Faculty of Rehabiliatation & Allied Health Sciences

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STUDY PROTOCOL

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Program	Ph.D Rehabilitation Sciences
Department	FRAHS
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Title of study	Comparative Effects of Concurrent Training and Soleus Push-Ups on Neurogenesis-Related Biomarkers in Patients with Diabetic Peripheral Neuropathy: A Randomized Controlled Trial
Objective (s) of the study	**1.** To compare the effects of soleus push-ups and concurrent training on circulating neurogenesis-related biomarkers (BDNF, NGF, and S100B) in patients with Type 2 Diabetic Peripheral Neuropathy. **2.** To examine whether exercise-induced modulation of neurogenesis-related biomarkers is associated with improvements in neuropathy symptoms, postural stability and balance, quality of life, and glycemic control in individuals with Diabetic Peripheral Neuropathy. **3.** To examine whether variations in glycemic control are associated with differences in neurogenesis-related biomarkers and corresponding changes in neuropathy symptoms, balance performance, and health-related quality of life in patients with Diabetic Peripheral Neuropathy. **4.** To assess and compare the acceptability, perceived appropriateness, and feasibility of soleus push-ups and concurrent training interventions.
Methodology **(Design, sample size, sampling technique, Study setting, inclusion and exclusion criteria and Tool)**	**Design** A randomized controlled trial (RCT) design with three parallel groups. Participants diagnosed with Type 2 Diabetic Peripheral Neuropathy (DPN) will be randomly allocated into one of three intervention arms: 1. Concurrent Training Group (CT): Participants will undergo a combined aerobic and resistance exercise program. 2. Soleus Push-Up Group (SPU): Participants will perform seated plantar-flexion exercises targeting the soleus muscle, using a controlled movement protocol modeled after the soleus push-up method.

	3. Control Group: Participants will receive standard diabetes care and general lifestyle advice without structured exercise intervention. **Sample Size** A total sample size of 99 participants (33 per group) was determined for this randomized controlled trial. The sample size was calculated using the G*Power software version 3.1.9.7 (Heinrich-Heine-University, Düsseldorf, Germany). As no previous study has evaluated the same population, intervention, and outcome measures, the effect size was conservatively set in the small-to-medium range (f = 0.25). This sample size provides a statistical power of 80% (1–β = 0.80) to detect an effect size of 0.25 at a 5% level of significance (α = 0.05) for the primary outcome measures. **Study Duration** The study duration would be 1.5 years after the approval. **Sampling Technique** Nonprobability Convenient Sampling would be used. **Study Setting** The study will be conducted in Pakistan Railways General Hospital and Mediplex Health Care Center, Rawalpindi. **Selection Criteria** **Inclusion Criteria** **• Age** 40–65 years **• Diagnosed Type 2 Diabetes Mellitus (T2DM)** **• Confirmed Diabetic Peripheral Neuropathy (DPN)** Diagnosis is based on clinical evaluation and scoring tools such as: o Michigan Neuropathy Screening Instrument (MNSI) Cut-off value of ≥4 for Part A (History)(104) Cut-off value of ≥2 from Part B (Examination)(105) o Vibration Pressure Threshold (≥25 Volts)(106) **• HbA1c between 6.5% and 9.0%** Reflecting moderate glycemic control, patients with extremely poor control were excluded for safety. **• Stable medication regimen** No changes in antidiabetic, antihypertensive, or neuropathy medications in the past 3 months. **• Sedentary or low physical activity level** Based on standard questionnaires (International Physical Activity Questionnaire – IPAQ), not currently engaged in structured exercise programs. **• Ability to walk independently** To ensure safety during aerobic or resistance exercise (for Group A). **• Able to understand and follow instructions** **• Consent to participate and comply with study protocol** **• Written informed consent, obtained before randomization.** **3.6.2. Exclusion Criteria**

