Review Article

# Evaluation of animal models and methods for assessing shoulder function after rotator cuff tear: A systematic review

Yang Liu [a], Sai C. Fu [a,b], Hio T. Leong [a], Samuel Ka-Kin Ling [a], Joo H. Oh [c],
Patrick Shu-Hang Yung [a,*]

[a] Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
[b] LuiChe Woo Institute of Innovative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
[c] Department of Orthopaedic Surgery, Seoul National University College of Medicine, Republic of Korea

A B S T R A C T

*Background and objective:* Restoring the shoulder function is a crucial demand of patients with rotator cuff (RC) tears. Most preclinical studies only focused on biological and mechanical measurements. Functional assessment was less investigated in the preclinical studies. This study aims to review the literature of shoulder function in animal models for RC tears and evaluate the strengths and weaknesses of different shoulder functional assessments and animal models.
*Method:* A literature search for studies used RC tear animal models to evaluate changes in shoulder function was performed. We searched databases of PubMed, Embase, Web of Science, and Scopus from inception to September 2019. Animal species, functional parameters, injury and repair types, and study durations were summarised. Cluster analyses were then used to separate animal models with different levels of injury and timings of repair. The reliability and clinical relevance of the included assessments and animal models were then discussed.
*Results:* Fourteen animal studies that related to shoulder function in animal models of RC tears were reviewed. Five methods (gait analysis, passive range of motion test, open field test, staircase test, and running endurance test) to assess shoulder function were identified. Single or massive RC tendon tears and immediate or delayed RC repair models were found. We reported and discussed factors to be considered when researchers would select assessments and animal models for different study purposes.
*Conclusion:* Based on current evidences, gait analysis is the most appropriate method to assess changes in shoulder function of animal models of RC tears. More studies are required to further elucidate the reliability of passive range of motion measurement, open field test, staircase test, and running endurance test. Models that use massive tears and delayed repair better represent the clinical condition found in humans.
*The translational potential of this article:* Using more clinically relevant animal models and assessments for shoulder function identified in this review may help to investigate the value of preclinical researches and promote translation of preclinical interventions into clinical practices.

## Introduction

The rotator cuff (RC) is an integrated structure of four muscle–tendon complex that arises from the scapular and whose tendons attach to the tuberosity of the humerus. It includes the supraspinatus (SS), infraspinatus, teres minor, and subscapularis muscles and their tendons. Most of the RC tendon tears are nontraumatic injuries due to tendon degeneration or repeated impingements. People with older age and the

shoulder of one's donimate arm will have higher risk to develop RC tears. [1]. RC tears are one of the most common musculoskeletal injuries that are encountered in orthopaedics. Almost half of the general population older than 60 years has experienced this injury, resulting in a range of shoulder function loss up to 48% [2]. Patients may feel pain in the shoulder region which makes it difficult to sleep on the injury side. They may also experience shoulder weakness in raising arms, changing clothes, or reaching stuffs from a high shelf. It has been reported that up

to 93% of patients with RC tears cannot lift heavy objects and 86% cannot sleep on the injured side [3]. Patients with massive RC tears are reported to have up to 60–75% loss of strength with shoulder abduction [4]. Therefore, multiple strategies have been proposed to improve shoulder function in patients with RC tears.

The standard treatment for RC tears is surgical reattachment of the torn tendon on to its anatomical insertion. This treatment is reported to result in approximately 50% improvement in shoulder function in 1.5 years [5]. Although recovery of shoulder function after conservative treatment has been reported to be comparable with surgical repair [6], healing of the injured tendons was less favourable. Rates for retearing are reported to range from 21 to 94% [7,8] after surgical repair, which may be due to compromised healing at the bone–tendon junction (BTJ). Many researchers have explored innovative treatments to improve the healing process in preclinical studies with different RC tear animal models.

Rats are the most commonly used species to model RC tears because their shoulder anatomy and pathology are relatively similar to humans [9,10]. In addition, the healing process can be evaluated with stand-ardised histological and biomechanical assessments. In the rat model, the repaired tendon typically attaches to the bone with scar-like tissue instead of well-differentiated fibrocartilage layers. Thus, they do not form a typical enthesis [11]. Typically, most studies evaluated the mechanical strength of the BTJ without considering the functional position [12,13]. Because it is difficult to accurately estimate the shoulder function solely with histological and mechanical measurements, assessment of shoulder function in animal models is needed.

