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Research Article

Assessment of whether the rabbit subscapularis tendon model is suitable for studying the human chronic rotator cuff pathology: Discovery of a new ligament connecting the glenoid and subscapularis tendon

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ABSTRACT

Objective: This study aimed to investigate the anatomical relationship between the subscapularis tendon and glenosubscapularal ligament (GSL) that we accidentally identified from our previous study on a rabbit shoulder model and to determine whether this anatomical relationship has an impact on the rabbit shoulder model for studying the human chronic rotator cuff pathology.

Methods: In this study, 15 male New Zealand rabbits aged 12 weeks and weighing 2.5 kg were used. Moreover, 3 rabbits were sacrificed for the anatomical and histological investigation of the relationship between the subscapularis tendon and GSL at baseline. The remaining 12 rabbits underwent the subscapularis tendon tenotomy from the lesser tuberosity using a standardized procedure. The GSL was cut on the left side and preserved on the right side. For histomorphometric analysis, 6 rabbits were first sacrificed at 6 weeks and then the remaining 6 rabbits at 12 weeks.

Results: In all the rabbits, GSL was identified, connecting the upper portion of glenoid and subscapularis muscle–tendon junction. The mean thickness of the middle portion of GSL was 1.1±0.2 mm; the mean length of GSL was 8.4±2.3 mm. The mean widths of the proximal and distal attachments were 2.4±0.3 and 4.2±0.5 mm, respectively. The mean size of the native subscapularis muscle fibers was 122.6±4.3 μm^2. The mean size of the muscle fibers in shoulders with tenotomy alone was 112.6±6.2 and 102.6±4.8 μm^2 at 6 and 12 weeks, respectively. The mean size of the muscle fibers in shoulders with tenotomy plus GSL cut severing was 88.3±9.7 and 56.4±5.2 μm^2 at 6 and 12 weeks, respectively. The significant muscle atrophy was observed both at 6 and 12 weeks in the shoulders with tenotomy plus GSL cut compared with those with tenotomy alone as well as those with the native subscapularis. However, the muscle atrophy was not significantly different in the shoulders with tenotomy alone at different time points.

Conclusion: Because GSL may prevent the subscapularis retraction, the rabbit subscapularis tendon model may not be suitable for studying the human chronic rotator cuff pathology if GSL is neglected or preserved.

Introduction

A rotator cuff tendon provides dynamic stability to the glenohumeral joint (1, 2). However, rotator cuff disease is a common musculoskeletal disorder with an incidence of 22.1% in the general population, and its prevalence increases with age (3). Several approaches are developed to treat rotator cuff tear, but the rate of failure is still estimated to be 70% (4, 5). Thus, rotator cuff repair remains a great challenge for orthopedic surgeons. To enhance the healing of the rotator cuff in humans, numerous authors focused on rotator cuff healing. Different kinds of animal models are developed because of the limitations in evaluating biological healing in humans to simulate the rotator cuff tear in humans and to enhance the understanding on the pathological changes in rotator cuff tear (5-11).

A rabbit model is mostly used to study the muscular changes associated with rotator cuff tears (5,12).The supraspinatus and infraspinatus tendons have been used as repair models of rabbits possibly because their tendon healing fashion is similar to humans and the size of rotator cuff tendon and footprint is reasonable for repair performance (5, 12, 13). However, other studies have suggested that the subscapularis may be a better option to mimic human pathology because it passes under an enclosed arch, fatty accumulation is prominent, and biomechanical characteristics are similar to those of the supraspinatus of humans (5, 6, 14). Thus, many studies have used the subscapularis tendon of rabbits to create a rotator cuff tear model (15-17). Considering some anatomic studies on the subscapularis of rabbits (6, 13, 14), theoretically, we recommend the subscapularis tendon as a better option, but knowledge about the subscapularis of rabbits to mimic the chronic rotator cuff tear model of humans is limited.

