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"COMPARATIVE EFFECTS OF EXERCISE AND NON-EXERCISE INTERVENTIONS ON ROTATOR CUFF FOOTPRINT INTEGRITY AT THE HUMERAL ATTACHMENT IN CONSERVATIVELY MANAGED PATIENTS"

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KEYWORDS

Shoulder pain, rotator cuff, external rotation lag sign, internal rotation lag sign, isometric strength, clinically important improvement, randomized controlled trial.

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Abstract

Background: Pain and disability questionnaires do not directly assess the tendon-to-bone footprint, but functional improvement has been used as a proxy for this. To our knowledge, no trial has compared structured exercise with a non-exercise conservative intervention using the External and Internal Rotation Lag signs (ERLS, IRLS) as imaging- independent clinical measures of footprint related function, together with objective strength testing.
Objective: To compare structured exercise and non-exercise conservative intervention on: Lag-sign performance, Isometric external rotation and abduction strength deficit, Clinically important improvement (CII) after 6 months.
Methods: Two hundred patients with clinically diagnosed rotator cuff-related shoulder pain (RCRSP) were randomly assigned to two groups: Group A (structured exercise, n=100), Group B (corticosteroid injection, ESWT and education, n=100). Strength deficits of ERLS, IRLS and handheld-dynamometry were evaluated at baseline, 12 weeks, 3 months and 6 months. CII, a composite clinician's judgement in four outcome domains, was evaluated at 6 months.
Results: Group A had an advantage at the first post baseline assessment and this increased with each time point, with no crossover. At 6 months, ERLS was 5.33° vs. 11.43° (difference −6.10°, 95% CI −7.06 to −5.14), IRLS 4.21° vs. 8.23° (−4.02°, 95% CI −4.85 to −3.19), external rotation strength deficit 9.48% vs. 24.86% (−15.38, 95% CI −17.16 to −13.60), and abduction strength deficit 11.69% vs. 27.06% (−15.37, 95% CI −17.25 to −13.49), all $p<0.001$. The proportion of people who achieved CII was 75.0% in Group A and 55.0% in Group B (continuity corrected χ^2 $p=0.005$; absolute difference 20 percentage points, 95% CI 7 to 33; number needed to treat ≈5).
Conclusions: Structured exercise resulted in improved and long-lasting lag-sign performance and isometric strength compared with a non-exercise conservative package without showing an early crossover with the self-reported pain and disability noted in the companion paper. Lag signs are clinical surrogates and the difference in strength part of training specificity. The findings do not demonstrate tendon healing.

1. INTRODUCTION

1.1 Morphometry of the Rotator Cuff Footprint

The footprint is not a homogeneous insertion. It is a tendon specific connection zone whose dimension differ between individuals an between the four cuff tendons . In a cadaveric

study of 80 shoulders, documented reproducible insertional shape was found with the subscapularis on the lesser tubercle (oval) and the supraspinatus and infraspinatus on the greater tubercle (rectangular and round)[1]. This supports using ERLS (infraspinatus/teres minor) and IRLS (subscapularis) separately from a composite score. The thickened band of the rotator cable is perpendicular to the fibres of the supraspinatus and infraspinatus. It is a load-distributing arch that redistributes the load from the anterior and posterior edges of the cable to the central tear, allowing a patient with a normal cable to remain relatively asymptomatic with a tear in the middle. Disconnection of structure and symptom in the tendinopathy continuum model is one anatomical explanation of this "suspension bridge" model[3]. A favourable lag sign result should therefore be read as evidence of maintained functional load transfer, not as proof that the tendon to bone interface is intact.

1.2 Natural History of Conservatively Managed Cuff Disease

Natural-history cohorts reveal the degenerative tears have a generally predictable, but not uniformly progressive, course. As tear size increase, fatty degeneration worsens more muscles involved and beyond a critical size humeral migration becomes apparent. Tears that are meaningful are either preserved or gradually deteriorate, and age, tear size, and existing muscle degeneration categorize risk for deterioration.[4] A review of imaging evidence in untreated tears has shown that tears usually start at the anterior infraspinatus posterior supraspinatus border and that the size of the tears is correlated with the degree of fatty degeneration and involvement of the number of muscles[5]. A meta-analysis of randomized trials that included trial populations, not just observational cohorts, was performed to study the natural history of full-thickness tears[6], and a systematic review of intermediate and long-term outcomes found that the natural history following repair might not vary significantly from that without repair[7]. Imaging follow-up has been used to quantify the number of high-grade partial-thickness tears that converted to full-thickness tears, in those managed conservatively.[8] However, prospective imaging cohorts of asymptomatic tears also show that tears enlargement and the later development of symptoms over time[9,10]. Almost all of this work depends on serial imaging in cohorts designed for structural

monitoring, which is rarely feasible in pragmatic outpatients trials. None compared different conservative treatments, which raises the question as to whether validated clinical measures might be used to expand footprint related questions into comparative-treatment trials.

1.3 Diagnostic Accuracy of the ERLS/IRLS Battery

A cohort study of fifteen shoulder special tests, using surgically confirmed tear status as the reference, found that lag-sign magnitude correlated with the extent of full- thickness tearing of the supraspinatus, infraspinatus and subscapularis. Specificity was as high as 98% for the external rotation lag sign, with a positive likelihood ratio of 6.06, which, at that specificity, suggests modest sensitivity[11]. The examiner was blinded to imaging and the reference standard was direct surgical visualisation. This evidence relates to full thickness tears, while the present sample included patients treated conservatively for tendinopathy or partial-thickness tears. The results of a pooled analysis of clinical tests for posterosuperior cuff pathology showed that none of the tests is sufficiently reliable on its own, as a paired battery, ERLS and IRLS is recommended[12]. Because the lag signs are low cost and equipment independent measure, they can be repeated at each subsequent visit; serial imaging cannot.

1.4 Strength Testing

Handheld dynamometry showed good to excellent interrater (ICC 0.914 for external rotation and 0.842,for internal rotation) and intrarater (ICC 0.865 and 0.901, respectively) reliability for isometric rotational strength with isokinetic dynamometry as refrence. This evidence is from healthy, non-symptomatic subjects[13]. Strength is a separate domain from pain, and from the lag-sign performance domain and including it allows the structural-surrogate findings are examined alongside a true measure of physical capacity.

1.5 Rationale and Objective

Randomised trials and meta-analyses of surgery versus conservative management have confirmed that conservative care as a durable option[14]. There are at least two trials using imaging follow-up of conservatively managed tears, but the majority of the larger literature focuses on pain and function[15]. Minimal comparators were used and no lag-sign battery was used in the factorial trials of exercise and injection.[19],[20] The trial is, to our knowledge, the first to include a lag-sign test,

objective strength testing, and composite clinically important improvement outcome in a conservatively treated patient population with a structured exercise and a specified non-exercise intervention package. This paper aimed to compare the two approaches in these outcomes after 6 months. It was hypothesized that structured exercise would be more effective. In a companion paper, pain (NPRS) and disability (SPADI) were reported.

