1. Study Protocol

Title: Chronotype-Aligned Exercise Timing in Middle-Aged Adults at Cardiometabolic Risk: A Randomized Controlled Trial

Objective:
To investigate whether aligning exercise timing with chronotype enhances cardiometabolic and sleep-related outcomes in sedentary adults with cardiovascular risk factors.

Study Design:

- 12-week, single-center, randomized controlled trial
- Participants stratified by chronotype (morning/evening) and cardiovascular risk level
- Randomized to:
    - Chronotype-Aligned Exercise (CAE) – exercise at preferred time
    - Chronotype-Misaligned Exercise (CME) – exercise at non-preferred time

Participants:

- Age: 40–60 years
- Sedentary adults with ≥1 cardiovascular risk factor (prehypertension, overweight/obesity, impaired fasting glucose, or family history of CVD)
- Exclusion: diagnosed CVD, diabetes, night-shift workers, irregular sleep schedules, certain medications affecting cardiovascular or circadian function

Recruitment:

- Outpatient departments of internal medicine and cardiology at 7 government hospitals in Lahore, Pakistan
- Additional recruitment via noticeboards, affiliated clinics, community initiatives, and digital media

Chronotype Assessment:

- Morningness-Eveningness Questionnaire (MEQ)
- 48-hour core body temperature monitoring (iButton DS1922L) to confirm chronotype

Randomization:

- Computer-generated permuted blocks (size 4 and 6)
- Stratified by chronotype and cardiovascular risk level
- Allocation concealed using sealed envelopes

Intervention:

- **Exercise type:** Moderate-intensity aerobic exercise (brisk walking or treadmill)

- **Duration:** 40 minutes/session (5 min warm-up, 30 min main, 5 min cool-down)
- **Frequency:** 5 sessions/week for 12 weeks
- **Timing:**
    - Morning exercise: 8–11 AM
    - Evening exercise: 6–9 PM
- Supervised at hospital gym with heart rate monitoring (Polar® H10)
- Physician present for safety monitoring

Outcome Measures:

- **Primary:** Systolic & diastolic blood pressure, heart rate variability (RMSSD)
- **Secondary:** VO_2 peak (estimated), LDL, fasting glucose, body weight, BMI, PSQI sleep score
- Measured at baseline (Week 0) and post-intervention (Week 12)

Blinding:

- Full blinding not feasible
- Outcome assessors were not blinded, but participants were unaware of study hypothesis

Statistical Analysis:

- Repeated measures ANOVA for group × time interaction
- Paired t-tests for within-group changes
- Independent t-tests for between-group comparisons
- Subgroup analyses by sex, chronotype, hypertension status
- Significance set at $p < 0.05$

Safety and Adherence:

- Adverse events monitored
- Adherence tracked via attendance logs and weekly reports
- ≥90% attendance considered compliant

Ethics:

- Conducted in accordance with the Declaration of Helsinki
- Institutional approval obtained
- Informed consent collected from all participants

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2. Statistical Analysis Plan (SAP)

Software: SPSS v24.0

Primary Outcomes:

- Systolic and diastolic blood pressure
- Heart rate variability (RMSSD)

Secondary Outcomes:

- VO_2 peak, total treadmill time, Bruce protocol stage
- LDL cholesterol, fasting glucose
- Body weight, BMI
- Sleep quality (PSQI)

Analysis Approach:

1. **Normality check:** Shapiro–Wilk test and Q–Q plots
2. **Baseline comparisons:**
    - Independent t-tests (continuous variables)
    - Chi-square tests (categorical variables)
3. **Main analysis:**
    - Repeated measures ANOVA to assess group × time interactions
    - Paired t-tests for within-group pre-post comparisons
    - Independent t-tests for between-group changes
4. **Subgroup analysis:**
    - Stratified t-tests and chi-square tests for sex, chronotype, hypertension status
5. **Regression analysis:**
    - Multiple linear regression to identify predictors of change in systolic BP
    - Covariates: group assignment, baseline values, age, sex, chronotype, medication use, exercise adherence
6. **Effect size:**
    - Cohen's d for t-tests
    - Partial eta squared (η^2) for ANOVA

Significance threshold: $p < 0.05$