Different methods to assess shoulder function in animal models have been developed in recent years. To prove that different injuries would influence gait performance differently, Moser et al. [14] used gait analysis using a mouse model to evaluate forelimb motions after SS tendon tears. They demonstrated that the walking gait was barely affected by partial SS tendon tears, but full SS tendon tears significantly impaired the walking gait. This result implied that gait analyses could be used to detect changes in forelimb function in RC injuries of different severities. Meanwhile, the passive range of motion (ROM) in a rat model was reported to have decreased 15% after RC tears. The investigators also examined the histological findings and suggested that the scars that formed in the subacromial space might restrain passive shoulder movement [15]. Testing movement in an open field is another approach for functional assessment, which could evaluate the changes in activities by analysing an animal's spontaneous movements within an arena. One study assessed movement using the open field test on a rabbit model to observe spontaneous movements after subscapularis tendon tears [16]. In that study, the distance walked was increased after injecting stem cells into the injury site. Although preclinical studies have established various animal models and performed different functional assessments, there is no reliable scientific evidence to show which assessment is superior and which model most accurately correlates with RC tears in humans.

Therefore, a review of the different animal models that are available to assess the repair of RC tears with reliable and clinically relevant functional tests is needed. This information can be used to develop new treatments that will allow functional recovery that can be translated into future clinical trials. The goal of this study is to systematically review the current functional assessments associated with animal models of RC tears and to identify the assessments and animal models that are the most reliable and clinically relevant for future studies.

## Materials and methods

### Search strategy

Published articles that inspected the evaluation of shoulder function in RC tear or repair models were reviewed. The current work followed the Cochrane Collaboration and preferred reporting items for systematic reviews and meta-Analyses. The research protocol has been published in the international prospective register of systematic reviews with registration number: CRD42019136659. An electronic database search was carried out using MEDLINE, Embase, Web of Science, and Scopus from inception to September 2019. The keywords in combinations for searching were as follows: (rotator cuff OR supraspinatus OR infraspinatus OR teres minor OR subscapularis) AND (tear OR torn OR injur* OR lesion OR damage OR rupture OR repair OR restore OR operation OR surg*OR suture OR tendin* OR overuse OR impingement) AND (animal OR rat OR mouse OR mice OR rabbit OR rodent OR canine OR dog OR bovine OR cow OR ovine OR sheep OR goat OR lamb OR porcine OR pig OR avian OR chicken OR kangaroo OR baboon OR monke* OR primate OR vervet) AND (function OR range of motion OR gait OR ambulatory OR locomotion OR behaviour). Supplementary searches were performed by hand searching the reference lists of the included studies. The search results were imported into Endnote X9 (Clarivate Analytics, Philadelphia, PA, USA) to remove duplication of records.

### Inclusion and exclusion criteria

The inclusion criteria were as follows:(1) original study performed on an animal model of RC tendon tear; (2) reported results of shoulder functional assessment; (3) published in English in peer-reviewed journals; and (4) before September 2019. The exclusion criteria were as follows:(1) a study that did not compare the functional changes to the baseline, uninjured control nor sham control; (2) in vitro, cadaveric or veterinary studies; and (3) a study reported as a review article, letter, conference abstract, or case report.

### Quality assessment

The methodological quality of the included studies was assessed with the criteria adapted from the checklist of Hooijmans et al. [17] and Fu et al. [18]. The criteria included unilateral or bilateral surgery; stand-ardisation of surgical procedure; acclimatisation and habituation; complications during follow ups; data normalisation; variable of coefficient; and randomisation and blindness. The total quality score was seven. A study received five points or above will be regarded as of good quality. The quality assessments were performed by two authors (Y.L. and H.T.L.) independently. Disagreements in scores were resolved by consensus between the two reviewers or third opinion (S.C.F.) when required. Intra-class correlation coefficient analysis was calculated using SPSS, version 24 (SPSS Inc, Chicago, IL).

### Data extraction and synthesis

Data extraction includes characteristics of the study (authors, years), characteristics of the animal (animal species and sample size), type of injury and surgical repair, timepoint with significant changes in shoulder functional evaluation, and follow-up period.

