIIb, MHCIIx, and MHCI in the absence of significant interaction effects with experimental group. The young adult group had a significantly greater proportion of MHCIIb than the middle-aged or old groups \([F(2,97) = 11.79, P < 0.0001]\). The middle-aged and old groups had a significantly greater proportion of MHCIIx than the young adult group \([F(2,97) = 8.69, P = 0.0004]\). The old group had a significantly greater proportion of MHCI than the young adult or middle-aged groups \([F(2,97) = 3.17, P = 0.0466]\) (Fig. 10).

Significant main effects for experimental group were found for MHCIIa, MHCIIb, and MHCI in the absence of significant interaction effects with age. The treadmill running group had a significantly lower proportion of MHCIIa \([F(2,97) = 11.14, P < 0.0001]\) and significantly higher proportion of MHCIIb \([F(2,97) = 6.34, P = 0.003]\) than the no exercise or targeted tongue exercise groups. The targeted tongue exercise group had a significantly greater proportion of MHCI than the no exercise or treadmill running groups \([F(2,97) = 7.30, P = 0.001; \text{Fig. 11}]\). No other significant age, experimental, or interaction effects were found.

### Table 1. Medial branch stimulation

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Maximal Twitch Tension, mN</th>
<th>Contraction Time, ms</th>
<th>Half-Decay Time, ms</th>
<th>Maximal Tetanic Tension, mN</th>
<th>Fatigue Index, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tongue exercise</td>
<td>112.9 (9.3)</td>
<td>10.3 (0.2)</td>
<td>38.3 (0.9)</td>
<td>256.1 (20.9)</td>
<td>81.2 (3.8)</td>
</tr>
<tr>
<td>Treadmill running</td>
<td>92.8 (6.9)</td>
<td>9.9 (0.4)</td>
<td>35.9 (1.1)</td>
<td>231.6 (15.7)</td>
<td>87.7 (2.3)</td>
</tr>
<tr>
<td>No exercise</td>
<td>122.7 (9.3)</td>
<td>9.7 (0.2)</td>
<td>37.4 (0.9)</td>
<td>281.6 (21.1)</td>
<td>75.9 (5.2)</td>
</tr>
<tr>
<td>Middle aged</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tongue exercise</td>
<td>95.4 (7.4)</td>
<td>11.8 (1.9)</td>
<td>38.1 (0.8)</td>
<td>215.3 (16.6)</td>
<td>79.7 (2.1)</td>
</tr>
<tr>
<td>Treadmill running</td>
<td>72.8 (6.8)</td>
<td>10.4 (0.2)</td>
<td>36.4 (1.0)</td>
<td>199.9 (15.4)</td>
<td>83.3 (2.3)</td>
</tr>
<tr>
<td>No exercise</td>
<td>81.0 (7.7)</td>
<td>11.2 (0.6)</td>
<td>41.3 (1.4)</td>
<td>176.5 (17.3)</td>
<td>83.5 (3.6)</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tongue exercise</td>
<td>92.4 (7.2)</td>
<td>10.3 (0.2)</td>
<td>37.4 (0.9)</td>
<td>213.9 (16.2)</td>
<td>90.9 (4.5)</td>
</tr>
<tr>
<td>Treadmill running</td>
<td>64.8 (6.4)</td>
<td>10.2 (1.0)</td>
<td>34.2 (2.6)</td>
<td>204.0 (14.4)</td>
<td>87.5 (2.2)</td>
</tr>
<tr>
<td>No exercise</td>
<td>80.0 (7.4)</td>
<td>10.6 (0.5)</td>
<td>38.9 (1.2)</td>
<td>184.2 (16.6)</td>
<td>70.8 (3.6)</td>
</tr>
</tbody>
</table>

Data are expressed as means (SE) of muscle contractile properties for each age group and exercise treatment during protrusive tongue action elicited by stimulation of the medial branch of the hypoglossal nerve.
DISCUSSION

The hypothesis of this study was that targeted tongue exercise and treadmill running would alter protrusive tongue muscle contractile properties and induce changes in biochemical properties of the GG muscle in young adult, middle-aged, and old rats. Additionally, we hypothesized that changes found within the tongue following targeted tongue exercise or treadmill running would be distinct because of the increased specificity of the tongue exercise condition. Our results support these hypotheses. Rats in the targeted tongue exercise and treadmill running groups showed alterations in both tongue muscle contractile and biochemical properties compared with the no exercise group.