2. MATERIAL AND METHODS

Design and setting: This was a prospective randomized parallel group, assessor-blinded, two-arm trial at the Department of Physiotherapy, along with the Department of Orthopaedics, Malwanchal University, Indore. The design, population, randomisation and interventions have been fully described in the companion paper and summarised here.

Participants and sample size: Participants were recruited by consecutive sampling from the outpatient departments, and consisted of 200 patients, aged 30-65 years, diagnosed with RCRSP (tendinopathy or partial-thickness tear) and treated conservatively, without surgical intervention planned. Using a computer-generated block randomisation with SNOSE concealment, they were grouped into two: Group A: structured exercise (n=100) and Group B: (n=100). The sample was capable of detecting the difference of about 0.40 at 80% power. Confirm primary or secondary measures of lag sign and strength (as in the companion paper).

Inclusion criteria:

- Age 30–65 years, both sexes
- A clinical diagnosis of RCRSP is a positive Jobe/empty-can test, Hawkins-Kennedy test, or painful arc.
- Duration of symptoms ≥6 weeks, and baseline NPRS ≥4/10
- No surgery scheduled
- Informed consent and willingness for 6-month follow-up

Exclusion criteria:

- A full-thickness tear is suspected or diagnosed and surgery is indicated.
- Adhesive capsulitis, glenohumeral instability, or cervical radiculopathy
- Shoulder surgery or injection within three months ago
- Contraindications for exercise or electrotherapy modalities.

- Pregnancy

Interventions: Group A received 12 weeks of three times a week supervised progressive programme of pain-free isometric holds and (weeks 1-4),Theraband resisted external rotation, internal rotation and abduction with scapular stabilisation, with clear guidelines for progression (week 5-8), and functional and endurance strengthening according to occupational demands for (weeks 9-12). Group B received a subacromial corticosteroid injection (methylprednisolone with lidocaine, second injection at week 6, if NPRS ≥4/10), six sessions of radial ESWT, two education sessions and was not encouraged to start a strengthening programme. There was a large difference between the amount of supervised contact in both arms.

Assessment procedure: Outcomes were recorded at baseline, end of week 12, 3 months and 6 months, with a protocol window of ±7 days. Baseline assessments were performed by an assessor who was blinded to allocation, follow-up assessments were undertaken by an assessor were blinded wherever feasible. The participants were given the instruction not to reveal their allocation. Self-report measures (described in the companion paper) were administered prior to the lag-sign tests and dynamometry within each visit to minimize the effect of the pain associated with maximal effort testing

Outcome measures:

- **ERLS**: participant seated, arm placed in passive 20° abduction, near full external rotation and 90° elbow flexion. The wrist was released while the elbow remained supported and the participants actively held the position. Lag was the angular difference between passive and active positions and was measured by goniometer. Lag was categorised as none (0°), minimal (1–15°), moderate (16–30°) or marked (>30°).
- **IRLS:** hand was passively placed behind the back at mid-lumbar levels in maximal internal rotation, and wrist was released, while the participant maintained the position. Lag was defined as being the distance from the spine that the hand had fallen, in an angular equivalent, and classified as for ERLS. Both lag tests were performed on both sides at each time point, and the mean of two trials per side was recorded.

- **Isometric strength:** handheld dynamometry ("make" test) and fixed dynamometer with examiner counter-resistance. External rotation was tested seated with elbow flexed 90° and arm at the side with the dynamometer at the wrist. The abduction was tested at 90° in the scapular plane, dynamometer located proximal to the elbow. The three trials were taken with ≥30 s rest, and the mean value was recorded. The deficit was expressed as the percentage difference from the unaffected side and categorized as minimal (<10%), mild (10–25%), moderate (26–50%) or severe (>50%).
- **Clinically important improvement (CII):** the composite judgement made by the assessing clinician at 6 months in the four outcome domains. The basis for the judgement was whether each domain fulfilled its own criterion: NPRS reduction ≥2 points, SPADI reduction ≥13 points, an improved ERLS/IRLS category, and an improved strength-deficit category.
- **Ethics and safety :** Approval was obtained from the Institutinal Ethics Committee of Malwancal University, with prospective CTRI registration. Written informed consent was obtained and adverse events were recorded on a structured log.

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Statistical analysis: Shapiro–Wilk test for normality; independent-samples t-test or Mann–Whitney U test for between-group comparison; continuity-corrected chi-square test for CII, with absolute risk difference and number needed to treat. Mean differences, 95% CIs and Cohen's d were calculated from summary statistics (Table 10). Significance was set at $p<0.05$ (two-tailed). Several outcomes and time points were tested without multiplicity adjustment, so the findings should be treated as exploratory. All randomised participants were analysed as randomised.

3. RESULTS

All 200 participants completed follow-up. Baseline ERLS, IRLS and both strength-deficit measures were similar between groups (all $p>0.25$).

TABLE 1: ERLS (MEAN ± SD, DEGREES)

Time Point	Group A (n=100)	Group B (n=100)	p-value	Interpretation
Baseline	12.85 ± 4.30	13.48 ± 3.91	0.280	Not significant
Post-12wk	8.96 ± 2.81	11.03 ± 3.04	<0.001	Favours Group A
3-Month	7.29 ± 3.14	10.73 ± 2.86	<0.001	Favours Group A
6-Month	5.33 ± 3.41	11.43 ± 3.49	<0.001	Favours Group A