## Results

A total of 914 published manuscripts were identified. After removing duplications, 499 abstracts were screened, and irrelevant publications were excluded. Sixty-seven full texts were obtained, and the inclusion criteria were applied. Fourteen articles ultimately were included in this review (Figure 1). The mean methodological quality score was 4.4 ± 1.0 (range from 3 to 6) (Table 1). The inter-rater agreement between the two reviewers was 0.78 (95% confidence interval: 0.72–0.84).

The rat was the most commonly used species for shoulder function assessment (used in 10 of the 14 articles). Five functional assessments including gait analysis (nine studies), passive ROM testing (two studies), an open field test (two studies), a staircase test (two studies), and a running endurance test (one study) were documented in the included publications. Descriptions of the included studies are listed in Table 2.
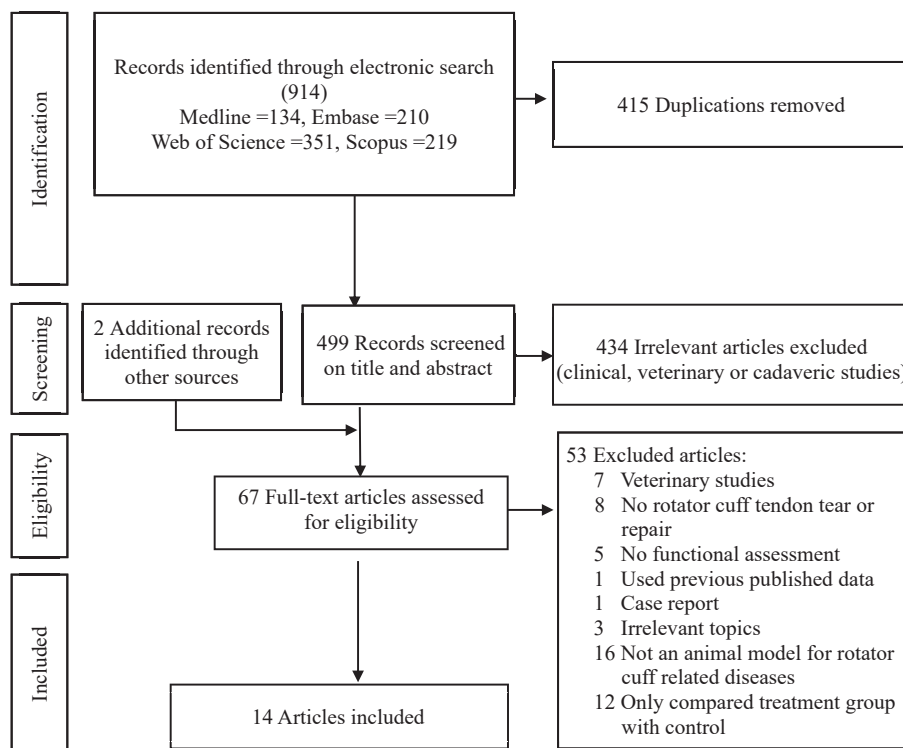
**Figure 1.** Flow chart of study selection based on the inclusion and exclusion criteria is shown.

**Table 1**
Quality assessment of included studies.

| Study | Unity | Surgery standardisation | Acclimatisation and habituation | Complications in follow-up | Data normalisation | Variable coefficient | Randomisation and blindness | Total score |
|---|---|---|---|---|---|---|---|---|
| Sarver et al., 2008 [19] | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Perry et al., 2009 [20] | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 3 |
| Sarver et al., 2010 [21] | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Hsu et al., 2011 [22] | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Yamazaki et al., 2014 [23] | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 5 |
| Bell et al., 2015 [24] | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 4 |
| Park et al., 2015 [16] | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 4 |
| Sahin et al., 2015 [25] | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 4 |
| Sevivas et al., 2015 [26] | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| Yamaguchi et al., 2015 [27] | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 5 |
| Kim et al.,2017 [28] | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 5 |
| Wang et al., 2018 [29] | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 4 |
| Sevivas et al., 2017 [30] | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 4 |
| Moser et al., 2018 [14] | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 3 |

*Functional assessments*

*Gait analysis*

Gait analysis was the most commonly used method to evaluate shoulder function (nine of 14 studies) (Table 2). It was conducted with the rat or mouse walking through a walkway while the paw prints were recorded and analysed. Stride length was the most frequently used parameter to assess gait (six studies). The next most common parameters that were used were step width (five studies) and paw print area (five studies). Other functional parameters, such as ground reaction forces (GRF) and contact intensity (three studies), stance assessment (three studies), and gait speed (two studies) also were investigated.