	• **History of recent cardiovascular events** Includes myocardial infarction, stroke, or any cardiac intervention within the past 6 months. • **Uncontrolled hypertension** Blood pressure ≥160/100 mmHg at rest. • **Severe musculoskeletal disorders or physical disability** Including joint deformities, fractures, or amputation that limit the ability to perform exercise. • **Severe diabetic foot ulcers or active lower limb infection** Increases risk during lower limb activity or exercise. • **End-stage renal disease (CKD stage 4 or higher)** Associated systemic complications may confound study outcomes or affect safety. • **Severe retinopathy or proliferative diabetic eye disease** Exercise may pose risks such as retinal hemorrhage. • **Current Smoker, tobacco or alcohol consumer.** That may affect the blood biomarkers. • **Cognitive impairment or psychiatric disorders** That may affect understanding, compliance, or safety during exercise. • **Participation in a structured exercise program within the last 3 months** To avoid training-induced bias and ensure a sedentary **baseline.** • **Chronic inflammatory or autoimmune conditions** Conditions like rheumatoid arthritis or lupus may alter neurotrophic biomarkers. • **Use of medications affecting neurogenesis or metabolism** Such as corticosteroids, antipsychotics, or immunosuppressants. • **Diagnosed sleep disorder** That may affect the outcomes of the intervention. • **Pregnancy or lactation** Due to altered physiology and ethical concerns. **Tools** **Primary outcomes:** Blood biomarkers of neurogenesis **Biomarker** — **Relevance** BDNF (Brain-Derived Neurotrophic Factor) — Neuroplasticity, nerve regeneration NGF (Nerve Growth Factor) — NGF is the hallmark neurotrophic factor for peripheral nerve regeneration S100B (Schwann Cell Marker) — S100B is secreted by Schwann cells, which are key mediators of peripheral nerve repair and remyelination. **Secondary outcomes:** • Michigan Neuropathy Screening Instrument (MNSI)

• Vibration perception threshold (VPT),
• Force plates
• HbA1c
• Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)
• Acceptability of Intervention Measure (AIM), Intervention Appropriateness Measure (IAM), & Feasibility of Intervention Measure

Intervention

Group A: Combined aerobic and resistance training

Total Session Duration: 50–60 minutes	**Frequency**	**Intensity**	**Time**	**Type**
Component				
Aerobic	3-4 days/week	Moderate (50–70% HR max)	20-30 mins	Walking, step-ups, ankle pumps, cycling
Resistance	3-4 days/week	Moderate (50–70% 1RM or RPE 5–6/10)	20-30 mins	Band/rope/ body-weight training

Warm-Up (5–7 minutes)
• Marching in place
• Arm circles
• Ankle circles
• Seated ankle pumps

Aerobic Exercises (20–30 minutes)	**Sets x Time**	**Equipment**
Exercise		
Treadmill/Indoor walk/ Static cycling	1 × 5-7 min	Body only
Step-ups on a low platform	3 × 10 reps × both legs	Step or box
Ankle pumping seated	2 × 20 reps	None
Rope pulling (rhythmic)	3 × 2 min	Wall rope station
Seated Gym Ball Bouncing	10 bounce × 3 sets	Gym Ball

Resistance Training (20–30 minutes)	**Exercise**	**Sets x Reps**	**Equipment**
Muscle Group			

Lower limb	Sit-to-stand (chair squats)	3 × 10–15 reps	Chair only
Lower limb	Resistance band leg extensions	3 × 10 reps/leg	Resistance band
Lower limb	Calf raises	3 × 12 reps	Body weight
Upper limb	Wall push-ups	3 × 10 reps	Wall
Upper limb	Bicep curls (band/rod)	3 × 10 reps	Band or weighted rod
Upper limb	Seated rowing with a Resistance band	3 × 12 reps	Resistance band
Core	Seated trunk rotations (with rod)	3 × 10 reps/side	Rod or stick
Core	Bird dog (on mat)	3 × 10 reps	Mat

Cool Down (5–7 minutes)
• Deep breathing
• Gentle hamstring and calf stretch
• Toe curling and spreading
• Ankle alphabet **Group B: Soleus push-ups**

• Soleus-dominant, moderate-intensity push-ups (SDMISPU) performed seated with ankle weights (30–40% of soleus muscle 1RM).

• Aim: improve soleus muscle endurance, glucose utilization, and postprandial metabolic response.

Weeks 1–2 (Adaptation Phase)

• Frequency (F): 5-7 days/week

• Intensity (I): 30% 1RM

• Time (T): Single session, three sets of Soleus Push-ups till the point where the muscle started to fatigue, after 30 minutes of every meal

Weeks 3–4 (Progression Phase 1)

• Frequency: 5-7 days/week

• Intensity: 35% 1RM

	• Time: Single session, three sets of Soleus Push-ups till the point where muscle started to fatigue, after 30 minutes of every meal **Weeks 5–6 (Progression Phase 2)** • Frequency: 5-7 days/week • Intensity: 40% 1RM • Time: Single session, three set of Soleus Push-ups till the point where muscle started to fatigue, after 30 minutes of every meal **Weeks 7–8 (Consolidation Phase)** • Frequency: 5-7 days/week • Intensity: 40% 1RM • Time: Single session, three sets of Soleus Push-ups till the point where muscle started to fatigue, after 30 minutes of every meal
Data collection procedure	• Consent will be taken before randomization. • Participants will be assessed for eligibility according to the Inclusion and Exclusion Criteria. • Randomization of participants will be done through the sealed envelope method, and allocation concealment will be observed. • Baseline measurements will be taken through outcome tools. • Intervention will be provided according to the allocated group. • Follow-up assessment will be done after 4 weeks and 8 weeks.
Ethical considerations	Participants will be informed and consent will be taken for: **Risks**: Mild fatigue, soreness, or discomfort during exercises. **Confidentiality:** All information will be kept strictly confidential; participants name will not appear in any reports. **Voluntary Participation**: Participation is voluntary, and participants may withdraw at any time without affecting medical care.

