Objective. The purpose of this study was to examine whether the diffusion tensor imaging (DTI) technique can be used as a modality to represent the structural deformation in the in vivo genioglossus (GG) muscle fibers with oral appliances (OAs).

Study Design. Three healthy subjects were recruited for the pilot study. A custom-made OA, which is modified from a tongue retaining device (TRD), was constructed for each subject before the DTI acquisitions. Recordings were made with and without OAs to compare the GG muscle fiber deformation.

Result. DTI provided good resolution of tongue muscle fibers in vivo and successful isolation of each muscle fiber bundle. In particular, the GG muscle fiber deformation due to OAs was clearly visualized.

Conclusions. This DTI technique may be used not only to identify the individual myoarchitecture, but also to assess muscle fiber deformations in vivo, such as constriction, dilatation, and rotation with OAs. Clinical studies for OSA patients will be the next step. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:e57-e63)

It is widely thought that the anatomical deformation of the upper airway muscles, especially the genioglossus (GG) muscle, might be important in the cause of obstructive sleep apnea (OSA).1,2 In a cephalometric study,3 OSA patients had hyoid bones that were located more inferiorly than normal and their tongues extended caudally toward the lower part of the pharynx. Endurance of GG muscles was found to decrease frequently with age, and was strongly associated with the increased prevalence of OSA.4 Carrera et al.5 found histologically that composition of fiber type (ie, types I and II) in GG muscles was different between healthy individuals and OSA patients. These changes influence fiber orientation, diameter, length, and distribution of muscle, fat, and connective tissues. However, there are no reports about the myoarchitecture of GG muscles in the in vivo tongue of OSA patients.

Oral appliances (OAs), such as a tongue retaining device (TRD)6-8 and a mandibular advancement device (MAD),9-11 are used as a therapeutic method for OSA patients. The TRD changes the tongue position and posture, whereas the MAD advances the mandible. Therefore, both of them help to compensate for impaired dilator muscle function (ie, posterior portion of the GG muscle) and to prevent the air passage from closing during sleep. Although the MAD is by far the most common OA in use today, the TRD is still an option for patients with temporomandibular dysfunction, edentulous dentition, or periodontal disease, as well as for those who cannot tolerate mandibular fixation.

Diffusion tensor imaging (DTI) was recently developed to investigate orientation and integrity of nerve structures in the central nervous system.12-14 The method involves the estimation of the anisotropy in the tissues using the diffusion of water molecules, which is commonly known as Brownian motion. The diffusion characteristics depend on the component and structure of the tissues. An effective diffusion tensor is measured in each voxel by using a set of diffusion-weighted images. The eigenvectors and eigenvalues derived from the tensor provide information on the local tissue geometry. Tractography is a component post-processing method and is based on the assumption that the eigenvector with the largest eigenvalue (ie, the principal eigenvector) of the tensor coincides with the local fiber orientation.15

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The DTI technique has also been used for muscle fiber imaging and characterization.\textsuperscript{16-23} In humans, the myocardial fiber orientation,\textsuperscript{16} human calf muscle,\textsuperscript{17} and the myoarchitecture of the human uterus\textsuperscript{18} were successfully defined using the DTI technique. In the tongue, there have been some ex vivo studies in bovine,\textsuperscript{19} mammalian,\textsuperscript{20} and calf tongues.\textsuperscript{21} In addition, the human tongue in vivo has been examined.\textsuperscript{22-24} In this study, we apply the DTI technique to visualize the deformation of in vivo GG muscles with a custom-made OA, which is a modified TRD.

**MATERIAL AND METHODS**

**Subjects**

Three volunteers (2 males + 1 female, aged; 26 to 38 years), with no history of neurological or functional disorders, were recruited from the dental school staff for the pilot study. These data provide baseline normative effects of the OA to compare to subsequent studies of OSA patients. OSA patients should be able to tolerate these procedures absent neurological disorders that preclude holding the tongue still. The experimental procedures were approved by the Institutional Review Board of the University of Maryland. Written informed consent was obtained from the subjects before the study.