**Table 2**
Functional changes in different animal models.

| Studies | Species | Rotator cuff injuries | Functional assessments | Follow-ups (day) | Functional changes |
|---|---|---|---|---|---|
| **Single tendon tears** | | | | | |
| Kim et al. 2017 [28] | Rats | SS tear | Gait analysis | 28 | No difference to the baseline |
| Perry et al. 2009 [20] | Rats | SS tear | Gait analysis | 56 | Paw width and stride length were less than those in the uninjured control |
| Moser et al., 2018 [14] | Mice | SS tear | Gait analysis | 28 | No difference to the sham group |
| Park et al. 2015 [16] | Rabbits | SSc tear | Open field test | 28 | Walking distance increased from the baseline |
| **Massive tendon tears** | | | | | |
| Wang et al. 2018 [29] | Mice | SS, IS tear | Gait analysis | 42 | Stride length, step width, paw print area decreased from the baseline |
| Yamazaki et al., 2014 [23] | Rats | SS, IS tear | Gait analysis | 56 | Stride length, print area and contact intensity were less than those in the sham group |
| Yamaguchi et al., 2015 [27] | Rats | SS, IS tear | Gait analysis | 27 | Stands and paw print area were less than those in the sham group |
| Perry et al. 2009 [20] | Rats | SS, IS tear | Gait analysis Passive ROM | 56 | Stride length, step width, speed, and passive ROM were less than uninjured control |
| Hsu et al. 2011 [22] | Rats | SS, IS tear | Gait analysis | 28 | GRF decreased from the baseline |
| Sevivas et al. 2015 [26] | Rats | SS, IS tear | Open field test, Staircase test | 112 | Travel distance and vertical count were not different from the sham group The number of eaten pellets was less than that in the sham group |
| Sevivas et al. 2017 [30] | Rats | SS, IS tear | Staircase test | 112 | No difference to the sham group |
| **Immediate RC tendon repair** | | | | | |
| Bell et al. 2015 [24] | Mice | SS repair | Gait analysis | 14 | Stride length decreased from the baseline |
| Sarver et al. 2010 [21] | Rats | SS repair | Gait analysis | 56 | Step length and GRF decreased from the baseline |
| Moser et al., 2018 [14] | Mice | SS repair | Gait analysis | 28 | Paw print area was larger than that in the uninjured control |
| Sarver et al. 2008 [19] | Rats | SS repair | Passive ROM | 56 | Passive ROM decreased from baseline |
| Sahin et al. 2015 [25] | Rats | SS repair | Running performance | 84 | Penalty score was higher than the uninjured control |
| Yamazaki et al., 2014 [23] | Rats | SS, IS repair | Gait analysis | 56 | Stride length, print area, contact intensity were less than the sham group |
| **Delayed RC tendon repair** | | | | | |
| Wang et al. 2018 [29] | Mice | SS, IS immediate repair/ delayed repair | Gait analysis | 42 | Stride length, stance width, and paw print area were higher in the immediate repair group than the delayed repair groups |
| Hsu et al. 2011 [22] | Rats | SS, IS delayed repair | Gait analysis | 28 | GRF decreased from the baseline |

RC: rotator cuff; SS: supraspinatus tendon; IS: infraspinatus tendon; SSc: subscapularis tendon; GRF: ground reaction force; ROM: range of motion

*Passive ROM*

The passive ROM was assessed with equipment that contained a sensor, which measured the torque and orientation of the glenohumeral joint during passive rotation. The reported parameters included internal and external passive ROM and rotational stiffness.

*Open field test*

The open field test evaluated the spontaneous locomotorbehaviour of an animal within an arena (3 × 3 m for rabbits and 27 × 27 × 20 cm for rats). The motions were monitored with a camera. The walking distance and numbers of vertical movements (vertical count) were the main parameters that were assessed. The walking distance was defined as the total distance moved in 5 min. The vertical count was defined as the frequency with which the animal raised its trunk and stood on its hind limbs. There were two studies that included this test. One study [16] observed a significant increase in the walking distance after a single RC tendon tear. At the same time, another [26] found no significant change in the walking distance after a massive RC tear.