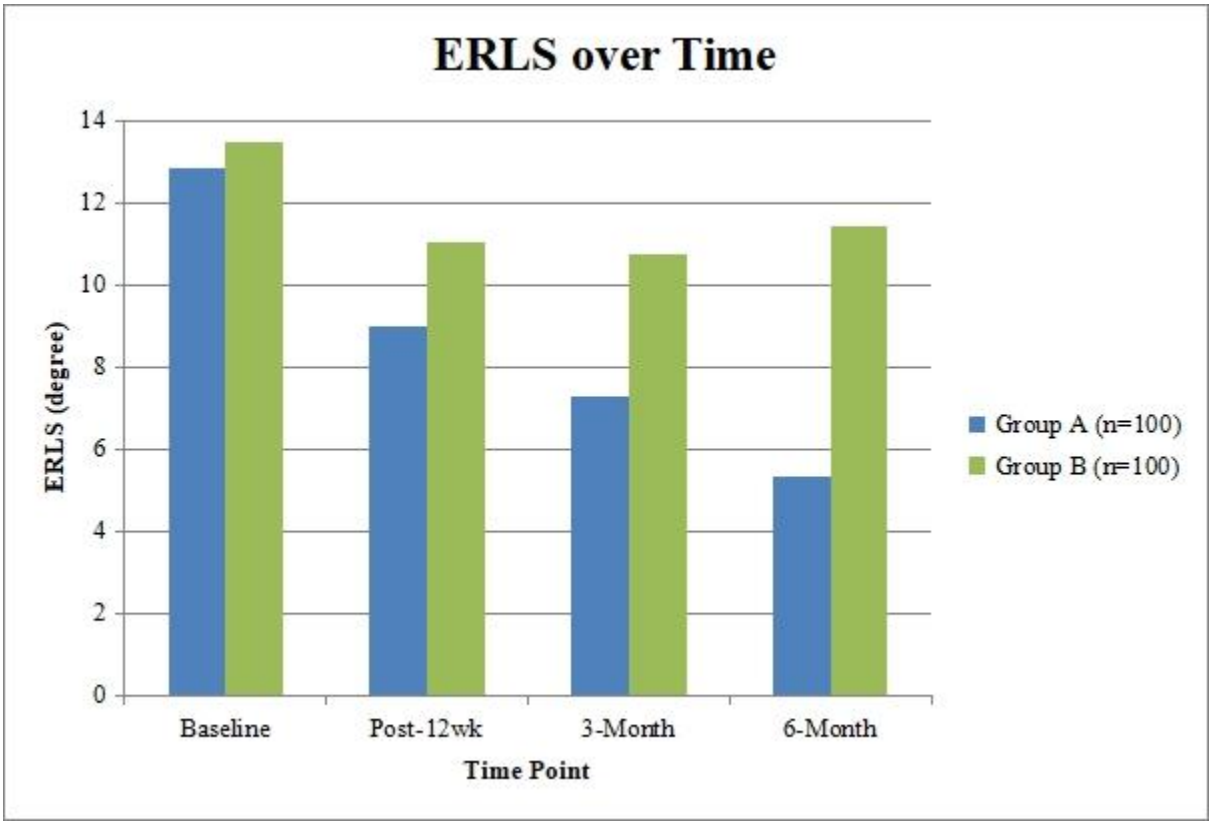


Figure -8 ERLS Mean Trend Across Four Time Points (Group A versus Group B)

TABLE 2: ERLS CATEGORY DISTRIBUTION, BASELINE vs 6 MONTHS

Lag Magnitude	Group A Baseline	Group A 6-Month	Group B Baseline	Group B 6-Month
0° (no lag)	0 (0.0%)	8 (8.0%)	0 (0.0%)	0 (0.0%)
1°–15° (minimal)	72 (72.0%)	92 (92.0%)	69 (69.0%)	86 (86.0%)
16°–30° (moderate)	28 (28.0%)	0 (0.0%)	31 (31.0%)	14 (14.0%)
>30° (marked)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

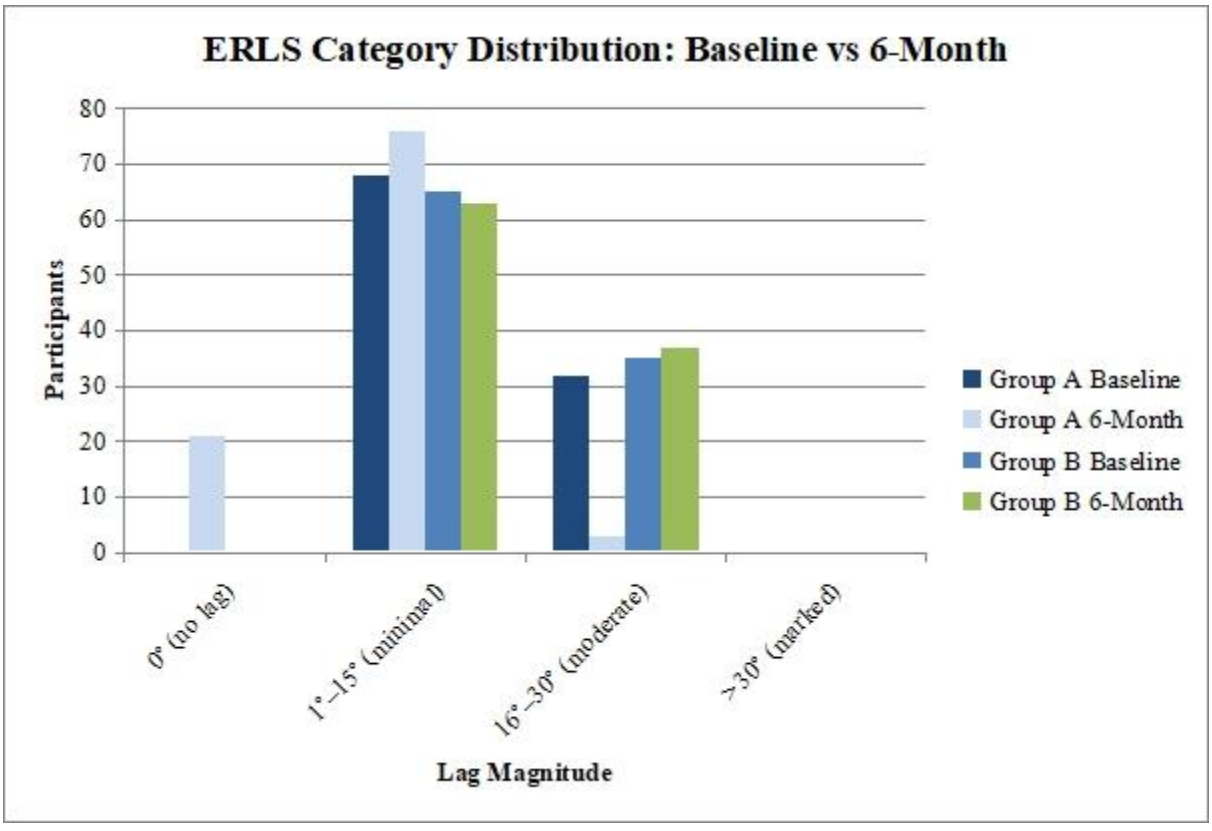


Figure 9 — ERLS Category Distribution: Baseline vs. 6-Month

TABLE 3: IRLS (MEAN ± SD, DEGREES)

Time Point	Group A (n=100)	Group B (n=100)	p-value	Interpretation
Baseline	9.65 ± 3.82	9.91 ± 3.27	0.606	Not significant
Post-12wk	6.65 ± 2.83	8.49 ± 2.63	<0.001	Favours Group A
3-Month	5.11 ± 2.52	7.77 ± 3.07	<0.001	Favours Group A
6-Month	4.21 ± 2.51	8.23 ± 3.35	<0.001	Favours Group A