**Material**

Custom-made OAs were constructed of flexible polyvinyl material (Raintree ESSIX, Metairie, LA) for all subjects from plaster casts (see Fig. 1).\textsuperscript{24} The OA shape was modified from the standard configuration of TRD to fix the tongue tightly because of the DT-MRI sensitivity to small motion. The subjects had silicone impressions made to transfer the tongue protrusion shape accurately to the cast. During the impression, each subject was instructed to protrude the tongue forward as much as possible with a comfortable mouth opening to create the shape of a tongue bulb to be built in the OA. This method of taking the impression was

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Fig. 1. Frontal (A) and oblique (B) views of a modified tongue retaining device (TRD), and lateral view (C) of a cast are shown. The black arrows in A and B denote a hole to which the vacuum hose is connected, thereby immobilizing the tongue and extracting saliva during MRI acquisitions. The cast (C) for fabricating the oral appliance (OA) was also used to measure the amount of tongue protrusion (2-headed white arrow) and mouth opening (2-headed black arrow), using a slide gauge.
designed to be comfortable for each subject and the quantitative effects of the protrusion on the tongue position and rotation were measured on MR images afterwards. The size of the tongue tip beyond the lower incisor (LI), which was measured in each cast (see Fig. 1, C) by using a slide gauge, was 24.6 mm for subject 1, 23.0 mm for subject 2 and 22.8 mm for subject 3. The finished tongue bulb was further modified by drilling a hole, with a diameter of about 10 mm, on the inferior surface, for suctioning saliva and creating a vacuum to fixate the tongue. The OA also fixed the jaws because it was attached to the upper and lower teeth by adhesive. The amount of the mouth opening was also measured at the distance between the upper and lower incisors in each cast (Fig. 1, C): 19.7 mm for subject 1, 20.6 mm for subject 2, and 25.5 mm for subject 3.

MRI acquisition
A 1.5-T MRI machine (Magnetom Avanto; Siemens, Erlangen, Germany) with 8 channel head and neck coil was used to obtain the MRI images. The subjects lay supine in the scanner. The head was fixed to the head coil with adhesive tape (AMD-Ritmed Inc., Plattsburgh, NY, USA). Recordings were made with and without the OA (hereafter, w/ OA and w/o OA). During the w/ OA task, a suction force of 150 to 200 mm Hg was used. A spin echo echo-planar imaging (EPI) pulse sequence with diffusion sensitizing gradients was applied in 6 collinear directions and b-value = 500 s/mm². Other imaging parameters were the following: FOV = 200 mm, slice thickness = 3 mm (no gap), voxel size = 3.1 × 3.1 × 3.0 mm, matrix size = 64 × 64, repetition time (TR) = 5000 ms, echo time (TE) = 66 ms, 4 averages. Twenty-three to 28 axial slices parallel to the skin surface of the mandible were chosen for each subject to cover the whole tongue volume. Total acquisition time was approximately 3 minutes.

T1- and T2-weighted high-resolution MRI images were also taken of sagittal, axial, and coronal sections, using the following parameters: T1: FOV = 160 mm, TR = 600 ms, TE = 14 ms, voxel size = 0.8 × 0.8 × 3.0 mm, matrix size = 256; T2: FOV = 160 mm, TR = 3000 ms, TE = 99 ms, voxel size = 0.8 × 0.6 × 3.0 mm, matrix size = 256. The high-resolution axial slices, matched in thickness and position to the DT slices, were used as anatomical references. In addition, the midsagittal slices w/ and w/o the device were used for morphological measurements.

Data analysis
The analysis and visualization of tongue fiber tracking were performed using dTV-II implemented in VOLUME-ONE software (University of Tokyo, http://www.ut-radiology.umin.jp). The threshold for the fiber tracking was set at fractional anisotropy (FA) greater than 0.18. Regions of interest (ROIs) for GG muscle fiber tracking were chosen manually, using both T2 axial and coronal images. In order to display 3D fiber...
tracking (ie, tractography), the principal eigenvector in each voxel was color-coded as demonstrated by a 3D colored sphere (Fig. 2):15,27 green is horizontal (anterior-posterior); blue is vertical (superior-inferior); and red is transverse (left-right).

