Cover letter

Title: Low-Load Blood Flow Restriction Training vs Traditional Resistance Training Exercises Following Anterior Cruciate Ligament Reconstruction Surgery: A Pilot Randomized Controlled Trial

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Purpose Statement: This document outlines the study protocol and statistical analysis plan for evaluating the effectiveness of low-load blood flow restriction training compared with traditional resistance training following anterior cruciate ligament reconstruction surgery (ACLR). The purpose is to ensure transparency, and adherence to ethical research practices throughout the study.

Ethical Considerations

This version of the Study Protocol and Statistical Analysis plan was reviewed and approved by the Rehman Medical Institute- Research ethics committee.

Approval No. RMI/RMI-REC/Approval/209, on 31-May-2024

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Study Protocol

Background

Anterior cruciate ligament reconstruction (ACLR) is a common surgical procedure for athletes and active individuals who suffer ACL tears. Despite successful surgery, significant challenges remain during rehabilitation, including persistent quadriceps muscle atrophy and strength deficits, which can delay functional recovery and return to sport (Ardern et al., 2011). Traditional resistance training is effective for muscle hypertrophy but may not be feasible for all patients in the early postoperative phase due to load restrictions and joint stress.

Low-load blood flow restriction (LL-BFR) training has emerged as an innovative rehabilitation strategy that combines low-intensity resistance exercise with partial vascular occlusion to enhance muscle growth and strength while using much lower loads than traditional resistance training (Hughes et al., 2017). Evidence suggests that BFR can safely stimulate muscle hypertrophy comparable to high-load training and may reduce joint stress, making it particularly suitable for patients recovering from ACLR (Scott et al., 2015).This pilot study aims to compare the effectiveness of LL-BFR and traditional resistance training in improving muscle size, strength, range of motion, pain, and joint effusion in patients following ACLR, contributing to the evidence base for optimal post-surgical rehabilitation strategies.

Objective

The main objective/s of the study is to compare the effectiveness of low load blood flow restriction training (LL-BFR) with traditional resistance training exercises (T-RT) at improving skeletal muscle hypertrophy, strength, Range of motion (ROM), pain and effusion in individuals who have undergone anterior cruciate ligament (ACL) reconstruction surgery.

Study Design

1. Study Type

Interventional: The study (Pilot randomized controlled trial) involves an active intervention (BFR-RT vs. T-RT) to evaluate treatment efficacy.

2. Primary Purpose

Treatment: The study aims to assess the therapeutic effectiveness of BFR-RT compared to traditional low load training in improving post-ACLR outcomes.

3. Study Phase

N/A: The trial does not involve investigational drugs or biologics.

4. Intervention Model

Parallel Design: Two distinct groups receive different interventions simultaneously:
Group 1 (BFR-RT): Low-load resistance training with blood flow restriction along with standard rehabilitation.
Group 2 (T-RT): Low-load traditional resistance training with standard rehabilitation.

- **Model Description:**

BFR-RT group undergoes 8 weeks of BFR training followed by standard rehabilitation protocol progression.
T-RT group receives standard rehabilitation alone for the entire duration.

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5. Number of Arms

2 Arms:
Experimental Arm: BFR-RT.
Active Comparator Arm: T-RT.

6. Masking (Blinding)

- Single-Assessor Blinded:

Investigator: The researcher assessing outcomes is blinded to group assignments.
Participant: Participants are aware of their group (open-label for participants).

Care Provider: Therapists administering interventions are unblinded.

None (Open Label): Not applicable; partial blinding is used.

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6. Allocation

Participants are randomly assigned to BFR-RT or T-RT groups.

Method: Opaque envelopes with coded assignments prepared by an independent team member.

Stratification: Not specified, but typically balanced for age, sex, or baseline function.

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7. Enrollment

- Target Sample Size: 32 participants.

Type: Actual (confirmed enrolled participants).

Justification: Based on power analysis to detect clinically significant differences in muscle hypertrophy/strength.