In our previous rabbit experiment, we accidentally found a ligament connecting the glenoid and the subscapularis tendon (the glenosubscapularal ligament [GSL]), which has not been reported in other studies. We thought that the structure can limit the subscapulris retraction after tear, possibly affecting the formation of a chronic rotator cuff tear model. In this study, we evaluated the anatomy of the subscapularis and

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the GSL from the view of creating a chronic rotator cuff tear to simulate the supraspinatus tendon tear in humans. We hypothesized that the subscapularis tendon of rabbits could not mimic the chronic rotator cuff tear in humans because the GSL restricted muscle retraction by pulling the middle portion of the subscapularis muscle-tendon junction.

Materials and Methods

Experimental design

In total, 15 male New Zealand rabbits (aged 12 weeks and weighing 2.5 kg) were used. This study was approved by the Institutional Animal Care and Use Committee and carried out in strict accordance with its regulations. Furthermore, 3 rabbits were sacrificed for the gross observation of the bilateral subscapularis tendon and its insertion, the GSL, and the histological morphology of the native subscapularis. In addition, 12 more rabbits had their subscapularis tendons detached from the insertion on the less tuberosity on the right side and their subscapularis tendons detached and their GSL severed on the left side; 6 rabbits each were sacrificed for histological morphology examination at 6 and 12 weeks.

One fresh human cadaveric shoulder was dissected to study and compare the anatomy of the subscapularis tendon and glenoid with the anatomy of the rabbit.

Surgical technique

A total of 12 rabbits had subscapularis tendon tenotomy from the less tuberosity. Anesthesia was induced by intramuscularly injecting 50 mg/kg zolazepam and tiletamine (Zoletil 50; Virbac, Carros, France) and 10 mg/kg xylazine (Rompun; Bayer HealthCare, Leverkusen, Germany). A skin incision was made over the anterior deltoid muscle, and the deltopectoral approach was used to expose the insertion of the subscapularis tendon on the less tuberosity. The subscapularis tendons from both the sides were then transected from the insertion and warped with a Penrose drain to prevent spontaneous healing (17). The GSL was cut on the left side and preserved on the right side at the same time. A 3-0 absorbable suture (Vicryl; Ethicon, Somerville, NJ, USA) was used to close the fascia and muscle septa, and a 3–0 Prolene (Ethicon, Somerville, NJ, USA) was used for skin closure.

After surgery, all the rabbits were housed individually at room temperature under a 12-h light/dark cycle. They were allowed to move freely within their cages until they were sacrificed 6 and 12 weeks after the operation.

Gross observation and measurement of the ligament between the glenoid and subscapularis tendon of rabbits

After being sacrificed, 3 rabbits, including 6 shoulders, were dissected, and the subscapularis was exposed while carefully protecting the GSL. After observation, the ligament was harvested for measurement with a digital caliper.

HIGHLIGHTS

- The glenosubscapular ligament connects the glenoid and subscapularis tendon.
- This ligament can limit the subscapulris retraction after tear, affecting the formation of a chronic rotator cuff tear model.

Histomorphometric analysis

Immediately after the rabbits were sacrificed, the subscapularis muscles, 1.5 cm proximal to the tendon insertion of both shoulders of all the rabbits, were harvested. The samples were fixed in 10% neutral buffered formalin for 24 h, processed, and embedded in paraffin. Sagittal 3-μm-thick sections of the subscapularis muscle were placed on glass slides and stained with hematoxylin and eosin. Images of the muscle histology were obtained using an inverted microscope (Nikon TS100; Nikon, Melville, NY, USA). The cross-sectional area of the muscle fiber was outlined and measured by 2 blinded investigators using 3 randomized views of each slide using ImageJ (NIH, Bethesda, MD, USA) under 200x magnification.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences statistical software version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). Data were reported as mean±standard deviation. Histological comparisons were conducted using the student's *t* test between 2 groups, and one-way analysis of variance was used for histological intragroup comparison at 3 time points. Statistical significance was set at $p<0.05$.