The results of this study demonstrated a relatively large capacity for plasticity within the GG muscle throughout the lifespan and as a function of two different exercise-based treatments. The middle-aged and old groups demonstrated significant reductions in tongue strength, as measured by twitch and tetanic tension, compared with the young adult group. In addition, there was a shift in GG MHC isoforms toward more slowly contracting isoforms with increasing age. Differential effects were observed in muscle contractile properties and biochemical profiles dependent upon which of the two exercise treatments was administered to the young adult, middle-aged, or old rats. Specifically, targeted tongue exercise was associated with increased twitch tension vs. treadmill running and an increased proportion of MHCI in the GG muscle. On the other hand, treadmill running induced shorter half-decay times, increased proportions of MHCIIb, reduced proportions of MHCIIa, and less fatigue in the young adult group. Both targeted tongue exercise and treadmill running were associated with fatigue reductions in the old group compared with no exercise. Therefore, musculoplasticity within the tongue was both age and activity dependent.

The targeted tongue exercise group completed a progressive resistance-training program that focused on strengthening the tongue musculature through a skilled motor action coupled with a water swallow. The overall goal of this prospective task was to approach a load near maximal capacity for the tongue musculature. In our study, all rats in the young adult, middle-aged, and old groups significantly increased their maximal voluntary tongue forces after the 8 wk of training. Human studies that have used progressive resistance exercises that target the lingual musculature have found improvements in strength and swallowing function as well (29, 58).
tary gains in strength observed in our study were also manifested on a contractile and biochemical level.

The targeted tongue exercise group generated the greatest maximal twitch tension. Because the specific contractile properties of muscle are defined by MHC composition (86), we also determined the MHC isoform composition in the GG muscle. The rat GG is primarily composed of MHCII isoforms, and fibers of this phenotype have the greatest capacity for force generation and the most rapid twitch response (66, 68, 74, 84). All groups in this study had a large proportion of MHCII isoforms in the GG muscle, whether or not exercise was provided. Thus, additional factors likely contributed to the gains in force seen in both the maximal voluntary tongue forces and twitch tension, such as increases in GG muscle fiber cross-sectional area (CSA) that were not measured in this study. In a previous study using a rat model of progressive resistance tongue exercise, there was a trend for increased muscle fiber CSA and a significant increase in variability of the CSA in the GG muscle after 8 wk of training (11). Muscle fiber hypertrophy may have increased force production in the targeted tongue exercise group. With progressive strength training exercise, fiber types generally shift to a more slowly contracting fatigue-resistant phenotype, from MHC type IIb to IIx to I to (24, 51, 52, 72). This shift also occurred in our targeted tongue exercise group, specifically an increased proportion of slowly contracting (MHCI; 2.0%) and fast-contracting fatigue-resistant (MHCIIa; 33.7%) isoforms and a decreased proportion of fast-contracting highly fatigueable isoforms (MHCIIb; 9.9%). The increase in MHCI and MHCIIa may have contributed to the significantly reduced amount of fatigue in the old targeted tongue exercise group. Accordingly, targeted tongue exercise, with its focus on training specificity and gains in strength, was associated with changes in structure and contractile properties.