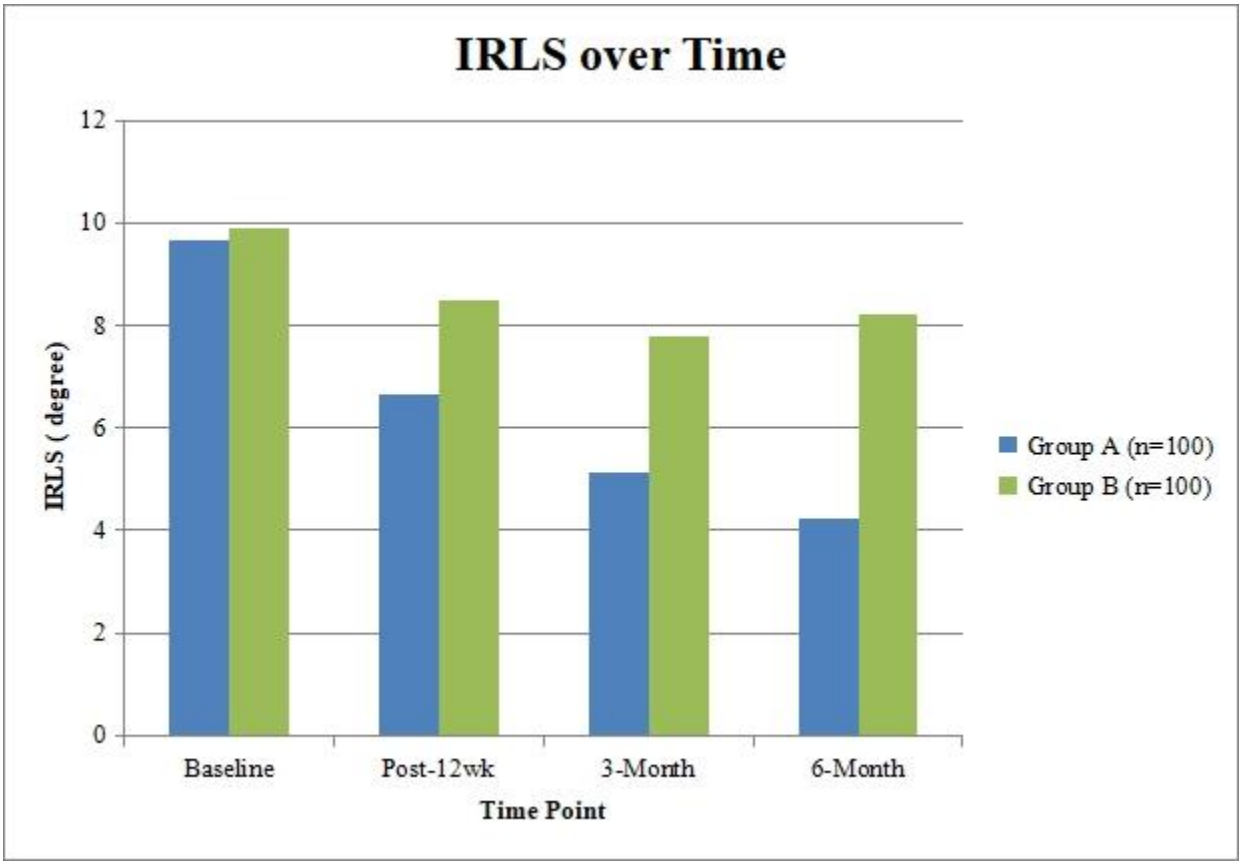


Figure -10 IRLS Mean Trend Across Four Time Points (Group A versus Group B)

TABLE 4: IRLS CATEGORY DISTRIBUTION, BASELINE vs 6 MONTHS

Lag Magnitude	Group A Baseline	Group A 6-Month	Group B Baseline	Group B 6-Month
0° (no lag)	0 (0.0%)	5 (5.0%)	0 (0.0%)	0 (0.0%)
1°–15° (minimal)	94 (94.0%)	95 (95.0%)	97 (97.0%)	100 (100.0%)
16°–30° (moderate)	6 (6.0%)	0 (0.0%)	3 (3.0%)	0 (0.0%)
>30° (marked)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

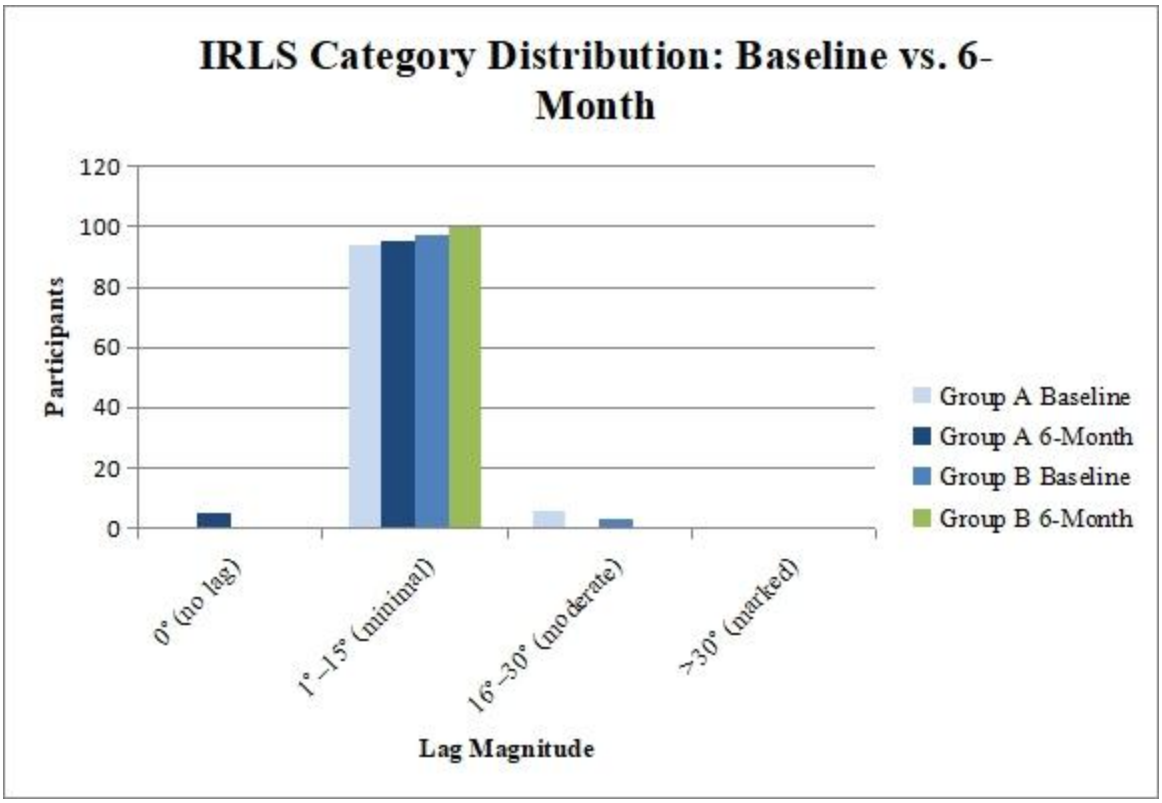


Figure —11 IRLS Category Distribution: Baseline vs. 6-Month

TABLE 5: EXTERNAL ROTATION STRENGTH DEFICIT (MEAN ± SD, %)