Results

Gross observation of the GSL

Occurrence rate

The GSL connecting the upper portion of the glenoid and the subscapularis muscle-tendon junction was found in all the shoulders of

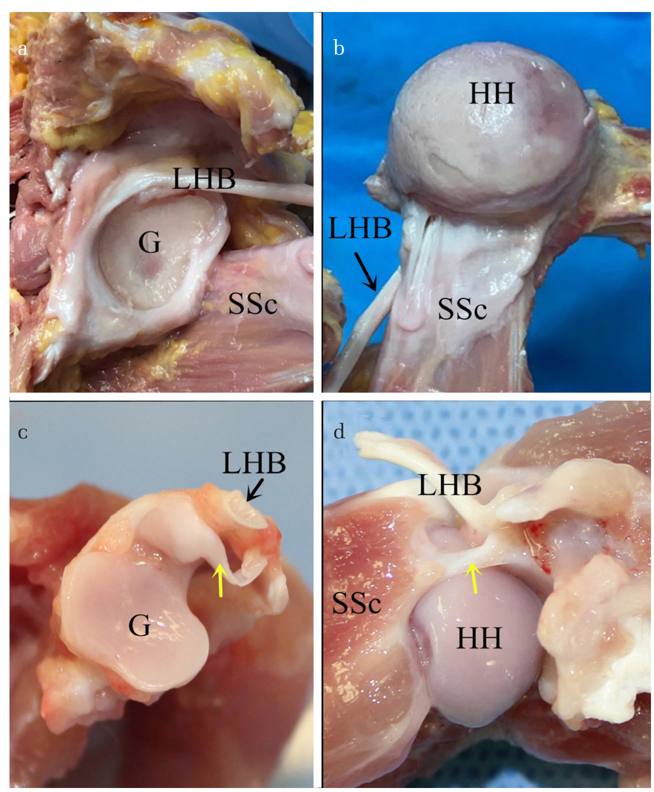

Figure 1. a-d. Comparison of the shoulder anatomy between humans and rabbits. (a) Glenoid of the shoulder of humans. (b) Humeral head and subscapularis tendon of the shoulder of humans. (c) Glenoid of the shoulder of rabbits; the glenosubscapularal ligament (GSL) originates under the insertion of the long head of the biceps. (d) Humeral head and subscapularis of the shoulder of rabbits; the GSL connects the glenoid and the middle portion of the subscapularis tendon
G: glenoid; LHB: long head of biceps tendon; SSc: subscapularis. The yellow arrow points out the GSL