The treadmill running group completed a high-intensity training program geared toward increasing running speed and endurance, with concomitant changes in respiratory capacity. With ventilatory changes of this kind, GG recruitment is increased to produce both phasic and tonic contractions for

### Table 2. MHC isoforms in the GG

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>I</th>
<th>IIa</th>
<th>IIx</th>
<th>IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young adult</td>
<td>1.6</td>
<td>33.7</td>
<td>50.2</td>
<td>14.4</td>
</tr>
<tr>
<td>Tongue exercise</td>
<td>1.4</td>
<td>34.1</td>
<td>54.3</td>
<td>10.3</td>
</tr>
<tr>
<td>Treadmill running</td>
<td>0.7</td>
<td>29.0</td>
<td>59.5</td>
<td>10.8</td>
</tr>
<tr>
<td>No exercise</td>
<td>0.2</td>
<td>37.0</td>
<td>52.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Middle aged</td>
<td>2.9</td>
<td>33.2</td>
<td>57.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Tongue exercise</td>
<td>0.6</td>
<td>29.4</td>
<td>54.7</td>
<td>15.3</td>
</tr>
<tr>
<td>Treadmill running</td>
<td>1.4</td>
<td>34.0</td>
<td>56.3</td>
<td>8.3</td>
</tr>
<tr>
<td>No exercise</td>
<td>0.6</td>
<td>34.0</td>
<td>56.3</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Data are expressed as average percents (SE) of myosin heavy chain (MHC) isoforms in the genioglossus (GG) muscle for each age group and exercise treatment.
creased tongue mobility (75), decreases in strength and force. Fatty and connective tissue have been found along with amyloid deposits in the tongue vasculature and increases in the reductions in strength and force common with aging. The young adult group also had the greatest proportion of MHCI, the fastest-contracting phenotype. With increasing age, we observed a transition from fast-to-slow MHC isoforms that manifested overall, more specifically a transition from MHCIb to MHCIx in the middle-aged and old groups, and a conversion to MHCI for the old age group.

A well-established principle in muscle training is specificity, but it is unknown whether this principle applies to the swallowing musculature. It has not been elucidated if the exercise performed must include the swallow to improve tongue function and swallowing, or if an exercise that targets muscles involved in the swallowing action is sufficient. Specific motor exercises that include the swallow and have been shown clinically to improve behavioral aspects of the swallow are the Mendelsohn Maneuver, the effortful swallow, and the Masako Maneuver (5, 8, 62). However, motor exercises that do not include a swallow such as progressive resistance tongue exercise with the Iowa Oral Pressure Instrument, expiratory muscle strength training, and the Shaker Head Lift have also led to improvements in strength and swallowing function (5, 8, 53, 62, 65, 79). Therefore, we included aspects of learning and specificity in our study to further investigate their effects on the tongue musculature.

Our study was the first to elicit changes in tongue muscle contractile properties and MHC isoform composition using a specific (tongue exercise) and nonspecific (treadmill running) exercise distinction. The targeted tongue exercise group findings reinforced the observation that the effects of training are most dramatic for movements that closely match the training specific (tongue exercise) and nonspecific (treadmill running) exercise distinction. The targeted tongue exercise group finding was reinforced the observation that the effects of training are most dramatic for movements that closely match the training exercise (64). Because the targeted tongue exercise group was trained to perform a tongue-strengthening task that incorpo-
rated the swallow, there is a possibility this specific exercise may have had a lasting neuroplastic effect and be translatable to functional improvements in the swallow (33, 62). However, the tongue is not just involved in swallowing; it also plays a key role in speaking and breathing. These functions share the same muscles and similar central and peripheral neural control elements (61). Thus, a promising translational pathway for tongue exercise may be through a route that upregulates breathing, such as treadmill running. Because the tongue is a muscular hydrostat with an interdigitated muscle fiber orientation, there may be a variety of muscle activation patterns to accomplish the same movement goal (8, 31). This suggests that, for the tongue, exercise training may not need to be as specific. In the targeted tongue exercise group, the rats exercised their tongue by protruding their tongue against a force-incremented disc. A similar action was accomplished via an indirect route in the treadmill running group. The increase in ventilation that resulted from running increased recruitment and activation of the GG muscle, the major muscle of tongue protrusion. This may be a reason why, in our study, treadmill running was capable of inducing changes in the tongue musculature. The GG’s role in maintaining the airway and improving airflow during treadmill running may have been sufficient enough to manifest in changes of tongue muscle strength and structure.