*Staircase test*

Two studies conducted the staircase test to estimate the forelimb function. Rats were trained to reach for the sugar pellets that were placed on seven different steps of a staircase inside a custom-designed box. The number of retrieved sugar pellets and the level of steps where the retrieved pellets were placed were the main parameters. Controversial results were noted in these two different studies. One study [26] reported significantly fewer pellets were eaten by the rats with massive RC tears, while the other study [30] reported no significant changes of the performance in rats with the same types of RC tears.

*Running endurance*

Only one study [25] used a running endurance test to assess shoulder function with a motorised treadmill. A low-dose electrical foot shock occurred at the end of the trial when the rat stopped running. The number of shocks (which was recorded as a penalty score) was reported to indicate running endurance.

*Animal models*

*Single RC tendon tear*

Four articles investigated the functional changes that occurred in the animal models that used a single RC tear (Table 2). Most of the parameters used in the gait analyses and the staircase test indicated that shoulder function was not significantly impaired by single RC tear. Only one study [20] found a decrease from baseline in gait when the stride length was assessed at one, 10, and 42 days after injury,while other studies [14,28] found no significant change in gait after a single RC tear. The passive ROM was decreased from 1 to 56 days post operatively [20].

In addition, the open field test [16] revealed a significant increase in walking distance at 28 days after injury.

*Massive RC tendon tears*

A tear that involves more than one RC tendon is considered as a massive RC tendon tear in animal studies. Six articles explored changes in shoulder function using this type of animal model. Four studies conducted gait analyses. The passive ROM test indicated distinctly compromised forelimb function. The stride length, step width, and paw print area in this model decreased at 14 and 42 days after injury [29]. The passive ROM was reduced from one to 56 days in follow-up assessments [20]. Meanwhile, the staircase test indicated that massive RC tears led to an inferior performance at 112 days after injury compared with the sham injury group [26]. However, in the same study, the open field test found no significant loss in the walking distance and vertical count after massive RC tear at 112 days after injury [26].

*RC tears with an immediate repair*

An immediate repair is defined as performing the repair surgery was on the same day as the injury. We included seven studies that used this model, and five of them used SS tendon to model RC tear and immediately repair. Two studies [23,29] modelled massive RC tendon tear and immediately repaired models. Three [14,21,24] of the five SS repair models conducted gait analyses and reported that the forelimb function was marginally changed. Only stride length and GRF demonstrated a significant decrease. Other gait parameters showed no differences from the uninjured controls or baseline. Another study [25] reported the penalty score (measured by the running endurance test) of a SS tear and the immediately repaired model had a significantly higher score than the healthy control group at three-month after operation. One study [19] evaluated the passive ROM of the injured shoulder after an immediate SS repair and found it decreased significantly at 28 and 56 days after operation.

Two studies investigated the shoulder function in massive RC tear and immediately repaired model. Only gait analyses data were available. One study [23] reported that the gait performance including stride length, contact intensity, and paw print area decreased at three and seven days after surgery and subsequently recovered to the same level as the sham group. However, another study [29] reported that there was no significant change in gait analyses in rats with similar operation.

*RC tears with a delayed repair*

A delayed repair is defined as a repair that was completed two or more weeks after the occurrence of the RC tear. Only two studies were included in this review that examined function using models of massive RC tendon tears with a delayed repair. Only gait analyses were reported in these two studies. One study [22] reported that the gait performance was significantly compromised up to 14 days after a four-week-delayed repair when compared with the baseline. The other study reported that the stride length, GRF, and paw print area were significantly decreased up to 42 days after a six-week-delayed repair when compared with the sham group and the immediate repair group [29].

## Discussion

Commonly used histological and biomechanical assessments of animal models of RC tears are only weakly correlated with the clinical assessments. Therefore, functional assessment of the shoulder is an essential component in preclinical studies to estimate the degree to which the results can be translated to the clinic. However, both the assessing methods and animal models that were used for the functional evaluation have not been standardised, and no consensus seems to exist. The present study systematically summarised the existing studies that included a functional evaluation of the shoulder joint. We found five functional assessments that were used in these animal studies, including gait analysis, passive ROM test, open field test, staircase test, and running endurance test. However, the degree of change in shoulder function varied in the different RC tear models. The most important findings from this study are that gait analysis is the most reliable assessment of shoulder function in preclinical studies. In addition, the animal model that used massive tears or delayed repair could better represent the clinical conditions seen in humans compared with single RC tendon tears or immediate repair models.