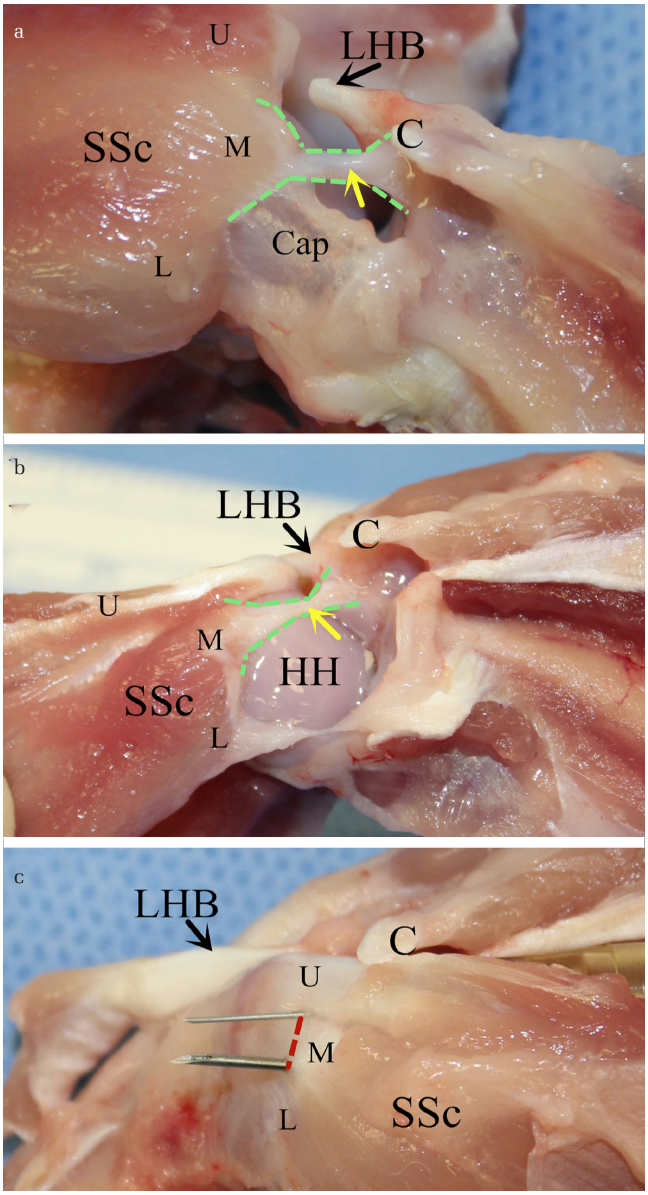

Figure 2. a-c. Gross observation of the glenosubscapularal ligament (GSL). (a) Relationship between the GSL and the anterior capsule. (b) After the capsule was removed, a clearer insertion and relationship with the capsule were observed. (c) The insertion margin was marked with a needle from the articular side and observed from the opposite side. The insertion was clearly marked, and the subscapularis tendon was divided into 3 parts: upper, middle, and lower parts
LHB: long head of biceps tendon; SSc: subscapularis; HH: humeral head; C: coracoid; U: upper portion of the subscapularis tendon; M: middle portion of the subscapularis tendon; L: lower portion of the subscapularis tendon. The yellow arrow points out the GSL. The double green lines depict the shape of the GSL

the 15 rabbits. During our dissection on the human cadaveric shoulder, we did not find the similar ligament connecting the glenoid and the subscapularis tendon (Figure 1).

Shape, exact location, relationship with the anterior capsule, and effect on the anatomy of the subscapularis

The GSL presents a funnel shape having 2 attachments. The mean thickness in the middle substance and the mean length of the GSL were 1.1±0.2 and 8.4±2.3 mm, respectively. The mean widths of the proximal and distal attachments were 2.4±0.3 and 4.2±0.5 mm, respectively. It originated from the upper portion of the glenoid under the insertion of the long head of the biceps, extended anteriorly, and widely inserted at the middle portion of the subscapularis tendon from the articular side (Figure 1, 2). The proximal attachment was on the glenoid side, and the distal attachment was on the subscapularis muscle–tendon junction (Figure 2). It was widely inserted into the subscapularis muscle–tendon junction laterally and connected to the anterior capsule medially (Figure 2a). Our observation showed that the subscapularis tendon could be divided into 3 parts according to the insertion of the GSL on the subscapularis tendon: upper portion (superior to the insertion of the GSL), middle portion (insertion of the GSL), and lower portion (inferior to the insertion of the GSL; Figure 2).

Table 1. Quantitative analysis of cross-sectional area of the subscapularis muscle fiber stained by hematoxylin and eosin

Time point (weeks)	Tenotomy (μm^2)	Tenotomy plus GSL severing (μm^2)	P1
0	122.6±4.3 (native)	122.6±4.3 (native)	–
6	112.6±6.2	88.3±9.7	<0.05
12	102.6±4.8	56.4±5.2	<0.05
P2	0.93	<0.05	–

GSL: glenosubscapularal ligament. P1 shows the result of comparison between tenotomy and tenotomy plus GSL severing group at 6 and12 weeks, respectively; P2 shows the result of intragroup comparison

Functional anatomy of the GSL

Study on the cadaveric rabbit

The GSL has a static attachment on the glenoid and a dynamic attachment on the subscapularis muscle-tendon junction. Similar to a sling, the ligament plays an important role in restricting the anterior and medial movement of the subscapularis after the tendon tears from the less tuberosity. Figure 3 shows the sling effect of the GSL of the fresh cadaveric rabbit. After the subscapularis tendon was cut from the humerus, the tendon slightly retracted medially (Figure 3a). However, the GSL was under tension. The subscapularis muscle was manually retracted, but the tendon site could not be retracted proximally further from the glenoid level (Figure 3b). However, once the GSL ruptured, the subscapularis could be further retracted proximally from the glenoid level through manual retraction mimicking the muscle force (Figure 3c).