Time Point	Group A (n=100)	Group B (n=100)	p-value	Interpretation
Baseline	36.42 ± 7.34	37.03 ± 7.96	0.574	Not significant
Post-12wk	22.55 ± 5.93	25.21 ± 7.11	0.005	Favours Group A
3-Month	16.77 ± 6.11	23.78 ± 7.23	<0.001	Favours Group A
6-Month	9.48 ± 5.25	24.86 ± 7.36	<0.001	Favours Group A

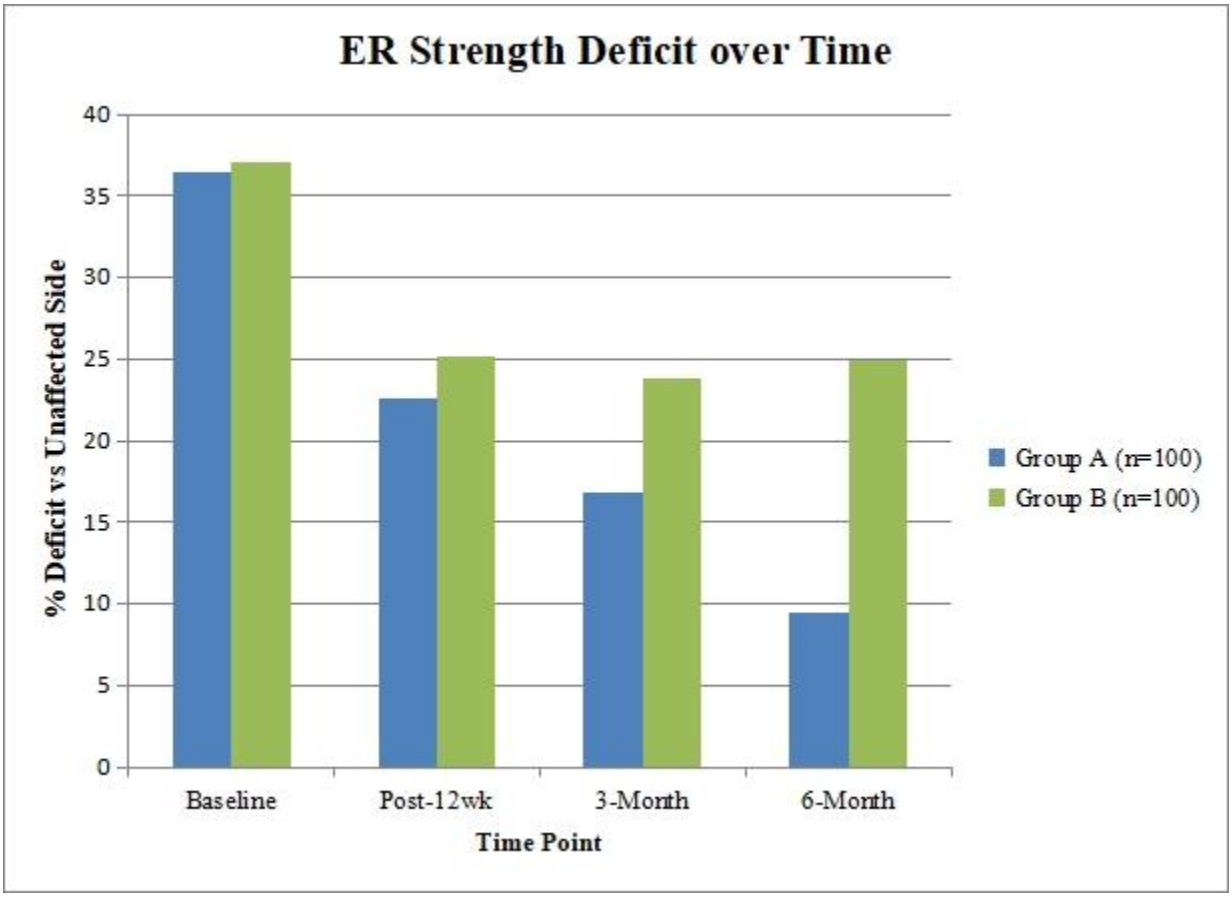


Figure -12 External Rotation Strength Deficit Trend Across Four Time Points

TABLE 6: ER STRENGTH DEFICIT CATEGORY DISTRIBUTION, BASELINE vs 6 MONTHS

Deficit Category	Group A Baseline	Group A 6-Month	Group B Baseline	Group B 6-Month
Minimal (<10%)	0 (0.0%)	55 (55.0%)	0 (0.0%)	5 (5.0%)
Mild (10–25%)	5 (5.0%)	45 (45.0%)	7 (7.0%)	49 (49.0%)
Moderate (26–50%)	90 (90.0%)	0 (0.0%)	89 (89.0%)	46 (46.0%)
Severe (>50%)	5 (5.0%)	0 (0.0%)	4 (4.0%)	0 (0.0%)

