DURING NORMAL DEGLUTITION the contraction of lingual muscles confers signature deformative changes on the tongue, which first contain and configure the bolus in a groove-like depression in the posterior portion of the tongue and then propel the configured bolus retrograde into the oropharynx (13, 15). The mechanical basis of deglutitive lingual deformation is not well understood largely because of the following issues. 1) The tongue consists of an intricate three-dimensional network of interwoven muscle fibers (14), and, consequently, it is difficult to associate regional movements of the tongue with the activity of specific muscles or muscle groups. 2) Because the tongue is effectively incompressible, tongue motion incorporates deformation in regions distinct from regions of active contraction (30, 33, 35). Accounting for the above, the development of a mechanical model of deglutitive tongue function should derive from a knowledge of region-specific muscle fiber organization. Although conventional histology provides information regarding the organization of fibers within a two-dimensional plane of section, this approach is unable to portray three-dimensional relationships among fibers.

To determine muscle fiber direction and distribution in the tongue, we have utilized a noninvasive NMR imaging technique, diffusion tensor imaging, that derives information regarding tissue myoarchitecture noninvasively through the measurement of direction-specific water diffusion (1–3, 12, 17, 18, 22, 23). With the use of this technique, the magnitude and direction of water diffusion within a discrete region of tissue are indicative of local structural orientation, i.e., the directionality of muscle fibers. Diffusion will be greatest in the direction parallel to fibers (least resistance to molecular movement) and least in the direction perpendicular to fibers (greatest resistance to molecular movement). Directionality of diffusion in fibrous tissues is dependent on the spatial disposition of macromolecular barriers such as cytoskeletal fibers or membranes. If there are no macromolecular barriers to affect water movement, molecular motion is equal in all directions (anisotropic condition). In contrast, when water diffusion is modified by the densities and orientations of macromolecular barriers, as is the case in muscular tissue, molecular motion is not equal in all directions (anisotropic condition). The latter condition therefore provides a rationale for the use of diffusion-weighted NMR imaging to assess local fiber direction for the skeletal muscle of the tongue.

In this study, we have used diffusion tensor imaging to assess the three-dimensional myoarchitecture of the excised sheep tongue. These data have provided new information regarding the structural relationships existing among lingual muscle fibers, which should allow for the development of improved models relating lingual structure with mechanical function.

METHODS

Preparation of tissue specimens. Magnetic resonance imaging (MRI) was performed on four ex vivo sheep tongues obtained from Blood Farms (West Groton, MA). The excision was performed by making an incision from the thyroid prominence to the angle of the mandible for exposure of the...
tongue and en bloc resection. The tongue specimens were rinsed in Halfirin solution and then placed in a bath of the same solution in a rubber container. The tongue was mounted in a manner to ensure that sagittal views of fiber orientation would be recovered. The container was sealed and covered with paraffin to minimize leakage of bathing solution during imaging.

Magnetic resonance imaging. We used diffusion tensor imaging to create discrete three-dimensional representations of tongue myoarchitecture in excised specimens. MRI was performed on a 1.5-T General Electric magnetic resonance (MR) scanner retrofitted for echo-planar imaging (Advanced NMR, Burlington, MA). With the use of diffusion tensor MRI, lingual fiber orientation can be identified as the direction of maximum diffusion mobility of water molecules for a given location within a tissue (2, 12, 22, 23). Direction-resolved measurement of water diffusion makes use of the relationship between MRI signal attenuation and the intensity, direction, and duration of an applied diffusion-encoding gradient (17, 31). Diffusion encoding is a spatial modulation of the proton spin phase (q) as a column vector with units of radians/cm) that persists for a duration Δ. Such an encoding causes the MRI signal of a chemical species to undergo a diffusion-dependent attenuation from an initial value (S0) to a lower value (Si) according to

\[ \log S_i/S_0 = -q^T D q \]  

(1)

where D is the diffusion tensor. From a mathematical perspective, D is a 3 × 3 matrix at each location that is symmetric, so that its nine entries only six are independent. From a physical perspective, D defines a three-dimensional ellipsoid of water diffusion at each location, for which the radius in any direction is proportional to the square of the diffusion rate in that direction. The longest axis of this ellipsoid, the direction of maximum water diffusivity, indicates the local fiber orientation to the midsagittal plane. These angles were color coded on a continuous scale, 0–90°, with 0° representing those fibers in the sagittal imaging plane and 90° representing those fibers perpendicular to the sagittal imaging plane. The angular accuracy for the principal orientations of these fibers, based on the inherent signal-to-noise ratio of the instrument and the observed strength of anisotropy, was estimated to be ±7.5°.

Tissue histology. Whole tongue tissue was sliced in a sagittal orientation and viewed using hematoxylin and eosin stains to depict myoarchitectural features. Sagittal slices were obtained from three tongues from a midline position to approximate the location of the NMR image slice. Sagittal images were viewed as a whole midline section at a low magnification (2-fold) or in regional segments at high magnification (4-fold). For the higher magnification images, photomicrographs were obtained from 10 square segments (2 rows of 5) that divided the sagittal section of tissue.