STATISTICAL ANALYSIS PLAN

I. Data Preparation and Screening

1. Data Entry and Cleaning

- Double-entry verification in SPSS.
- Range and consistency checks.
- Handle missing data using **multiple imputation** (if <10%) or **intention-to-treat (ITT)** approach.

2. Normality Tests

- Shapiro–Wilk test and visual methods (histograms, Q-Q plots).
- Log transformation for skewed variables (e.g., BDNF or HbA1c).

3. Homogeneity of Variance

- Levene's Test for equality of variances.
- Box's M test for multivariate homogeneity.

II. Descriptive Statistics

Variable Type	**Analysis**	**Output**
Continuous (e.g., BDNF, HbA1c)	Mean ± SD	Tables & graphs
Categorical (e.g., gender, treatment adherence)	Frequency & percentage	Tables
Baseline comparison	One-way ANOVA or Chi-square	Verify randomization success

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III. Inferential Statistical Analysis

1. Primary Analysis — Neurogenesis Markers (BDNF, NGF, S100B)

- **Design:** 3 groups × 3 time points
- **Test: Two-way Repeated Measures ANOVA (Mixed ANOVA)**
    - Between-subject factor: Group (SPU, CT, Control)
    - Within-subject factor: Time (Baseline, 4th, 8th week)
- **Post-hoc:** Bonferroni correction for pairwise comparisons.
- **Effect Size:** Partial Eta Squared (η^2).
- **Interpretation:**
    - Significant Group × Time interaction = differential effect of exercise on neurogenesis.

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2. Secondary Analyses

a. Neuropathy Severity and Quality of Life

- **Tests:**
    - Repeated Measures ANOVA (for MNSI, VPT, QoL).
    - If data not normal → Friedman test (non-parametric alternative).
- **Post-hoc:** Wilcoxon signed-rank or Mann–Whitney U with Bonferroni adjustment.

b. Glycemic Control (HbA1c)

- **Test:** Paired t-test (within-group) and One-way ANOVA (between groups).

- **Effect Size:** Cohen's d.

c. Acceptability, Feasibility, Appropriateness

- **Test:** Kruskal–Wallis H test (non-parametric, ordinal data).
- **Follow-up:** Dunn's multiple comparison test.

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3. Correlation and Regression Analysis

a. Correlation

- **Pearson's correlation** (if data are parametric) or **Spearman's rho** (if not) Between:
    - Neurotrophic markers (BDNF, NGF, S100B)
    - Functional outcomes (MNSI, VPT)

b. Regression

- **Multiple Linear Regression** to determine predictors of neurogenesis (BDNF) using HbA1c, exercise adherence, and neuropathy scores as independent variables.

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4. Intention-to-Treat (ITT) and Per-Protocol Analysis

- Conduct **ITT analysis** including all randomized participants using last observation carried forward (LOCF).
- Conduct **Per-protocol analysis** on participants completing ≥80% of sessions.
- Compare both to ensure robustness.

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5. Significance Level

- **Alpha level (p):** 0.05 (two-tailed)
- **Confidence Interval:** 95%
- **Statistical Power:** 0.80 (sample = 99 adequate for 3×3 design)

Consent form (English)

Comparative Effect of Soleus Push-Ups and Concurrent Training on Neurogenesis in Patients with Type 2 Diabetic Peripheral Neuropathy (DPN): A Randomized Controlled Trial

Purpose: You are invited to participate in a research study to understand how physical activity and rehabilitation affect symptoms of diabetic neuropathy and overall quality of life.

Procedures: You will fill out a questionnaire, perform supervised physical activities, and attend follow-up visits.

Risks: Mild fatigue, soreness or discomfort during exercises.

Benefits: Possible improvement in symptoms or quality of life; help researchers understand better ways to manage diabetic neuropathy.

Confidentiality: All information will be kept strictly confidential; your name will not appear in any reports.

Voluntary Participation: Participation is voluntary and you may withdraw at any time without affecting your medical care.

Consent Statement: I have read and understood the information above. I voluntarily agree to participate.

Participant Name: _____ Signature/Thumb Impression: _____ Date: _____

Investigator Name: _____ Signature: _____ Date: _____