

Molecular Transducers of Physical Activity Consortium (MoTrPAC) Sedentary Adult Study

Statistical Analysis Plan for ClinicalTrial.gov Hypotheses

Version 1.1

April 8, 2026

TABLE OF CONTENTS

1	Summary of Study Design.....	3
2	Study Outcomes.....	3
2.1	Primary Outcomes	3
2.2	Secondary Outcomes	4
3	General Approach	4
3.1	Intention to Treat.....	4
3.2	Control of Type I Error.....	4
3.3	Covariate Adjustment	4
3.4	Missing Data	4
4	Analytical Plan for Specific Outcomes	5
4.1	Primary Outcome: Peak VO2	5
4.2	Primary Outcome: Isometric Knee Peak Torque.....	5
4.3	Secondary Outcomes	6
4.4	Exploratory Outcomes	6
5	References.....	7

1 Summary of Study Design

The Molecular Transducers of Physical Activity Consortium (MoTrPAC) is a mechanistic randomized controlled trial (RCT) that will enroll ~1,500 sedentary adults. After screening the sedentary volunteers to determine eligibility, participants are randomized to one of three intervention groups: endurance exercise (EE), resistance exercise (RE), and no exercise (Control). All sedentary participants undergo phenotyping visits and familiarization visits to prepare them for the acute exercise/rest visit, which is when biospecimens are collected for the characterization of molecular responses to exercise. After completing the acute exercise/rest visit, participants undergo the exercise/no exercise control interventions for 12 weeks. The phenotyping visits and acute exercise/rest visit with biospecimen collections are then repeated.

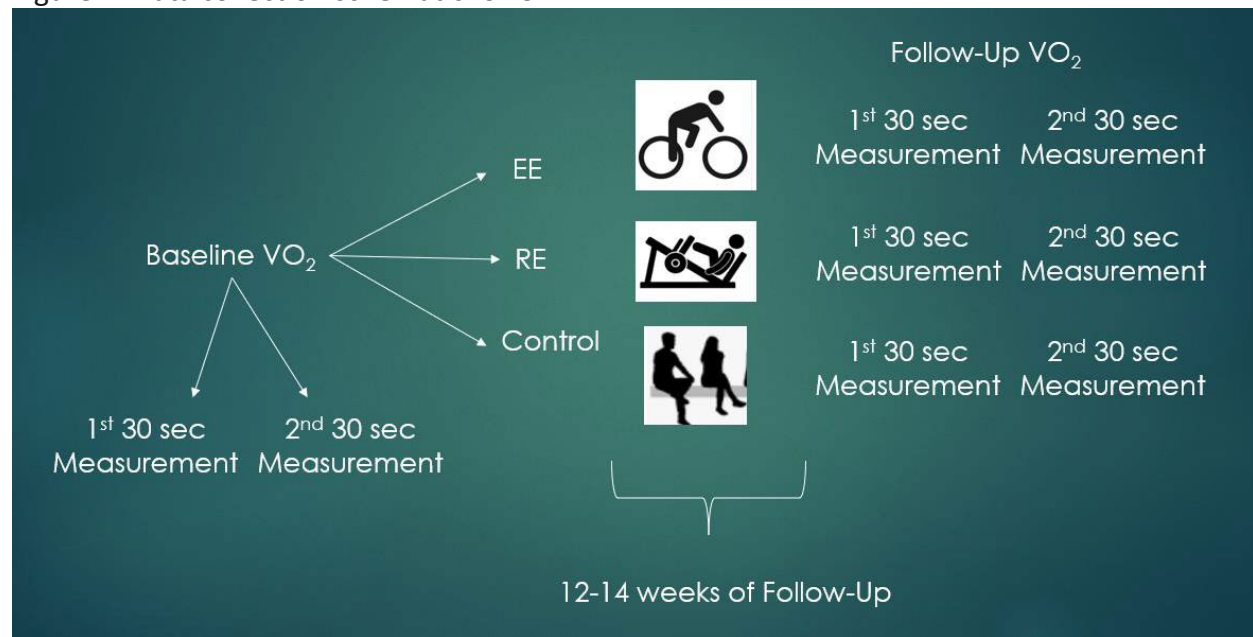
2 Study Outcomes

2.1 Primary Outcomes

The primary aim is to test the hypotheses that EE, RE and Control will have differential effects on cardiorespiratory fitness and muscular strength. The primary outcomes include peak oxygen consumption (Peak VO₂) and isometric knee peak torque.

