# Dependence on Diffusion Time of Apparent Diffusion Tensor of Ex Vivo Calf Tongue and Heart

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## Introduction

Diffusion time dependency of water diffusion is related to the physical size of the restricting compartment. In the brain, the diffusion time dependency can be observed with very short diffusion time (< 6 ms) which is difficult to implement in conventional clinical MR scanners [1]. Since muscle fibers are generally larger than neuronal axons, it is expected that diffusion time dependency can be observed with longer diffusion time in such tissue. The purposes of this study are to investigate restricted diffusion of water in muscle tissues with diffusion times achievable in conventional clinical scanners and to evaluate a novel analysis framework for correlating the measured diffusion time dependency to morphological factors such as fiber size and volume fraction.

## Method

The experiments were performed on four excised calf tongues and four excised calf hearts within 24 h of harvest. The specimens were scanned with a standard quadrature head coil in a 1.5T GE Signa MR scanner (GE Medical Systems, Milwaukee, WI) equipped with a whole-body gradient coil producing gradient pulses up to 50 mT/m. A custom diffusion-sensitive stimulated-echo pulse sequence was used with 8-shot echo-planar spatial encoding (resolution: 2.03 × 2.03 × 2 mm$^3$, TR=3.0s, diffusion gradient amplitude = 44.5 mT/m, diffusion gradient directions: [1, 1, 0], [1, 0, 1], [0, 1, 1], [1, -1, 0], [1, 0, -1], and [0, 1, -1]). The diffusion time was varied by changing the mixing time from 10 ms to 800 ms, resulting in diffusion times ranging from 32 ms to 810 ms, while the trace of the b-matrix was kept constant at about 1400 s/mm$^2$ by varying the width of the diffusion weighting gradients from 14.4 ms to 2.4 ms (TE = 47.9 ms to 23.9 ms). To improve S/N, we repeated each acquisition 8 times. Ten coronal images were acquired for the tongue specimens and four short-axis images for the heart.

Van Gelderen *et al* [2] have derived the following expression for the attenuation of an MR signal in a Stejskal-Tanner type experiment due to the diffusion of spins confined to the interior of an infinite cylinder:

$$\ln\left(\frac{A_\perp}{A_0}\right) = -2\gamma^2 g^2 \sum_{m=1}^{\infty} \frac{2D_i\alpha_m^2\delta - 2 + 2\exp\left(-D_i\alpha_m^2\delta\right) + 2\exp\left(-D_i\alpha_m^2\Delta\right) - \exp\left(-D_i\alpha_m^2(\Delta-\delta)\right) - \exp\left(-D_i\alpha_m^2(\Delta+\delta)\right)}{D_i^2\alpha_m^6\left(R^2\alpha_m^2 - 1\right)} = -F_{g,\delta,\Delta}(D_i, R) \quad [1]$$

where $A_\perp$ is the signal measured with diffusion weighting gradient perpendicular to the cylinder axis, $A_o$ the signal without diffusion weighting, $\gamma$ gyromagnetic ratio, $D_i$ the intracellular diffusion coefficient, $R$ the radius of the cylinder, $\delta$ gradient pulse duration, $\Delta$ gradient pulse separation, $g$ the gradient pulse amplitude, and $\alpha_m$ the $m$th root of the equation $J_1'(\alpha_m R) = 0$ ($J_1'$ = derivative of the Bessel function of the first kind and order one). While the above model appears adequate to describe the restricted diffusion in the intracellular compartment, it does not include the diffusion in the extracellular compartment and the exchange though the permeable membrane. To include the extracellular spins in our model, we propose that the MR signal is the sum of the signal from restricted spins described by Eq.[1] and non-restricted spins described by the Stejskal-Tanner formula:

$$A_\perp/A_0 = \rho\exp\left(-F_{g,\delta,\Delta}(D_i,R)\right) + (1-\rho)\exp\left(-\gamma^2 g^2\delta^2(\Delta-\delta/3)D_n\right) = \rho\exp\left(-F_{g,\delta,\Delta}(D_i,R)\right) + (1-\rho)\exp\left(-bD_n\right) \quad [2]$$

