Noninvasive assessment and validation of rotator cuff architecture with diffusion tensor imaging

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INTRODUCTION: Skeletal muscle has a well-characterized structure-function relationship, where the arrangement of muscle fibers can predict functional capacity[1]. Skeletal muscle architecture is defined as the organization and arrangement of muscle fibers within a muscle. Architectural data, especially in humans, are used to model joint behavior and help inform surgical decisions. Key components of muscle architecture can be noninvasively estimated through an imaging technique called tractography, which is a post-processing algorithm applied to diffusion tensor magnetic resonance imaging (DT-MRI) data[2]. Several groups have utilized tractography to approximate muscle fiber length and orientation (i.e. pennation angle) in a number of muscles including leg, spine, shoulder in adult, adolescent, and infant populations[2-5]. While widely used, there remains a lack of studies exploring the accuracy of tractography based architecture measurements with the tissue that is physically being scanned, with most studies comparing tractography measurements to previously reported architecture measurements in different cohorts[6]. Two studies have directly compared tractography to measured architecture[2,7], however both were performed in the leg of animal models and not in human tissues, which can be more complex due to larger size and potential pathology. Thus, the goals of this study are to 1.) validate tractography for noninvasive assessment of muscle architecture in an intact rotator cuff against the current gold standard (dissection) in cadaveric shoulders and 2.) describe an imaging and post processing protocol using open-source analysis tools that can be used to assess muscle architecture in vivo.

METHODS: *Cadaveric Specimens:* Two formalin-fixed cadaveric human shoulders were used in this study. Both specimens were confirmed to have intact rotator cuffs from MRI and direct visualization. The samples were scanned using a 3T MRI scanner (GE, MR750, Milwaukee, MI). Fat-water separation (IDEAL) (TE=2.97msec, TR=6.01msec, 1x1x1mm^3 voxel size), and diffusion weighted imaging (TR=10sec, TE=50.5msec, b-value = 400s/mm^2, 2x2x4mm^3 voxel size, 60 diffusion directions) sequences were acquired. After scanning each sample, the supraspinatus (SS), infraspinatus (IS), and subscapularis (Sub) muscles were excised, and the fiber lengths and pennation angles were measured by dissection in the regions as previously described[8]. *Human Volunteer:* One male (24 years old) without a history of rotator cuff tear volunteered for this study. The subject was scanned on a 3T MRI scanner (Siemens Prisma, Erlangen, Germany). A fat-water separation sequence (Dixon) (TE=2.46msec, TR=5.89msec, 1x1x1mm^3 voxel size) and a diffusion weighted sequence (TR=5.06sec, TE=48msec, b-value = 500s/mm^2, 2x2x4mm^3 voxel size, 60 diffusion directions) were acquired. *Post-processing:* The SS, IS, and Sub muscles were manually segmented based on their respective fascial planes using Horos. Diffusion weighted images were denoised using a principal component analysis filter[9] and the DT was calculated using AFNI[10]. A secondary mask was generated to exclude any voxels with a fat fraction greater than 25% from inclusion in tractography[11]. Tractography was performed using Diffusion Toolkit (Propagation algorithm: FACT, angle threshold: 25°, spline filter). Using the ROI tool in TracVis, regional measurements of muscle fiber length and pennation angle were made for each muscle (Figure 1d,e). *Statistical Analysis:* To compare the agreement between physically measured and tractography measured muscle architecture, an intra-class correlation coefficient (2,1) (ICC) was calculated.

RESULTS: Excellent agreement between architecture measured and tractography calculated fiber length (ICC = 0.890) and pennation angle (ICC = 0.986) was found for the cadaveric shoulder muscles (Figure 2). Tractography was generally more accurate for calculating smaller fiber lengths than larger ones, potentially due to exclusion of voxels for exceeding the fat fraction threshold. For in vivo tractography, excellent maps of the rotator cuff muscles were produced, clearly demonstrating the length and orientation of muscle fiber tracts in a pattern consistent with what is observed *in vivo* (Figure 1a-c). Furthermore, tractography calculated fiber lengths and pennation angles from human subjects were similar to those found in dissection from the cadavers (Table 1).

DISCUSSION: In this preliminary study, we demonstrate initial agreement between muscle fiber length and pennation angle calculated with a noninvasive imaging technique (DT-MRI with tractography) and dissection-based measurements of muscle architecture measured in cadaveric shoulders. In addition, this study suggests that this technique can be applied to living human tissue to accurately measure shoulder architecture *in vivo*. These initial experiments were performed in shoulders with intact rotator cuffs to minimize the likelihood of muscle degeneration and fatty infiltration, both of which can affect tractography measurements. Future development of this study will include additional cadaveric shoulders with and without the presence of rotator cuff tear, which can result in increased pennation angles and decreased fiber lengths, altering the native functional capacity of the rotator cuff.

SIGNIFICANCE/CLINICAL RELEVANCE: This study describes a noninvasive imaging technique and analysis approach to calculate key features of muscle architecture in the human shoulder. Understanding shoulder muscle architecture in the context of healthy and pathologic rotator cuffs has implications for diagnosis, guiding treatment decisions, tailoring rehabilitation strategies, and predicting functional outcomes.

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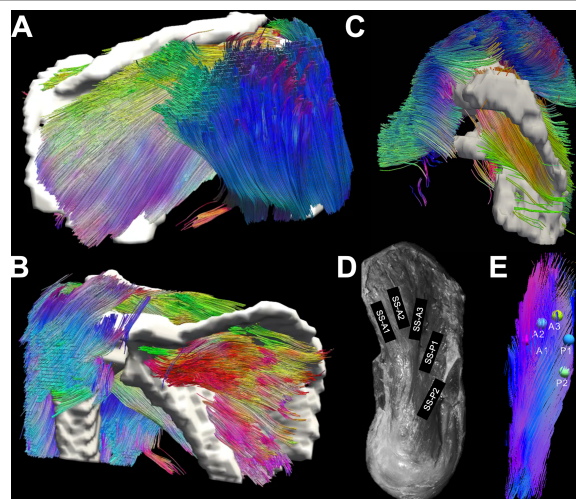
Figure 1. 3D tractography reconstruction of a human shoulder A) Posterior-Anterior. B) Anterior-Posterior. C) Superior-Inferior. D) Example of fiber length and pennation angle regions in SS from Ward et al . E) Tractography of SS with regions identified.
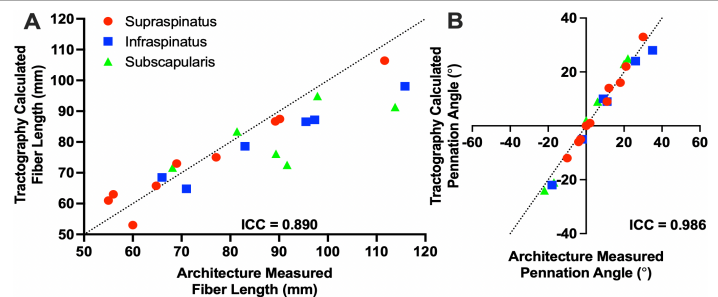
Figure 2. Agreement between architecture and tractography for A) fiber length and B) pennation angle. Dotted line indicates perfect agreement.

Table 1. Ranges of tractography measured architecture in cadavers and approximated from tractography in a live volunteer

	Fiber Length (mm)			Pennation Angle (°)		
	SS	IS	Sub	SS	IS	Sub
Cadavers	55-112	66-116	68-114	-10-30	-18-35	-22-22
In Vivo	53-93	79-98	40-44	-8-13	-13-18	-14-19