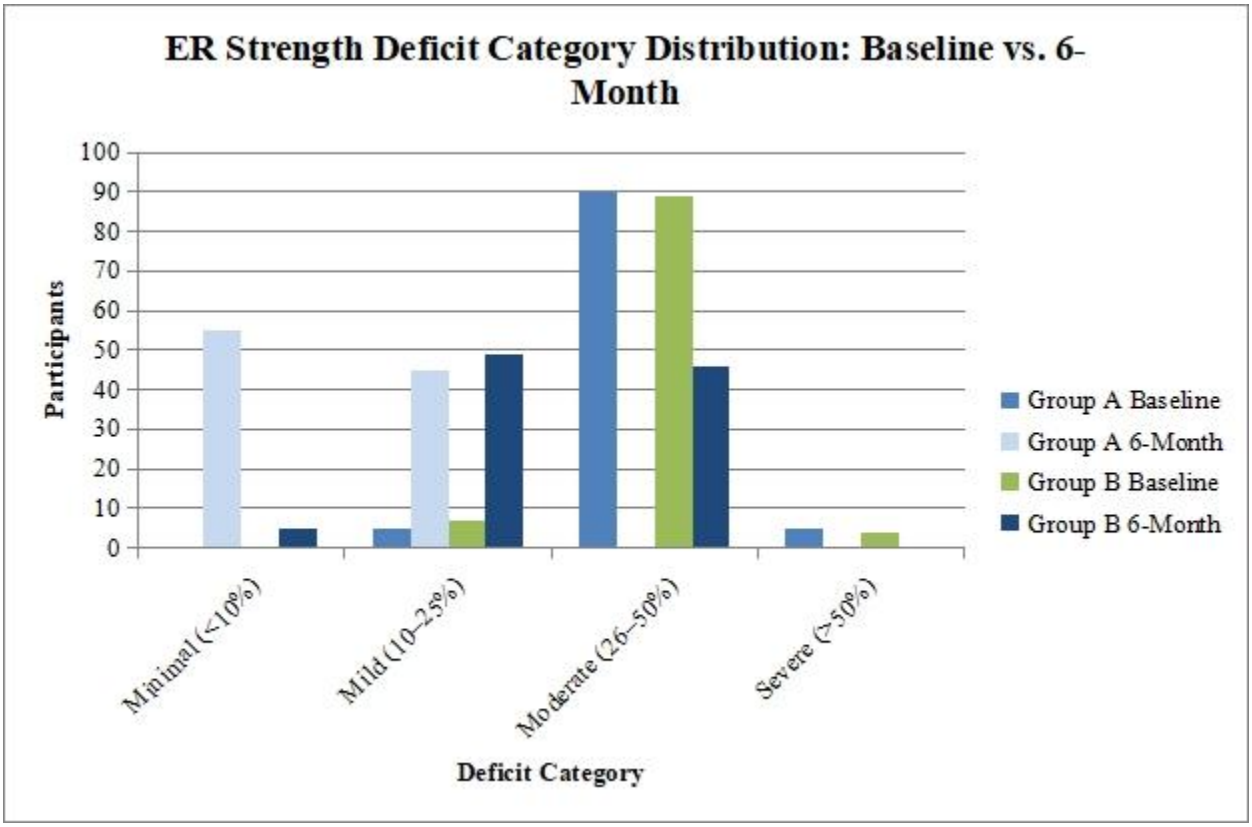


Figure -13 ER Strength Deficit Category Distribution: Baseline vs. 6-Month

TABLE 7: ABDUCTION STRENGTH DEFICIT (MEAN ± SD, %)

Time Point	Group A (n=100)	Group B (n=100)	p-value	Interpretation
Baseline	39.24 ± 7.43	39.75 ± 7.34	0.625	Not significant
Post-12wk	24.81 ± 5.97	28.01 ± 7.42	<0.001	Favours Group A
3-Month	16.89 ± 6.13	26.75 ± 6.76	<0.001	Favours Group A
6-Month	11.69 ± 4.93	27.06 ± 8.14	<0.001	Favours Group A

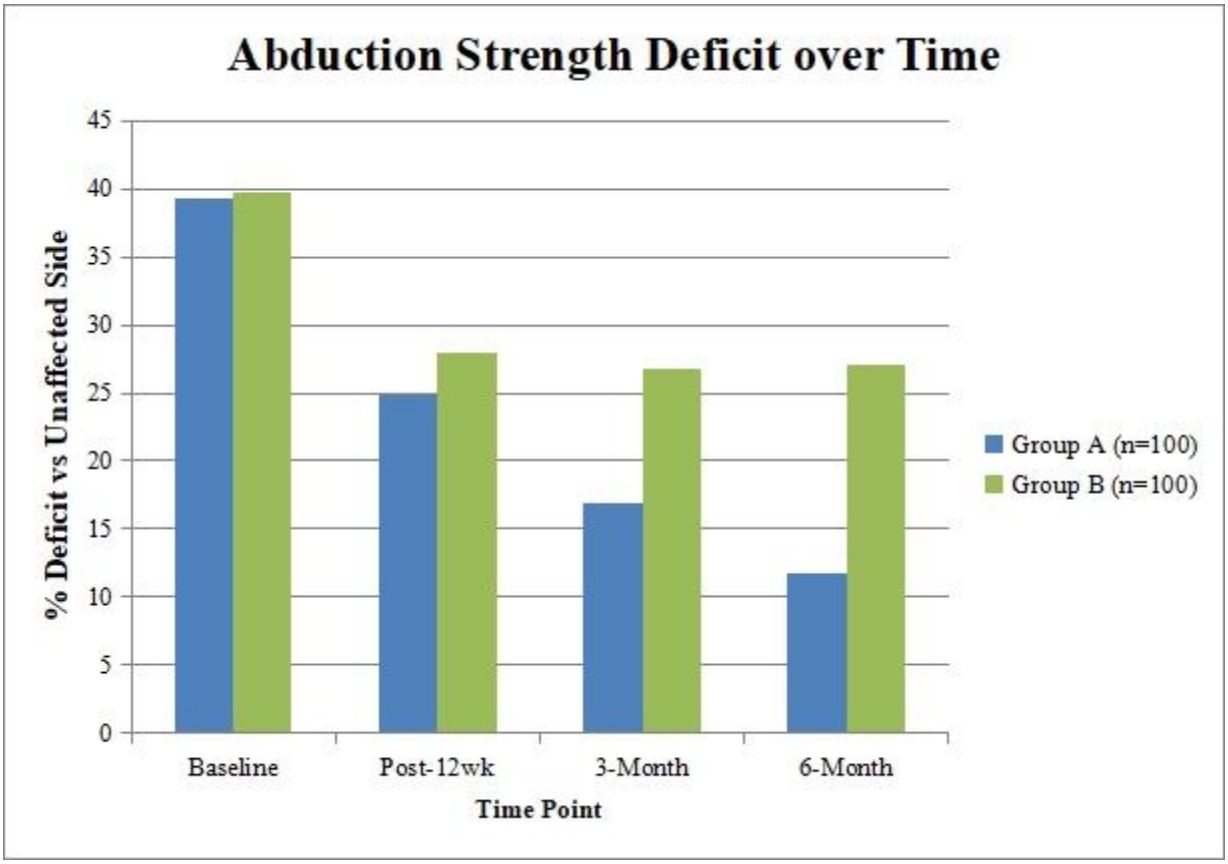


Figure -14 Abduction Strength Deficit Trend Across Four Time Points

TABLE 8: ABDUCTION STRENGTH DEFICIT CATEGORY DISTRIBUTION, BASELINE vs 6 MONTHS

Deficit Category	Group A Baseline	Group A 6-Month	Group B Baseline	Group B 6-Month
Minimal (<10%)	0 (0.0%)	34 (34.0%)	0 (0.0%)	1 (1.0%)
Mild (10–25%)	3 (3.0%)	66 (66.0%)	2 (2.0%)	41 (41.0%)
Moderate (26–50%)	90 (90.0%)	0 (0.0%)	88 (88.0%)	58 (58.0%)
Severe (>50%)	7 (7.0%)	0 (0.0%)	10 (10.0%)	0 (0.0%)

