Official Title:

Neuroimmune Response to Physical Exercise Training in Chronic Primary Low Back Pain

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Statistical analyses

This study is a mechanistic clinical trial aiming to examine the biological mechanisms underlying the effects of physical exercise (PE) training on chronic low back pain (CLBP), with a focus on immune responses, brain function, and neuroimmune interactions.

In June 2025, we initiated a pilot phase to determine the feasibility of our recruitment strategies (recruitment of patient cohorts of 5–8 patients who will receive the PE intervention or be waitlisted according to block randomization) and compliance with the exercise program and waitlist protocol, and all intermediary data collection points. Feasibility was confirmed in February 2026 and recruitment continued with subsequent cohorts beginning in March 2026. The analyses detailed below are those related to the primary aims of the study, which are mechanistic in nature.

At the time of this registration, no inferential analyses have been conducted on the biological or neuroimaging outcomes described below. Only preliminary descriptive analyses of the primary clinical outcome (pain intensity) have been conducted for feasibility-monitoring purposes. Because the primary objective of this project is mechanistic (i.e., understanding how PE training influences neuroimmune processes), the analyses described below will focus on biological and neurophysiological outcomes rather than intervention effectiveness.

Aim 1: **Characterize immune responses to acute and long-term exposure to physical exercise**

The first aim is to examine immune responses to PE training by evaluating changes in immune-related gene expression using blood transcriptomic analyses (RNA sequencing). Blood samples will be collected at baseline, and at multiple time points following the first exercise session (14 hours, 48 hours, and 2 weeks) to characterize acute immune responses over time, with an additional collection at the primary endpoint for patients only (end of the intervention period).

RNA sequencing data will first undergo standard preprocessing and normalization, followed by differential gene expression analyses using established RNA sequencing statistical frameworks. Gene-level and pathway-level results will then be integrated into longitudinal analyses.

Changes in gene expression across time points will subsequently be examined using longitudinal statistical models (e.g., linear mixed-effects models) with time, group (PE

vs. waitlist), and their interaction as fixed effects, and participant as a random effect to account for repeated measures. These models will evaluate differences in immune responses over time at both the gene and pathway levels between intervention and waitlist groups. Exploratory analyses will compare these responses between healthy controls and CLBP patients.

These analyses are contingent on the availability of funding for transcriptomic sequencing and downstream bioinformatic processing. Funding applications for these analyses are currently under review.

Aim 2: **Examine neuroimmune mechanisms associated with exercise-induced pain relief**

The second aim is to examine relationships between immune responses and changes in brain function and autonomic regulation. Brain outcomes include measures of functional connectivity and diffusion-weighted MRI, while autonomic function will be assessed through heart rate variability (HRV).

Primary MRI analyses will prioritize corticostriatal circuitry, particularly functional connectivity between the nucleus accumbens and medial prefrontal cortex, as well as the structural integrity of the white matter tract connecting these regions. Additional imaging metrics, including whole-brain connectivity measures and multivariate pain signatures, will also be examined to characterize broader neural changes associated with pain and exercise. HRV analyses will include RMSSD (Root Mean Square of Successive Differences) as a primary metric of vagal function.

Associations between changes in gene expression, brain measures, HRV, and pain outcomes will be explored using multivariable and mediation-based statistical approaches to examine potential relationships between immune, neural, and autonomic variables.

These analyses are contingent upon available funding and may be conducted once sufficient resources are obtained for the processing and analysis of the neuroimaging and transcriptomic data.

Aim 3: **Compare neuroimmune function in CLBP patients and healthy controls**

The third aim is to examine differences in neuroimmune function between individuals with CLBP and healthy controls. Baseline differences in gene expression, brain imaging measures, and HRV will be assessed using independent t-tests or equivalent non-parametric tests.

To examine the effects of PE training on these outcomes, linear mixed-effects models will be used with time (baseline vs. endpoint), group (PE vs. waitlist), and their interaction as fixed effects. These analyses will assess whether changes over time differ between intervention and control groups.

The recruitment of healthy controls will be conducted after all patient data collection has been completed, contingent on available funding.

General Analytical Approach

All analyses will be conducted using *R* and *RStudio*.

Analyses of the primary clinical outcome (pain intensity) will follow an intention-to-treat (ITT) approach. However, given that the primary aims of this study are mechanistic rather than effectiveness-focused, the biological and neurophysiological analyses described above will primarily be conducted using a per-protocol (PP) approach. Participants assigned to the PE intervention may be excluded from mechanistic analyses if adherence to the prescribed training sessions is insufficient (e.g., completion of less than 80% of sessions) or if substantial missing data (over 50%) occur during the final weeks of the intervention.

Because recruitment will occur in cohorts and biological analyses may be conducted as data become available, some analyses may be conducted and published before completion of the full planned sample size. Finally, given the multidisciplinary nature of the project and the large number of biological and neuroimaging outcomes, different outcomes described in this registration may be reported in separate publications or as part of different manuscripts.

Additional exploratory analyses may be conducted to further investigate relationships among clinical, immune, autonomic, and neuroimaging variables.