Measurements
Fig 3 shows the reference points, lines, and angles used for the 7 measurements shown in Table I.28-31 The reference points shown in red at Fig. 3 are as follows: LI, the lower incisor; Me, Menton; Vi, the most inferior velar point; Es, the most superior epiglottal point; CI, the inferior edge of the first cervical vertebrae; and CII, the inferior edge of the second cervical vertebrae. Each line through the pharynx (ie, WVi, WEs, and WCII) was drawn perpendicular to the line between the CI and CII. The measurements were performed by one rater 3 times at a few days’ interval for each image by using ImageJ version 1.38p software (NIMH, http://rsb.info.nih.gov/ij/). The averaged values (Mean) and standard deviation (SD) of the 3 trials were calculated. To assess the intra-rater variability, intra-class correlation coefficient (ICC) and the coefficient of variation (CV) were also calculated. The analysis yielded ICC greater than 0.95 and CV between 0.2% and 3.0% for all measurements.

RESULTS
Configuration of the tongue muscles without OA
DTI provided good resolution of all tongue muscles and successful isolation of GG muscle fibers to reveal OA-induced rotation, and subject differences. Fig. 4, A shows the tongue in subject 1 w/o OA. The fiber tracks of multiple muscles are shown in Fig. 4, B. Superior longitudinalis has circumferential fibers coursing along the upper surface (green) and angling downward at the pharyngeal surface (blue). In the tip, the transverse bundles (red) of transverses are seen between the vertical fibers (blue) of GG-anterior (GGA). Inferior longitudinalis originates at the tongue tip (green) and courses back through GG. The fan shape of GG, originating at the mandible, is visualized as half blue (vertical) and half green (horizontal). The horizontal fibers of geniohyoid are at the bottom. Thus, the relation between intrinsic and extrinsic muscle fibers in vivo was correctly observed.

Comparisons of GG muscle fiber orientation without and with OA
Fig 5 shows the tongue fiber orientation w/o and w/ OA, respectively, in the midsagittal slice of subject 1. The orientation of the GG muscle, which is fan shaped, clearly differs in the 2 conditions. More fibers of GG are vertical w/ OA (blue). The tongue protrusion seen in the MRI images is 21.6 mm for this subject. This is similar to the protrusion measured from the cast to create the OA.

Morphological comparisons between the without and with OA conditions
Morphological measurements showed that all tongues were protruded forward (ie, LTP) in the w/ OA condition by about 2 cm (Table II). Marked enlargements were observed in pharyngeal width (ie, WVi and WEs) and area (ie, OPA) in subjects 1 and 2 w/ OA, but not subject 3 (Table II). The GG muscle fiber angles for w/ and w/o OA are also shown in Table II. All the angles, especially in subjects 1 and 2, were rotated upward into a more oblique orientation w/ OA. Subject 3 had less rotation than the others (Table II). Also none of the subjects had more than 3 degrees change in Sp, which meant no change in radial spread of GG muscle fibers.

DISCUSSION
This is the first study to depict the GG muscle deformation effects of OAs, using the DTI technique. The technique successfully extracted the complex structure of intrinsic and extrinsic muscle fibers in vivo (Fig. 4) and correctly visualized fiber deformation of the GG muscles (Fig. 5). The data appear cluttered in

Table 1. Summary of the measurements, abbreviations, and definitions

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Abbreviations</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, widths, and area</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) Tongue protrusion length</td>
<td>LTp</td>
<td>Length between the LI-Me line and the parallel line that touches the tongue tip</td>
</tr>
<tr>
<td>(2) Velopharyngeal width</td>
<td>WVi</td>
<td>Width at the height of the most inferior velar point (Vi)</td>
</tr>
<tr>
<td>(3) Oropharyngeal width</td>
<td>WEs</td>
<td>Width at the height of the most superior epiglottal point (Es)</td>
</tr>
<tr>
<td>(4) Oro-hypopharynx area</td>
<td>OPA</td>
<td>Area between WVi and WCII</td>
</tr>
<tr>
<td>Angles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) Anterior GG muscle</td>
<td>Ga</td>
<td>Angle between the front edge of GG muscle and the LI-Me line</td>
</tr>
<tr>
<td>(6) Posterior GG muscle</td>
<td>Gp</td>
<td>Angle between the bottom edge of GG muscle and the perpendicular to the LI-Me line</td>
</tr>
<tr>
<td>(7) Spread of GG muscle</td>
<td>Sp</td>
<td>Angle between the Ga and Gp</td>
</tr>
</tbody>
</table>

*Hypopharyngeal width (WCII): Width at the height of the inferior edge of the second cervical vertebrae (CII). The data were not shown.
Fig. 4 because fibers for the entire 3D volume are superimposed; nonetheless, muscle fibers can be visually extracted. In Fig. 5, GG muscle fibers were especially well visualized because they are bundled at their origin, which led to very effective seeding for fiber tracking. The rotation of GG muscle fibers between the w/ and w/o OA was shown clearly. In particular, the number of vertical GG fibers increased noticeably w/ OA, but the spread of fibers remained constant (Fig. 5). This was especially true in subjects 1 and 2.