*Functional assessment*

Based on the evidence identified in this review, five functional assessments demonstrated different degrees of reliability and efficiency with respect to modelling the deterioration of shoulder function after RC tears.

*Gait analysis*

Gait analysis was the most commonly used functional assessment in the studies included in our review. Gait analysis describes the kinematic and kinetic changes observed in walking gait. Stride length, step width, GRF/contact intensity, stance, paw print area, and speed were the most commonly reported parameters. Each parameter represented different aspects of gait, but only the stride length and GRF/contact intensity were reliably and specifically observed to reflect the changes in shoulder function after RC tears or repair.

The forward stride of the forelimb in a rat could be analogous to shoulder abduction in humans when the scapular plane is taken as a reference [19]. Stride length has been defined as the distance between paw strikes [20], which represents the forelimb's ability for active forward flexion. The stride length decreased in the massive RC tear/repair models up to 42–56 days but presented no change in the single RC tear/repair models [20,21,23,24,29]. These results indicated that the RC tendon injury reduced the active forward flexion, and the extent of injury correlated to the extent of functional loss. These changes also were similar to the clinical observations that decreases in active ROM are more commonly seen in patients with massive RC tear than in patients with nonmassive tears [31]. This observation indicated that the stride length could resemble the human clinical condition by demonstrating active ROM loss in RC injury models. On the other hand, the step width (distance between the front paws) usually was found not affected in the cases where the stride length was drastically reduced. It was suggested that the stride width was impaired because the normal forelimb shifted medially to support more body weight, instead of being caused by the limited ROM of the injured forelimb [22]. Therefore, it is reasonable to postulate that step width may not be a reliable parameter to estimate the degree of function of an injured shoulder.

Because strength is another important aspect of shoulder function, researchers have developed several methods to indirectly measure the shoulder strength. In rats, the body weight is loaded on the shoulder joints and transmitted to the ground during walking, which helped the GRF to reveal the loading capacity of the shoulder [32]. Similarly, the light intensity that is generated in a fully automated gait analysis system (CatWalk XT, Noldus Information Technology, Netherlands) [23] could reflect the loading capacity of the shoulder because the light intensity correlates well with GRF [33]. Investigators have used the light intensity of a rat's footprint to assess its shoulder's loading capacity. Three studies measured the GRF/light intensity, and they demonstrated a notable decrease in the shoulder loading capacity in the RC tear/repair models [21–23]. A substantial decline in GRF values was reported with no change in the temporal and spatial gait results in the model with massive RC tears and delayed repair [22]. Based on a comprehensive comparison between GRF and the temporal and spatial parameters, the GRF was acknowledged to be the most sensitive parameter to reveal impairment of shoulder function [34]. Moreover, the decrease in loading capacity correlates with human clinical outcomes that indicated patients lost 60–70%

of their shoulder strength after RC tears [4]. Thus, GRF and light intensity are reliable and representative parameters that can be used to reveal the shoulder loading capacity in RC injury models.

Pain is another crucial factor that modifies the functional performance, and clinically, pain is reported by patients. Although pain cannot be assessed directly in animal studies, it can be reflected in changes in walking gait [35]. The influence of pain on the shoulder function was limited to the first four days postoperatively [32].

Although the animal's four-limb walking gait is different from the activities of the human shoulder, the influencing factors and the reactions to injuries are similar between animals and humans. Both species reduce the active ROM due to massive RC tendon injuries. With a torn RC tendon, both animals and humans minimise loading the shoulder joint and shift weight bearing to the contralateral side. However, animals have faster recovery times and greater tolerance to injuries than humans. Therefore, specific and sensitive assessment methods are required when evaluating animal models. Task-related functional assessments performed without weight bearing such as the skilled reaching test [36] or the handle pulling test [37] are more relevant to humans. However, due to the limited number of studies available to investigate, the applicability and reliability of these tests are not clear.