RESULTS

To display the three-dimensional organization of lingual muscle fibers, diffusion-weighted NMR data were obtained in contiguous sagittal slices through the excised sheep tongue. Local muscle fiber orientation was represented as a three-dimensional diffusion tensor, which was displayed graphically as an octahedron resolved to the level of the individual voxel.

Diffusion tensor data derived from the tongue, as shown in Fig. 1A, depicted the principal orientation of lingual fibers in the midsagittal plane. Two distinct families of muscle fibers were identified, those fibers aligned parallel to the sagittal imaging plane and those that are aligned perpendicular to the sagittal imaging plane. The fibers parallel to the sagittal plane originated in the posterosuperior region of the tongue and radiated anteriorly and superiorly with a fanlike projection (corresponding to the genioglossus muscle), ultimately merging with vertically oriented intrinsic fibers near the superior surface of the tongue. Fibers in the posterosuperior region (corresponding to the base of the genioglossus) displayed a highly uniform and parallel alignment, becoming less uniform in regions close to the superior surface of the tongue. Cross-plane fibers, on the other hand, were located predominantly in the anterior and superior regions of the tongue and were positioned at right angles to the vertically oriented fibers (corresponding to the transver and vertically aligned core intrinsic muscles). Laterally situated extrinsic lingual muscles were not identified, owing to the method of tissue dissection and the midsagittal location of the image slice.

Muscle fiber angles relative to the sagittal imaging plane are shown in Fig. 1B. To achieve these data, the fiber angle associated with each diffusion tensor was color coded, with brown equivalent to an angle of 0° (in

...
plane), blue equivalent to an angle of 90° (cross plane), and green/light blue equivalent to angles intermediate between 0 and 90°. This resulted in a map of lingual fiber orientation in the midline, which consisted of in-plane fibers (0° relative to the sagittal) radiating from the interoposterior base of the tongue or vertically oriented, and cross-plane fibers (60–90° relative to the sagittal) located in the superior and anterior tongue. There was, additionally, an interface region of intermediate fiber orientation (~45° relative to the sagittal) at the intersection between the in-plane and cross-plane fibers.

To quantify the structural differences in the postero-inferior (base of genioglossus) and anterosuperior (core intrinsic) regions, we compared (Table 1) directional water diffusion for each of the three principal directions of the diffusion tensor as a function of tissue location and depicted these values, in descending order of magnitude, as $\lambda_1$, $\lambda_2$, and $\lambda_3$ (eigenvalues). The direction corresponding to the largest magnitude of water diffusion ($\lambda_1$) was indicative of fiber direction, and the directions corresponding to the lesser magnitudes of diffusion ($\lambda_2$ and $\lambda_3$) were indicative of the shape of the diffusion tensor. The extent to which the largest diffusiv-
Regional comparison of principal diffusivities in midline plane of tongue

<table>
<thead>
<tr>
<th>Tongue Region</th>
<th>Directional Water Diffusion</th>
<th>Mean ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_1$</td>
<td>$\lambda_2$</td>
</tr>
<tr>
<td>Base genioglossus</td>
<td>1.19 ± 0.05</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>Core intrinsic</td>
<td>0.95 ± 0.06</td>
<td>0.66 ± 0.05</td>
</tr>
</tbody>
</table>

Values (in 10^{-3} mm²/s) are means ± 95% confidence interval; $n = 20$ voxels for each value. Mean apparent diffusion coefficient (ADC) represents $(\lambda_1 + \lambda_2 + \lambda_3)/3$.

DISCUSSION

The containment and propulsion of the ingested bolus by the tongue during swallowing is optimally described as a sequence of muscular tissue deformations. Previous investigations regarding the mechanics of lingual deformation have been limited by factors associated with the complex anatomy and material attributes of the tissue. In a structurally heterogeneous tissue such as the tongue, mechanical assessment requires knowledge of regional fiber properties, such as location, orientation, and contractility. In an effort to more completely define the structural organization of the tongue, we have utilized an NMR method to characterize the three-dimensional architecture of the muscle fibers present in the intact tissue.

Classical anatomic studies of the tongue emphasize the role of discrete muscle groups active during swallowing, each possessing linkage with other muscles, cartilage, or bone. Much of the bulk of the tongue is made up of fibers of the genioglossus, which is a fanlike muscle that originates at the superior mental spine and widens as it extends backward into the tongue. Superior fibers of the genioglossus pass to the tip of the tongue, middle fibers pass to the dorsum of the tongue, and inferior fibers attach to the hyoid. The fibers of the genioglossus integrate with the network of intrinsic muscle fibers, which are believed to contribute primarily to the dynamic reconfiguration of the tongue during deglutition. Also important in regulating lingual configuration during swallowing are the styloglossus, originating from the styloid process and inserting into the sides of the tongue and responsible for drawing the tongue upward and backward, the hyoglossus, originating from the upper border of the hyoid and inserting into the body of the tongue and responsible for the depression of the tongue base, and the palatoglossus, originating from the palatine aponeurosis and inserting into the body of the tongue and responsible for the retraction of the tongue upward and backward.