In vivo histological results of the muscle

The native size of the subscapularis muscle fiber was 122.6±4.3 μm^2. The sizes of the muscle fibers in the only tenotomy group were 112.6±6.2 and 102.6±4.8 μm^2 at 6 and 12 weeks, respectively. The sizes of the muscle fibers in the tenotomy plus severed GSL group were 88.3±9.7 and 56.4±5.2 μm^2 at 6 and 12 weeks, respectively. The sizes of the muscle fibers in the only tenotomy group were not significantly different at 0, 6, and 12 weeks after surgery (Figure 4). However, the muscle fiber in the tenotomy plus severed GSL group was significantly smaller from 0 to 12 weeks. Furthermore, the muscle fiber in the tenotomy plus severed GSL group was significantly smaller than that in the only tenotomy group at 6 and 12 weeks, respectively. Additionally, after 12 weeks, even 2 of the 6 samples with a cut GSL showed more tendon-like cells rather than pure muscles, indicating that the muscles were well retracted without restriction from the GSL (Figure 4c). The detailed statistical information is shown in Table 1.

Discussion

This study revealed a new anatomic structure, namely, the GSL, which originated from the upper portion of the glenoid, under the insertion of the long head of the biceps, and inserted into the middle portion of the subscapularis muscle–tendon junction from the articular side of rabbits. Considering that the subscapularis tendon of rabbits is a

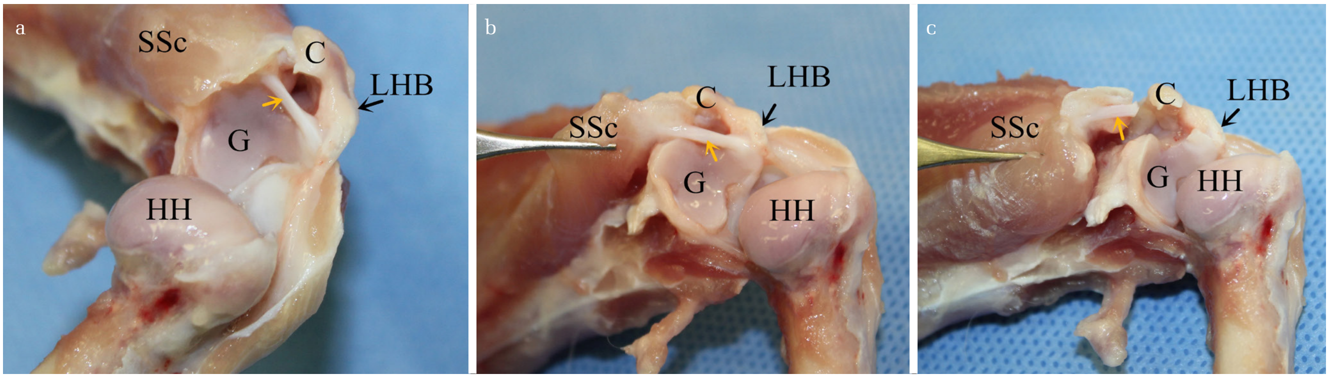

Figure 3. a-c. Functional anatomy of the glenosubscapularal ligament (GSL) in a cadaveric rabbit. (a) Subscapularis tendon tenotomy forms the humeral head. (b) The torn subscapularis tendon manually and medially retracts, and the GSL pulls the subscapularis, preventing retraction. (c) The subscapularis is well retracted manually after an additional severing on the GSL

LHB: long head of the biceps tendon; SSc: subscapularis; HH: humeral head; C: coracoid. The yellow arrow points out the GSL