The OA produced tongue rotation, and increased the percentage of vertical GG fibers in subjects 1 and 2, not in subject 3. The degree of rotation was approximately consistent with the percent of enlargements in the upper airway. Subject 1 had almost twice as much rotation and enlargement as subject 2 (see Table II). Subject 3 had little rotation and enlargement in comparison with subjects 1 and 2. In this data set GG muscle rotation was the most salient effect of OAs.

Fujita et al.32 stated that there are 2 types of mechanisms to protrude the tongue forward: one is the elongation of the tongue, which is done by co-contraction of verticalis (V) and transversalis (T) muscles, and the other is the tongue body protrusion, which is generated by activating the posterior portion of the GG muscle. In this study, subject 1 and 2 protrude their tongues bodily and the tongue dorsa were flat. On the other hand, subject 3 elongated his tongue narrowly. These results were also correlated to the amounts of mouth opening (ie, 19.7 mm for subject 1, 20.6 mm for

### Table II. Measurements for each subject

<table>
<thead>
<tr>
<th>Subject</th>
<th>(1) LTp</th>
<th>(2) WVi</th>
<th>(3) WEs</th>
<th>(4) OPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>w/o OA</td>
<td>w/OA</td>
<td>Ratio</td>
<td>w/o OA</td>
</tr>
<tr>
<td>1</td>
<td>21.6 ± 0.2</td>
<td>5.6 ± 0.12</td>
<td>16.4 ± 0.15</td>
<td>3.1 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>19.4 ± 0.36</td>
<td>10.4 ± 0.12</td>
<td>20.4 ± 0.12</td>
<td>2.0 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>19.3 ± 0.15</td>
<td>9.2 ± 0.23</td>
<td>7.5 ± 0.06</td>
<td>0.8 ± 0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>(5) Ga</th>
<th>(6) Gp</th>
<th>(7) Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>w/o OA</td>
<td>w/OA</td>
<td>Diff</td>
</tr>
<tr>
<td>1</td>
<td>−14.8° ± 0.65</td>
<td>+15.2° ± 0.29</td>
<td>30.0° ± 0.70</td>
</tr>
<tr>
<td>2</td>
<td>−2.8° ± 0.29</td>
<td>+9.5° ± 0.25</td>
<td>12.3° ± 0.36</td>
</tr>
<tr>
<td>3</td>
<td>−8.4° ± 0.50</td>
<td>−3.5° ± 0.62</td>
<td>4.9° ± 0.49</td>
</tr>
</tbody>
</table>

**Ratio** is w/OA divided by w/o OA. **Diff** is w/OA minus w/o OA. The scales are mm for LTp, WVi, and WEs, mm² for OPA, and degrees (°) for Ga, Gp and Sp. Clockwise is − and counter clockwise is +.
subject 2, and 25.5 mm for subject 3). The tongues in subject 1 and 2 might be protruded by the activation of the GG muscles, whereas the tongue in subject 3 might be protruded by the activation of V and T muscles when the impressions were taken. This might be a hint to fabricate effective TRDs.

However, this study had several limitations. First, all participants were healthy volunteers and awake, unlike the conditions under which OSA occurs. Second, the parameters such as the amount of tongue protrusion and mouth opening, and the shape of the protruded tongue were not strictly defined for each subject when the OAs were fabricated. Third, tractography was performed by one rater and the intra-rater reproducibility was not accessed. Further studies with OSA patients who have had successful or unsuccessful OA treatment, and a larger sample size, are needed to fully evaluate effectiveness of the tongue response to OAs.

In conclusion, GG fiber orientation changes w/ OA were visualized in vivo with the noninvasive and non-radiative DTI technique. The DTI has the potential to reveal the deformation of the myoarchitecture of the tongue muscles in vivo. This study showed that DTI may be applied to OSA research to help us understand the fiber deformation such as compression, expansion and translation in tongue muscles in OSA patients in vivo, since it is possible to clarify the changes in GG muscle fiber orientation due to OAs.

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REFERENCES


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