### Passive ROM

Another way to measure shoulder function is with the passive ROM test, which was conducted with the rats under general anaesthesia [19]. According to the protocol, the passive movement of the rat's forelimb would simulate the internal and external rotation of the human shoulder at 90 degrees of abduction, which is a standard position for functional assessment of RC tears in patients [4]. The studies included here all found a significant decrease of passive ROM after RC tears. The trend of change is comparable with that observed with human patients [38]. This may be explained by scar formation around the shoulder joint, followed by contraction of the soft tissue [15]. However, there are some challenges in testing passive ROM. Because the rats' bodies are soft and covered by thick fur and loose skin, it is difficult to precisely define the neutral position for the shoulder. In addition, during shoulder rotation, the scapula cannot be fixed. The measured rotational angle became the total rotation of the glenohumeral joint together with the scapula [19]. Besides, both RC tear and repair models presented changes in passive ROM to a similar extent and duration. Thus, the test may not differentiate the impact of different injuries or repairs. Also, attention should be paid to both the hardware and software, which was custom made for these animal models, which may limit the applicability of this assessment. Therefore, passive ROM is regarded as a useful parameter to reveal the flexibility of the shoulder joint. However, the equipment and settings necessary to assess passive ROM need to be improved to more reliably obtain valid and repeatable results.

### Open field and staircase tests

In clinical practice, to carry out functional evaluations, patients with RC tear were asked to complete several shoulder abduction and rotational movements. These assessments largely depend on the cooperation of the patients [39]. The open field and staircase tests were used in previous studies to the assess strength and activity levels in animals. These tests were used in expectations of achieving repeatable results that genuinely represented the movement capacity of the forelimbs. However, one study reported that animals with bilateral massive RC tears walked similar distance as the sham of injury group [26] in open field test at 16 weeks after injury. The observation indicates that the open field test might not be sensitive to reveal the changes in rats' shoulder function. Another probable explanation is that the injuries may have been healed spontaneously when the rats were assessed. Meanwhile, the results of two studies [26,30] that used staircase test indicated that this test may not effectively reveal the difference in shoulder function between rats with massive RC tear and sham injury. In fact, both assessments were

originally designed to assess neurological or psychological disorders [40, 41] and were secondarily adapted in recent years to assess the kinematics of the shoulder joint. These behaviour-related assessments are highly sensitive to the testing environment. Noise, temperature, odours, circadian rhythms ,and handling all need to be carefully controlled, which is challenging for researchers new to this area of research. Inconsistent results observed in the studies included in this review [16,26] were likely due to the influence of emotional reactivity and the physical activity of the animals. More studies are required to justify the reliability of open field and staircase tests.

### Running endurance

Running endurance [25] is more demanding than walking. However, this test generated just one nonspecific parameter, namely a penalty score. The penalty scores only represented the degree of endurance in the running, which is not related to the common assessment parameters used in human studies.

In summary, the gait analysis can provide a comprehensive evaluation of shoulder function by revealing degree of changes in kinetic and kinematic aspects. This assessment revealed differences in gait of animals with different levels of injury and repair. There is also commercially available testing equipment for gait analysis. The passive ROM assessment probably cannot differentiate the degree of changes between animals with RC tendon tear or repair. The open field and staircase tests are more likely to be influenced by emotional reactions. Running endurance could only provide a parameter that is not relating to a specific limb. This review found that based on the current evidence, gait analysis is a more appropriate assessment of shoulder function than other assessments. Further studies with various functional tests in multiple time points and a range of injury levels are required to elucidate the reliability of passive ROM measurement, open field test, staircase test, and running endurance.

### Animal models

Regarding animal modelling of human RC tears, two major types of tear simulation and two major types of surgical intervention were found. RC tear models were divided into studies that used a single RC tear which reflected medium to large RC tears in human and those that used massive RC tears which reflected massive RC tears in human. The interventions were divided into those that used immediate surgical repair (the acute tear model) and those with delayed surgical repair (the chronic tear model). All included studies used similar surgical techniques to tear and repair the RC tendons. Typically, RC tendons were sharply crosscut at their insertions with scalpel. In RC tendon repair models, the tendons were repaired in a transosseous manner. In this repair technique, a suture was passed through the tendon stump and bone tunnels that were drilled through greater tuberosity. The repair site received no antiadhesion treatment. The relevance of these animal models to the human clinical situation is as follows.