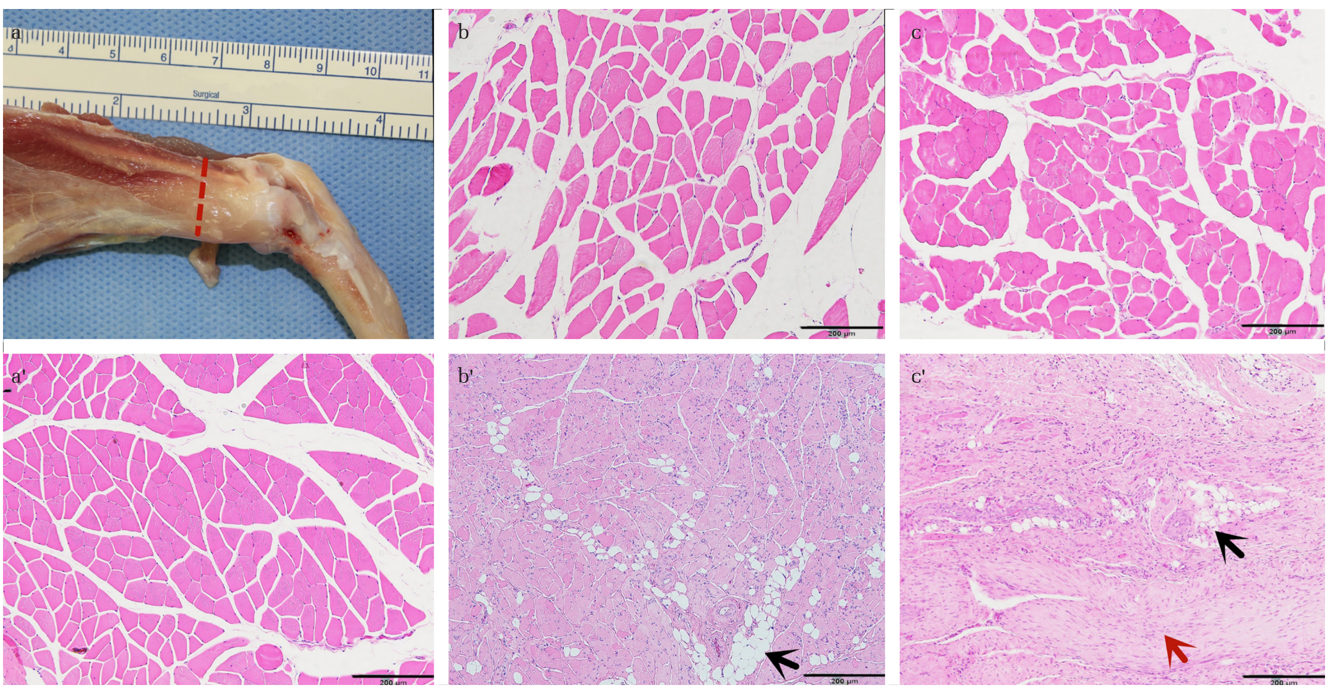

Figure 4. a-c. Representative hematoxylin and eosin staining of the muscle at each time point on both sides. (a) The figure shows our sectional location for histological evaluation, approximately 1.5 cm proximal to the lesser tuberosity. (a') Native subscapularis cross section. (b and c) The subscapularis cross section 6 and 12 weeks after subscapularis tenotomy only. The muscle fiber is similar to the native one. (b' and c') The subscapularis cross section 6 and 12 weeks after subscapularis tenotomy plus an additional severing on the glenosubscapularal ligament. Muscle atrophy and fatty infiltration are observed at 6 weeks. Muscle atrophy, fatty infiltration, and several tendon-like tissues are detected at 12 weeks, indicating that the muscle is more retracted at 12 weeks. The black arrow points out fatty infiltration. The red arrow shows tendon-like tissues

favorable option for researchers to simulate the rotator cuff pathology of humans in animal studies, we confirmed that the GSL played a sling-like function affecting the subscapularis retraction *in vitro* and even atrophy and fatty infiltration after rotator cuff tear *in vivo*. Thus, the subscapularis tendon of rabbits was not recommended to make a proper chronic rotator cuff tear model when the GSL was disregarded.