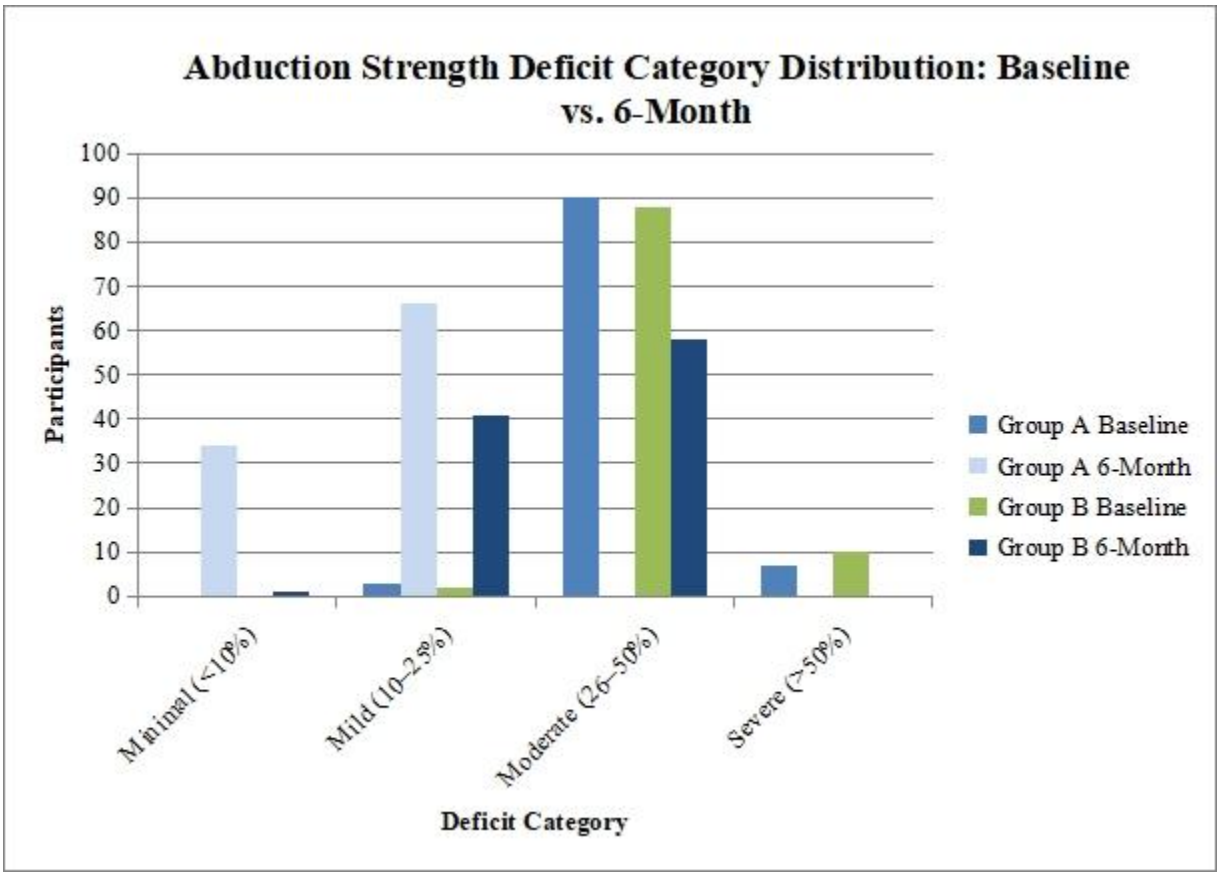


Figure-15 Abduction Strength Deficit Category Distribution: Baseline vs. 6-Month

TABLE 9: CLINICALLY IMPORTANT IMPROVEMENT BY GROUP

	CII: Yes	CII: No	Total
Group A (Structured Exercise)	75 (75.0%)	25 (25.0%)	100
Group B (Non-Exercise Conservative)	55 (55.0%)	45 (45.0%)	100
Total	130 (65.0%)	70 (35.0%)	200

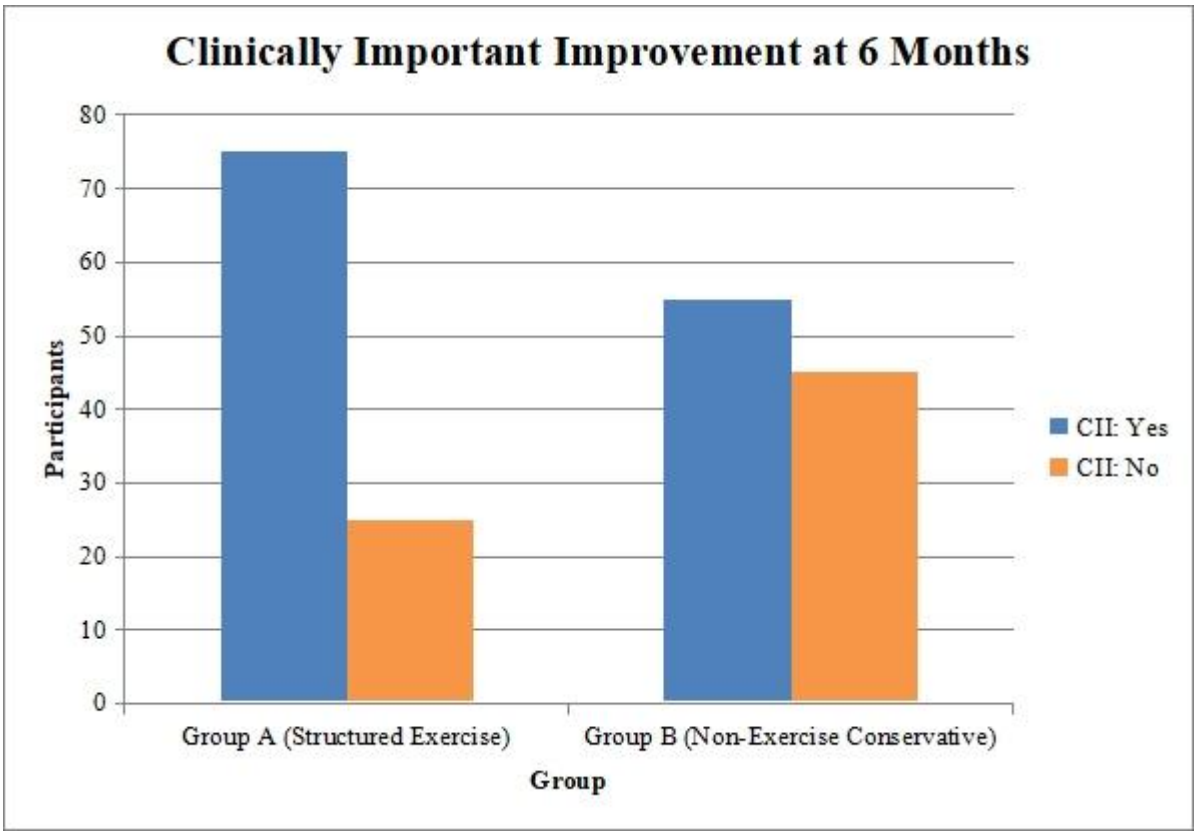


Figure - 16 Contingency Table: Clinically Important Improvement by Treatment Group

Continuity-corrected χ^2 p=0.005 (uncorrected p=0.003). Absolute difference

20 percentage points (95% CI about 7 to 33); number needed to treat ≈5 (approximate 95% CI 3 to 14).