8. Additional Protocol Details

Intervention Protocol

Frequency: Biweekly sessions (16 total over 8 weeks).

BFR Technique:

- Occlusion band placed proximally on the affected limb.
- Tightness standardized to allow one finger beneath the band.

Standard Rehabilitation:

- Includes range of motion (ROM) exercises, strengthening, and functional training per hospital protocol.

Outcome Assessments

Time Points: Baseline (Week 0-1), Mid-Intervention (Week 4-5), Post-Intervention (Week 9).

Measures:

Primary: Muscle hypertrophy (ultrasound/MRI), strength (manual muscle testing).

Secondary: Pain (KOOS), knee ROM, effusion (circumference), adherence rates.

Safety Monitoring

- Adverse Events: Documented throughout; none reported in this trial.
- Exclusion Safeguards: No vascular pathologies or anticoagulant use.

9. Ethical Considerations

Informed Consent: Obtained from all participants.

Approval: Ethical approval was taken from the Institutional Review Board (IRB) of Rehman Medical Institute.

10. Participants:

- **Inclusion criteria:** Individuals who have undergone ACL reconstruction surgery within the past 4 weeks, free of any neurological impairments or significant cardiac, pulmonary, or metabolic conditions.
- **Exclusion criteria:** Individuals with contraindications to exercise, any other comorbidity, or systemic inflammatory conditions.

Outcome Measures:

- **Primary Outcome**: Muscle strength (measured using manual muscle technique). Muscle size (measured via measuring tap), ROM (Using goniometer), pain (using a Visual Analog Scale), and joint effusion (assessed clinically).

Statistical Analysis Plan

This Statistical analysis plan (SAP) compares postoperative rehabilitation outcomes between an experimental training group (n=16) and a control group (n=16) across three key timepoints: immediate post-surgical baseline (0-1 week), mid-intervention (4-5 weeks), and post-intervention (8-9 weeks). The study employs a repeated measures design to evaluate five primary outcome domains: muscle size (continuous, cm), range of motion (continuous, degrees), muscle strength (ordinal, 0-5 scale), pain perception (ordinal, VAS 0-10), and joint effusion (ordinal, 0-3 clinical scale).

In contrast, This SAP includes descriptive statistics, normality assessments, inferential statistical tests with corresponding assumptions, multiple comparison adjustments, missing data handling procedures, and software implementation to ensure reproducibility.

1. Descriptive Statistical Approaches

Comprehensive descriptive analyses will precede all inferential testing to characterize the dataset and inform appropriate test selection. For continuous variables (muscle size, ROM), we will report means with standard deviations (SD) for normally distributed data and medians with interquartile ranges (IQR) and full ranges for non-normal distributions, supplemented by graphical representations including boxplots with superimposed individual data points to visualize both group trends and individual trajectories. Ordinal variables (strength, pain, effusion) will be summarized using median scores with IQRs and frequency distribution tables showing counts/proportions at each ordinal level, with stacked bar charts employed to visualize proportional changes across timepoints. These descriptive analyses will be stratified by group (experimental vs control) and timepoint to enable preliminary assessment of intervention effects and data distributions prior to formal hypothesis testing.

2. Data Characteristics and Variable Classification

The dataset comprises both continuous and ordinal variables requiring distinct analytical approaches. Continuous variables including muscle circumference (measured via standardized tape measure protocol) and active range of motion (measured via goniometry by blinded assessors) will be analyzed for both central tendency and variability. Ordinal variables including manual muscle testing scores (Medical Research Council 0-5 scale), visual analog pain scores (recorded as integers 0-10), and clinician-rated effusion scales (0=none, 3=severe) will be analyzed using non-parametric methods due to their inherent rank-based properties and expected non-normal distributions. All variables will be assessed at three protocol-specified timepoints with identical measurement protocols maintained across groups to ensure comparability.