### Severity of injury

Single tendon tears have a mild, adverse effect on shoulder function in animal models of RC tears. Only one of the three studies revealed a reduction in stride length after an SS tear [20]. No significant functional loss was found in the other two studies. These findings demonstrated that the animals could easily compensate for the injury. Furthermore, the healing outcomes of a single RC tear in humans are distinct from that of animals. Spontaneous healing of the injury was always achieved in rats, while further deterioration of the tear was commonly observed in humans [42]. It has been reported that 50% of RC tears in human will worsen within 18 months and 8.8% of previously asymptomatic patients with single RC tears will become symptomatic each subsequent year [43, 44]. Therefore, the animal models with single RC tears represented limited clinical relevance to humans because animal models have a vastly better capacity to heal than human patients. In addition, gait analysis and

passive ROM revealed significantly compromised shoulder function in models with massive RC tears. This trend was comparable with that observed clinically in humans in which the active and passive ROM loss may be 70% and 30%, respectively, in patients with massive RC tears [45,46]. Although the massive RC tear animal model did not involve surgical repair, it was still useful to investigate the risk factors for shoulder function or effectiveness of other treatments, such as biceps tenotomy [47,48].

*Timing of repair*

One clinical study [49] reported that among 510 patients with RC repairs, only 8% of the tears were acute RC tears. These patients lost approximately 83% of active abduction. Surgical repair was mostly conducted at 28.5 months, on average, after the onset of symptoms. Shoulder function was restored in these patients 3–6 months after surgery [50,51]. However, most of the preclinical studies included in this review used an immediate repair animal model. The surgical procedure in these studies included the tendon transection and repair during the same operation.

Three of the four immediately repaired studies reported that the gait returned to normal at seven to 10 days postoperatively [21,23,24]. This period was overlapping with the phase of postoperative pain and acute inflammations that caused by surgical trauma. No functional difference was observed in the immediate repair group when compared with the sham group, except function was worse on the third day postoperatively [23].

The immediate repair models seemed to depict just acute surgical trauma instead of simulating the chronic RC tears commonly seen in clinical practice. If we evaluated the pathological changes in the immediate repair models with clinical classification, this model may be classified as Goutallier stage 0 (a commonly used clinical classification of muscle atrophy and fatty infiltration after RC tear [52]) because it showed no sign of muscle degeneration. Although immediate repair is a popular model which is quick and easy to perform, it should be chosen according to the aim of each study.

To better simulate the clinical situation in humans, two animal studies produced chronic RC tears by repairing the torn RC tendons at two to six weeks after tendon transection [22,29]. In these chronic models, the BTJ was in chronic inflammation with features of degeneration, including persistent muscle atrophy and fatty infiltration [29]. These features are likely to reflect the Goutallier stage 1–2 degeneration in patient with chronic RC tears. In addition, these two studies showed significantly compromised gait parameters up to 14–42 days after surgery.

In conclusion, the animal models that simulate massive RC tears or that use delayed repair appeared to better represent human clinical conditions. Thus, these models are likely to more accurately simulate the healing conditions and functional changes that occur in human patients with RC tear.

*Future studies*

To achieve a more reliable and practical animal model for functional shoulder assessment, other factors including age, gender, and comorbidities also should be considered. Most animals used in these studies were young adult males with no other diseases. However, in human clinical conditions, most patients with RC tear were women and older than 50 years [2]. One animal model study compared the healing process between young (2.5–6.5 months) and aged (14–18 months) animals and noted that the aged animals presented worse histological results compared with the young animals [37]. Thus, future studies should try to use aged animals to more accurately represent human clinical populations.

Because different walking speed is one of the main sources of variation in the gait assessment, we recommend that the gait analysis should be performed at a consistent speed by putting the rats on a treadmill [14].

In addition, future studies should consider using other advanced equipment or techniques to observe the movement of the shoulder in animal models with marker-less optical tracking systems to analyse the movement of specific limbs [53,54].

*Limitations*

The current work summarised existing studies that focused on the functional assessment of the animal models of RC injury. Except for studies that used gait analysis, the number of studies that used other assessments is very small, which may limit the evaluation of the repeatability of less reported assessments. Most studies did not report the absolute values of the parameters, which made it difficult to compare the extent of function loss across the studies. Few articles performed multiple functional assessments on the same batch of animals, which made it difficult to verify the relationships among different assessments.

## Conclusions

Based on current evidences, gait analysis is the most appropriate method to assess changes in shoulder function of animal models of RC tears. More studies are required to further elucidate the reliability of passive ROM measurement, open field test, staircase test, and running endurance test. Models that use massive tears and delayed repair better represent the clinical condition found in humans.

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## Conflict of interest

The authors have no conflicts of interest to disclose in relation to this article.

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