4. DISCUSSION

4.1 Lag-Sign Findings

In contrast to NPRS and SPADI in the companion paper, neither lag sign showed an early crossover. The advantage of Group A was seen at 12 weeks and became more at each assessment (ERLS difference −2.07°, −3.44°, −6.10°). Group A's ERLS fell from 12.85° to 5.33° (7.52° improvement), and 8.0% of participants had no lag at 6 months. Group B's ERLS fell by only 2.45° during treatment (13.48° to 11.03°), then did not improve further and was slightly higher at 6 months (11.43°) than at 3 months (10.73°). IRLS showed the same pattern : Group A improved by 5.44° and Group B by 1.68 ° more than a threefold difference despite similar baseline (p = 0.606).Group B also drifted slightly upward between 3 and 6 months on ERLS, IRLS and ER strength deficit, so its course was a plateau with slight late worsening.

Group B pain relief during treatment was not accompanied by comparable gains in lag sign performance. This is consistent with the loosely coupled symptoms and structure in the continuum model[3]. The lag signs will be clinical surrogates. Their validation include full thickness tears[11], the present sample did not include surgically indicated tears, and there is no MCID for goniometric lag magnitude. Within each category there are also small differences that could be due to measurement error. An improving ERLS or IRLS is simply confirmation of the maintenance or improvement of functional load transfer across the footprint[2] and not histological healing of the tendon to bone interface. By 6 months, most of the participants in both groups were within the "minimal lag" range (92% of Group A and 86% of Group B for ERLS, and 95% and 100% for IRLS). The difference between the groups is therefore more appropriately summarized by the means rather than the number of cases in the groups.

4.2 Strength Outcomes

The strengths findings also have the same direction of effect. The deficits measured at 6 months were 9.48% external rotation (minimal range) and 11.69% abduction (mild range) in Group A. The percentages for Group B's were: 24.86% (mild range, upper boundary) and 27.06% (moderate range).

There was absolute improvement from baseline: A group 26.94 points, B group (external rotation) 12.17, and B group (abduction) 12.69 points. None of the participants in Group A were in the moderate or severe deficit range for either external rotation or abduction at 6 months compared to 46.0% of children in Group B for external rotation and 58.0% for abduction.

The strength and lag-sign result should not be interpreted as such a complete confirmation. Group A trained external rotation and abduction strength and Group B was instructed not to start strengthening. Therefore, there is a degree of expected strength-specificity differences in the training group, and reliability of handheld dynamometry has been demonstrated in healthy subjects only against the isokinetic reference[13]. However, the agreement across measures reinforces the notion that the exercise arm effect was more enduring on measures of physical capacity, rather than on any particular tissue mechanism.

4.3 Clinically Important Improvement

A 20 percentage point absolute difference found 75.0% of Group A and 55.0% of Group B (continuity corrected χ^2 p=0.005) to reach CII, equivalent to a number needed to treat of approximately[5]. The risk difference is rather large (95% interval approximately 7 to 33 points or an NNT of approximately 3 to 14).CII was a clinician 's composite judgement rather than a calculated score, and the all or any rule is not stated , so the result should be read as an overall clinical impression. It also is not comparable to the responder analysis reported in the companion paper, in which all Group A participants achieved NPRS and SPADI thresholds. This means that CII had to rely on the lag-sign and strength domains, or on the judgement of the clinician.

The between-group lag-sign and strength differences have no established minimal important difference. For the self-reported outcomes, the companion paper compares the 6-month between-group differences with the thresholds used in this trial: the SPADI difference (15.6 points) exceeded the 13-point threshold, whereas the NPRS difference (1.49 points) was below the 2-point threshold. These thresholds describe within-patient change, so this comparison is interpretive.

4.4 Positioning Within the Literature

Surgical vs. conservative treatment trials have shown that conservative treatment is a

long-term option[14]. Evidence comparing competing conservative strategies is less developed. Large factorial trials found no advantage of exercise over advice, or no added benefit of injection[19,20] but they did not use a lag-sign or similar battery and were not designed to detect divergence after the treatment phase. Imaging follow-up of conservatively managed tear does exist[15], but not in a head to head comparison of two conservative packages. The results here are consistent with two accounts, one a load-tolerance account for the exercise arm and one a short-lived local pharmacological account for the non-exercise arm. This is a trial that didn't have any biopsy, imaging or biomarker data, meaning that these were not tested. The hypothesis of superiority of exercise was not pre-specified, only the mechanism-specific predictions.

4.5 Limitations

- The baseline age imbalance (see companion paper) was not adjusted for. Companion paper's subgroup analysis revealed no age band effect on 6-month NPRS, but not investigated strength or lag sign.
- ERLS and IRLS are clinical surrogates, validated for full-thickness tears , with no MCID. Lag was present in all participants at baseline, which is unusual for tendinopathy and may reflect measurement error or a different case mix than expected.
- No imaging provided to confirm either tear status or tendon healing.
- Group A was trained the strength outcomes directly, whereas Group B was instructed not to strengthen.
- CII was a clinician's composite judgement with no stated all or any rule.
- **E**vidence for dynamometry reliability is provided by healthy subjects.
- Participants and clinicians were not blinded, but outcome assessors were.
- Several outcomes and time points were not multiplicity corrected.
- The trial was single centre and performed for 6 months.

5. CONCLUSION

In 200 conservative patients with rotator cuff-related shoulder pain, structured exercise produced better lag sign performance and lower isomeric strength deficits than a non- exercise conservative package at 6 months (ERLS 5.33° vs. 11.43°; IRLS 4.21° vs. 8.23°; external rotation deficit

9.48% vs. 24.86%; abduction deficit 11.69% vs. 27.06°; all $p<0.001$). None of these measures had an early crossover, as compared to the companion paper on pain and disability. Group A's advantage remained from 12 weeks and increased at each assessment while Group B's showed no improvement following end of treatment and a slight increase on some measures between 3 and 6 months.

Clinically important improvement, a clinician's composite judgement at 6 months, was achieved by 75.0% of Group A and 55.0% of Group B, a 20-percentage-point difference corresponding to a number needed to treat of about 5. The confidence interval for this difference is wide, and the rule for combining the four outcome domains was not specified, so the result should be read as an overall clinical impression. The findings should not be read as proof of tendon healing. ERLS and IRLS are clinical surrogates validated for full-thickness tears, and no imaging was used. The strength difference also partly reflects training specificity, since Group A trained these movements and Group B was instructed not to.

Taken together with the companion paper, these findings support for structured exercise as the primary conservative treatment, and possibly a bridging conservative treatment for the short term using the non-exercise package. They have some limitations because of the single-centre design, the absence of blinding of participants and clinicians, the lack of adjusted baseline age imbalance, the lack of multiplicity correction, and a 6-month horizon. Multi-centre replication that includes imaging confirmation, a defined CII rule and longer follow-up is the priority for future research.

DECLARATIONS

Ethical approval: Obtained from the Institutional Ethics Committee, Malwanchal University, prior to commencement; registered with the Clinical Trials Registry of India (CTRI) [registration number to be inserted].

Informed consent: Written informed consent was obtained from all participants prior to enrolment.

Conflict of interest: The authors declare no conflict of interest.

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Data availability: Data are available from the corresponding author on reasonable request.

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