There are some limitations to consider when translating the findings of our study to the swallow. First, there were no functional swallowing measures. Because we did not include behavioral or videofluoroscopic swallowing measures, it is difficult to attribute the changes in contractile and biochemical properties to improvements in the functional swallow. Second, because we only studied tongue protrusion, and swallowing requires both protrusive and retrusive actions of the tongue, we may be missing an important component of tongue muscle contraction. It would be advantageous to evoke tongue retrusion through whole hypoglossal stimulation to study additional contractile properties, and determine the MHC isoform composition of the tongue retractors, the hyoglossus and styloglossus. Third, because of the length of time required to complete the studies, animal batch difference may have occurred. Although the animals were genetically identical, there is a possibility that there were slight differences in animal care or other factors throughout the length of our study.

Because swallowing is considered a submaximal muscular activity, it is not known whether targeted tongue exercise, a training program geared toward generating improvements in maximal force, or treadmill running, an endurance exercise aiming to increase tongue activation, led toward an improved functional swallow. Progressive resistance tongue exercise is a current clinical treatment of dysphagia that is associated with improvements in the swallow (29, 58). However, no prior studies have examined the effects of a nonspecific exercise, such as treadmill running, on the swallow. Most of the literature concerning the effects of treadmill running on the GG muscle comes from the study of obstructive sleep apnea in respiratory physiology, and they have found that the GG muscle is activated to maintain airway patency and improve airway resistance. During exercise and hypercapnia, increased ventilation was associated with a progressive rise in GG EMG activity during both oral and nasal routes of breathing (21, 73, 88). Furthermore, activation of the GG muscle, even through hypoglossal stimulation or direct muscle stimulation, dilates the oropharynx to increase inspiratory flow rates and thus improves upper airway flow mechanics, specifically within the pharynx (16, 19, 23, 70). Although we did not assess GG muscle activation or inspiratory flow rates during treadmill running, our results, because of alterations in muscle contractile and biochemical properties, suggest that the GG was activated and played a role to improve respiratory mechanics.

The results of this study do not address the optimal dose or type of exercise to best rehabilitate the swallow. To make this determination, we must first have a clear understanding of the goals for therapy. We must determine whether achievements in strength, endurance, or both are necessary for the treatment of dysphagia. It may be advantageous to develop a treatment that combines strength and endurance training, for instance, a treatment protocol that includes both targeted tongue exercise and treadmill running. Another important consideration is that not all people are capable of running or even walking on a treadmill. In these instances, a different approach such as respiration therapy may be appropriate.

In conclusion, our results indicate that both targeted tongue exercise and treadmill running induce positive changes within the tongue musculature. The gain in muscle strength and the reduction in fatigue as a result of exercise may help combat dysphagia in the elderly population. Treadmill running, as a result of increased tongue muscle activity to maintain the airway, may be an alternative treatment for swallowing disorders for those who are able. In the future, our goal is to develop studies geared toward optimizing dose and manner of targeted tongue exercise and treadmill running.

ACKNOWLEDGMENTS
We acknowledge the contributions of Heather Mosley, Fatima Salem, Allison Schaser, and Ben Becker in the completion of this work. Dr. Hao Wang is also acknowledged for SDS-PAGE analyses.

GRANTS
This work was funded by grants from the National Institute on Deafness and other Communication Disorders (R01DC-005935, R01DC-008149).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: H.K., J.A.R., and N.P.C. conception and design of research; H.K. and J.A.R. performed experiments; H.K. and J.A.R. interpreted results of experiments; H.K. prepared figures; H.K., J.A.R., and N.P.C. approved final version of manuscript; G.E.L. analyzed data.

REFERENCES