3. Normality and Parametric Assumption Testing

Formal statistical testing for normality assumptions will be conducted for all continuous variables prior to selecting inferential methods. The Shapiro-Wilk test ($\alpha=0.05$) will be applied to residuals from all continuous outcome measures within each group-by-time combination, supplemented by visual inspection of Q-Q plots and histograms to detect deviations from normality that may not reach statistical significance in small samples. Homogeneity of variance between groups will be verified using Levene's test for equality of variances when parametric between-group comparisons are planned. For instances where normality or homogeneity assumptions are violated (Shapiro-Wilk $p \leq 0.05$ or Levene's $p \leq 0.05$), pre-specified non-parametric alternatives will be implemented as detailed in subsequent sections. All assumption testing results will be documented in supplementary materials to demonstrate the appropriateness of selected analytical methods.

4. Primary Inferential Statistical Methods

4.1 Within-Group Longitudinal Analyses

For evaluating changes over time within each experimental arm, distinct approaches will

be employed based on variable characteristics. Continuous variables meeting normality assumptions will be analyzed using repeated measures ANOVA with Greenhouse-Geisser correction for sphericity violations, followed by paired t-tests with Bonferroni-adjusted α levels for post-hoc timepoint comparisons when omnibus tests reach significance. Non-normal continuous data and all ordinal outcomes will utilize the Friedman test (non-parametric equivalent to repeated measures ANOVA) followed by Wilcoxon signed-rank tests for post-hoc comparisons with appropriate multiplicity adjustments. Effect sizes will be reported as Cohen's d for parametric analyses and rank-biserial correlations for non-parametric tests to quantify the magnitude of observed changes independent of sample size considerations.

4.2 Between-Group Comparative Analyses

Experimental versus control group comparisons at each timepoint will employ independent samples t-tests for normally distributed continuous variables with equal variances, Welch's t-tests for unequal variances (as determined by Levene's test), and Mann-Whitney U tests for non-normal continuous or ordinal data. Between-group effect sizes will be reported as mean differences with 95% confidence intervals for parametric tests and Hodges-Lehmann median differences for non-parametric analyses. To account for baseline imbalances in this pilot study, exploratory analyses of covariance (ANCOVA) may be conducted for continuous outcomes using baseline measurements as covariates if substantial pre-intervention group differences are detected despite randomization.

5. Missing Data Handling Protocol

Missing data patterns will be formally evaluated using Little's MCAR test prior to primary analyses. Given the pilot nature of this study with limited sample size, primary analyses will employ complete case approaches with explicit reporting of missing data proportions by group and timepoint. Sensitivity analyses will compare complete case results with multiple imputation approaches using chained equations (MICE)

incorporating baseline characteristics and observed follow-up data as predictors. Participants with missing baseline data will be excluded from all analyses, while those missing later timepoints will be retained for analyses where their available data permits. A participant flow diagram will document attrition at each study phase.

6. Software Implementation and Reproducibility

All analyses will be conducted using Stata MP 18.0. Graphical outputs will adhere to STROND guidelines for transparent reporting of non-normal data distributions.

Reference:

Ardern, C. L., Webster, K. E., Taylor, N. F., & Feller, J. A. (2011). Return to sport following anterior cruciate ligament reconstruction surgery: A systematic review and meta-analysis of the state of play. *British Journal of Sports Medicine*, *45*(7), 596–606. https://doi.org/10.1136/bjsm.2010.076364

Hughes, L., Paton, B., Rosenblatt, B., Gissane, C., & Patterson, S. D. (2017). Blood flow restriction training in clinical musculoskeletal rehabilitation: A systematic review and meta-analysis. *British Journal of Sports Medicine*, *51*(13), 1003–1011. https://doi.org/10.1136/bjsports-2016-097071

Scott, B. R., Loenneke, J. P., Slattery, K. M., & Dascombe, B. J. (2015). Exercise with Blood Flow Restriction: An Updated Evidence-Based Approach for Enhanced Muscular Development. *Sports Medicine*, *45*(3), 313–325. https://doi.org/10.1007/s40279-014-0288-1