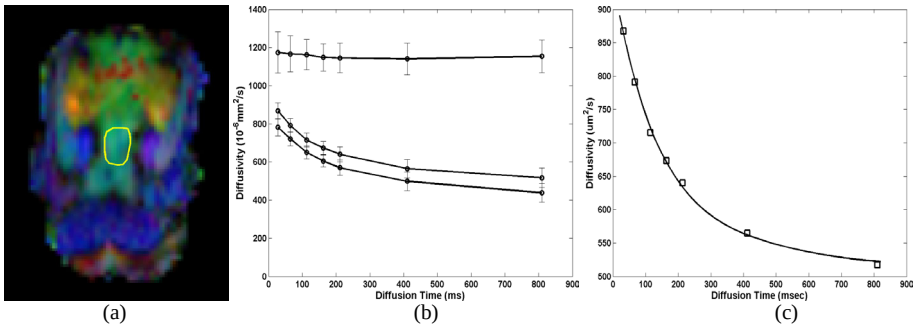
where $\rho$ is the volume fraction of the intracellular compartment and $D_n$ the non-restricted diffusion in the extracellular space. Although it is difficult to measure $A_\perp$ directly for individual muscles and muscle groups with different orientations, it is straight forward to formally compute $D_\perp$, the apparent diffusion constant perpendicular to the cylinder axis, from the data by diagonalizing the diffusion tensor. The above model was fitted to the diffusion tensor eigenvalues showing diffusion time dependency.

## Results & Discussions

Our results show evidence of restricted diffusion in both specimen types. Fig.1 shows an example of tongue muscle. In regions where the myofibers are parallel, the primary eigenvalue of the diffusion tensor remains the same for all diffusion times ($\tau_d$) measured, while the other eigenvalues decrease by 29%-36% between $\tau_d$ = 32 ms and $\tau_d$ = 400 ms. In regions where the fibers cross, the $\lambda_1$ also changes, decreasing by 17% between $\tau_d$ = 32 ms and $\tau_d$ = 400 ms. The restricting compartment size and volume fraction were estimated by fitting the time courses of the eigenvalues with diffusion-time dependency to the proposed model consisting of non-restricted and cylindrically restricted compartments (Table 1). The results are in good agreement with the literature [3-5].

We successfully performed several checks in our experiments and confirmed that the diffusion time dependency detected was not a spurious finding caused by systematic errors in the STEAM sequence, temperature changes during the long scan, unaccounted contributions of imaging gradients to diffusion sensitization, effects of variable echo time, or failure of the tensor model to fit the data.

To our knowledge, the present study represents the first to substantiate the selective dependence of water diffusivity on diffusion time in *ex vivo* calf tongue and heart muscle tissues. With improvement in imaging technology, future studies may permit non-invasive *in vivo* detection of changes in the biomechanical properties of muscles due to disease progression, drug effect, and exercise.




(a) (b) (c)

**Fig.1**. (a) Coronal DEC map of tongue showing an ROI in genioglossus. (b) Eigenvalues of diffusion tensor depending on diffusion time. (c) Proposed model (line) fitted to the secondary eigenvalues (squares).

| | | $\rho$ | $R$(μm) | $D_i$($10^{-6}$ mm$^2$/s) | $D_n$($10^{-6}$ mm$^2$/s) |
|---|---|---|---|---|---|
| Heart | $\lambda_2$ | 0.63 ± 0.05 | 6.55 ± 0.20 | 477 ± 78 | 1110 ± 223 |
| | $\lambda_3$ | 0.67 ± 0.04 | 6.25 ± 0.18 | 497 ± 80 | 770 ± 97 |
| Genio-glossus | $\lambda_2$ | 0.42 ± 0.01 | 16.38 ± 0.15 | 1162 ± 32 | 945 ± 18 |
| | $\lambda_3$ | 0.43 ± 0.02 | 17.76 ± 0.34 | 1173 ± 71 | 772 ± 30 |
| Tongue-core | $\lambda_1$ | 0.21 ± 0.01 | 13.72 ± 0.20 | 1160 ± 46 | 1092 ± 13 |
| | $\lambda_2$ | 0.25 ± 0.04 | 15.73 ± 1.16 | 1302 ± 271 | 931 ± 85 |
| | $\lambda_3$ | 0.45 ± 0.01 | 14.65 ± 0.07 | 1030 ± 13 | 725 ± 7 |

**Table 1**. Compartment radius $R$, intracellular diffusivity $D_i$, extracellular diffusivity $D_n$, and volume fraction $\rho$ derived from fitting the eigenvalues of the diffusion tensor as a function of diffusion time with the proposed model for *ex vivo* heart, genioglossus and the tongue core.

## References

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