Measurements of molecular diffusion within a tissue can be used experimentally to derive information about its microstructure, i.e., myoarchitecture. The underlying basis for these measurements in tissues constituted of anisotropic cells, such as muscle, is that fiber orientation may be inferred from the direction of principal diffusivity. With this approach, a spatially oriented gradient pulse is delivered and molecular motion is derived from the extent of signal attenuation, from which a diffusion coefficient is calculated (17, 18, 31). The extent of water diffusion in a given orientation is related to the location and characteristics of the macro-molecular barriers present, such as membranes or cytoskeletal proteins (4, 9). In our study, we have portrayed local three-dimensional diffusion data as a diffusion tensor. The diffusion tensor was depicted graphically at each location as an octahedron whose principal axis constituted the direction of maximum water diffusivity and whose minor axes reflected the extent of diffusion anisotropy. Such an approach has previously been applied to study the structure of ex-
cised and living myocardium (11, 12, 28), brain (5, 10, 22, 23), skeletal muscle (6), kidney (25), and the ocular lens (34) and may also have implications regarding the integrity of intracellular membranes and membrane transport during pathological conditions (24, 32).

We have used diffusion tensor NMR to probe the muscular architecture of the tongue in the midline sagittal plane. We defined two broad populations of muscle fibers.

1. Sagittal in-plane fibers, originating in the posteroinferior portion of the tongue, spread in a fanlike projection to the anterior and superior region (consistent with the genioglossus) and merge anatomically with vertically oriented in-plane fibers (consistent with the vertically oriented intrinsic muscles). From the perspective of fiber angle, these fibers were most uniform and parallel in the inferior portion of the tongue (base of the genioglossus) and least uniform in the superior and anterior regions (periphery of the genioglossus). 2) Axially oriented fibers, cross-plane to the sagittal fibers, were localized predominantly in the anterior and superior regions of the tongue (consistent with the core intrinsic fibers of the tongue). We additionally defined a region of the tongue at which the muscle fibers from the intrinsic and extrinsic muscles merged to comprise an interface zone of intermediate fiber angle. This was evidenced by the gradual change from uniformly radiating fibers at the posteroinferior region to nonuniform and orthogonally oriented fibers at the superior and anterior regions of the tongue. The architectural distinction between the fibers at the base of the genioglossus and those in the core intrinsic muscles was shown quantitatively as differences of diffusion for the principal directions of the diffusion tensor, i.e., the extent of anisotropy. These data demonstrated that the fibers located in the base of the genioglossus (parallel alignment) displayed higher diffusion anisotropy than
those in the core intrinsic region (orthogonal alignment). The fibers that formed the interface between these zones displayed an intermediate degree of diffusion anisotropy, reflecting a graded change of net fiber alignment between these regions.

An essential criterion for defining structure-function relationships in the tongue is a knowledge of the tissue’s three-dimensional geometry. As shown in the current study, diffusion tensor imaging depicts the geometric properties of lingual muscle fibers in terms of the direction of greatest diffusion (i.e., principal fiber direction) and the degree of fiber alignment (i.e., diffusion anisotropy). Because the fibers associated with specific muscles are extensively interwoven (Fig. 2), this approach provides a method for characterizing the architectural differences between regions of the tissue at the resolution of the individual voxel. Although the mechanical relationship between muscle fiber architecture and contractility is not known for the tongue, we hypothesize from our data that diffusion tensor imaging, through its representation of local fiber orientation and anisotropy, constitutes a basis to predict local contractile activity.

Several potential image artifacts must be considered in the interpretation of the diffusion tensor images (for review, see Ref. 7); these include system factors such as gradient calibration, directional accuracy, eddy currents, imperfections of system and hardware instability, and microscopic intravoxel effects caused by biophysical tissue properties. The latter may include partial volume effects and compartmentation of molecular diffusion and may be particularly important in the tongue given the complexity of its anatomy. The coexistence of different cell types and orientations comprising a voxel could result in partial volume effects that would result in an underestimate of the degree of anisotropy in that region of the tissue. Although partial volume effects are not necessarily regarded as a compromise of accuracy, such effects should be considered in the interpretation of individual diffusion tensor attributes within structurally heterogeneous tissues.

The in vivo application of these techniques may be instrumental in determining the mechanical role of local fiber bundles in the production of regional tongue contraction. This application will necessitate a technical solution to the effects of local deformation on MR signal characteristics. Principals of continuum mechanics and MR physics predict that gross movement of a segment of tissue may obscure other relevant molecular diffusions. Consequently, the recovery of fiber orientation during lingual motion will require methods to accurately gate MR acquisition and to cancel the effect of gross motion on local diffusion. It may be feasible to suppress the principal effects of motion on the NMR signal by adjusting the measurement periods relative to the motion cycle, as well as factoring in the effect of local deformation on NMR spin properties (28).

In summary, we have assessed the myoarchitectural patterns of lingual muscle fibers in an ex vivo specimen based on the orientation-dependent differences of water diffusion. These studies depict the patterns of fiber organization in the whole tissue and provide insight into the basic structural and functional relationships existing among the muscle fibers that constitute the lingual musculature.

REFERENCES