A rotator cuff tear is a very common skeletal disorder in the general population. A considerable number of patients have to receive a rotator cuff repair surgery after the conservative treatments fail. However, the retear and failure of repair are quite high, especially in chronic tear cases (3). As such, the chronic tear of rotator cuff becomes a hot issue among orthopedic surgeons. Several animal models have been created to mimic the rotator cuff pathology in humans and to enhance healing (5-11). However, creating a chronic animal model is complicated because of the differences in healing potential and anatomy (5-11). Nowadays, orthopedic surgeons and researchers value the chronic rotator cuff tear model more than an acute model. With in-depth understanding on the rotator cuff pathology, the acute model cannot meet the demand for good insights into chronic rotator cuff tear, including tendon degeneration, muscle atrophy, and fatty infiltration (5-11). Hashimoto et al. and Sevivas et al. suggested the use of a chronic rotator cuff tear model of rats (18, 19). However, a rabbit model is mostly used to study the muscular changes associated with rotator cuff tear rather than a rat model (5, 12). Rowshan et al. and Gupta et al. even proved that muscle atrophy, fatty infiltration, and healing fashion of rabbits are similar to those of humans (20, 13). Additionally, considering the economical expense and convenience for surgical manipulation, rabbits may be the suitable species for research on rotator cuff pathology (12).

Some studies have successfully used both supraspinatus and infraspinatus tendons as rotator cuff tear models of rabbits (21-23). However, recent studies have shown that the subscapularis may be a better approximation of human pathology (5, 6, 12, 14, 20). Anatomically, Grumet et al. reported that the subscapularis tendon and the scapular channel of rabbits are similar to the anatomy of supraspinatus of humans, both traveled beneath the acromion and inserted into the greater tuberosity of the humerus (14, 12). Otarodifard et al. provided additional evidence that the subscapularis of rabbits may be an appropriate model to explore rotator cuff repairs by verifying the biomechanical similarities between the different methods of rotator cuff repair in the subscapularis of rabbits compared with those in the supraspinatus of humans (6). Rowshan et al. established the subscapularis muscle of rabbits as a valid model to investigate the muscular changes associated with rotator cuff tears (20). From these aspects, the subscapularis of rabbits may be more comparable with the supraspinatus of humans. However, in this study, the GSL played an important role in preventing subscapularis retraction after subscapularis tear *in vitro* and *in vivo* by pulling the middle portion of the muscle-tendon junction of the subscapularis.

Our study further confirmed that the subscapularis would retract if the GSL was cut to make a chronic tear model, leading to muscle atrophy and fatty infiltration, which were similar to a clinical scenario of chronic rotator cuff tear. Our results revealed that the GSL was attached to the muscle-tendon junction of the middle portion of the subscapularis rather than the lesser tuberosity (Figure 3). Hence, detachment from bone-tendon junction of the subscapularis tendon to make a chronic rotator cuff tear model is not sufficient. The GSL must be further severed to create a proper model by using the subscapularis of rabbits.

No study has reported a similar ligament in between the glenoid and the subscapularis tendon of humans. The only possible similar structure is the superior glenohumeral ligament (SGHL) of humans. Both the ligaments originate from the upper portion of the glenoid and extend anterolaterally to the lesser tuberosity. However, the insertions are different in terms of the following: the insertion of the SGHL is on the lesser tuberosity of humans, whereas the insertion of the GSL is on the subscapularis tendon of rabbits. The differences in insertions causes additional severing on the GSL during the preparation of a chronic rotator tear model of rabbits by using the subscapularis tendon. Furthermore, rats are popularly used as a rotator cuff tear model, but no study has presented a similar ligament working as a GSL of rabbits. In this study, the subscapularis of rats was not dissected, but our previous experience with rat rotator cuff studies did not reveal a similar ligament. In our future studies, we will try to find a ligament with functions similar to those of the GSL in other animals and reveal the relationship with rotator cuff tear.

Several limitations should be acknowledged in the studies. First, our study focused on the gross anatomy and functional anatomy, and we did not investigate the major components of the ligament. Second, the tensions after the tear and the tear plus a severed GSL *in vivo* were not recorded, although they could directly reveal the difference.

In conclusion, The GSL plays a sling-like role in preventing subscapularis retraction during the preparation of a chronic tendon tear model. The subscapularis is not recommended for making a chronic tear model of rabbits if the GSL is neglected and preserved.

Ethics Committee Approval: Ethics committee approval was received for this study from the Animal Experimental Ethical Inspection of Gansu University of Chinese Medicine.

Informed Consent: N/A.

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