The Cardiopulmonary Exercise Test (CPET) to assess Peak VO₂ is completed on all sedentary participants at baseline and 12 weeks post-intervention. The CPET is to occur after at least a 4-hour fast from food and beverages except for water. The same CPET protocol is used at baseline and follow-up. Peak VO₂ is defined as the average VO₂ of the final two valid 30-second intervals during the CPET.

Figure 1: Data collection schematic for CPET



The isometric knee extension test is completed on all sedentary participants at baseline and 12 weeks post-intervention. Participants will perform a total of three Maximal Voluntary Contractions (MVC) trials. Peak torque is defined as the peak torque of 3 valid trials with Coefficient of Variation (CoV) $\leq 10\%$.

2.2 Secondary Outcomes

The secondary aim is to test the hypotheses that EE and RE relative to Control will improve metabolic biomarkers. The secondary outcomes include HDL-C, LDL-C, triglyceride, and HbA1c. These blood biomarkers are on all sedentary participants at baseline and 12 weeks post-intervention.

3 General Approach

3.1 Intention to Treat

The analysis of the primary and secondary outcomes will be according to the principle of intent- to-treat, i.e., all randomized participants will be analyzed according to their original intervention assignment regardless of adherence to protocol.

3.2 Control of Type I Error

To avoid inflation of the family-wise Type I error rate for the primary outcomes, Peak VO₂ and isometric knee peak torque will each be tested at 0.025 level (2-sided). For each primary outcome we will perform pairwise comparisons between EE, RE, and control groups. Bonferroni's method will be used to adjust for multiple comparisons (3 pairwise comparisons) and control type I error rate for each primary outcome at 0.025 for these comparisons. No adjustments for multiplicity will be applied on the secondary and exploratory outcomes. Accordingly, each secondary and exploratory outcome will be tested at 0.05 level (2-sided) and Bonferroni corrections will be made to adjust for the 3 pairwise comparisons between randomized groups. Results of analyses of secondary and exploratory outcomes will be interpreted as supportive evidence relative to the overall impact of the different modes of physical activity.

3.3 Covariate Adjustment

All primary analyses will adjust for clinical site and sex that are used as stratification factors in randomization. In sensitivity analyses, baseline age, baseline weight, and follow-up weight if appropriate, will also be included as covariates to increase precision of parameter estimates in sensitivity analysis.

3.4 Missing Data

Missing data are inevitable and statistical methods must take this into account to draw valid conclusions. Information collected during the study related to reasons that values are missing

will be helpful in examining assumptions about missing data, e.g., whether data are missing completely at random (MCAR), missing at random (MAR), or missing not at random (MNAR). In general, our analytical plans follow the recommendations of the 2010 National Academy of Sciences report regarding the treatment of missing data in clinical trials [National Research Council, 2010]. To identify factors that provide information as to the probability of missing outcomes, we will first compare the baseline characteristics of participants who do and do not have specific follow-up measures. Sensitivity analyses to determine how conclusions from primary outcome models may be affected by missing data initially will be performed by including covariates predictive of missing observations in such models. Such sensitivity analyses are intended as a conservative reexamination of data to explore whether reasonable assumptions placed on missing data might alter an observed finding, but primary consideration will be given to the original analysis of the aim. Additionally, the linear mixed effects model fitted by maximum likelihood estimation, which will be used for all the primary and secondary outcomes, is unbiased if missing data are unrelated to outcomes; i.e., if the data are considered MCAR or MAR.

4 Analytical Plan for Specific Outcomes

4.1 Primary Outcome: Peak VO₂

The primary analysis for Peak VO₂ will use the average VO₂ (L/min) of the final two valid 30-second intervals during the CPET as specified by the protocol. We will employ constrained linear mixed effect models (cLMM), a longitudinal data analysis approach, to test the group differences while constraining the baseline means to be equal between groups (Coffman, Edelman, Woolson 2016). Preliminary data indicate that log transformation improves the normality (of residuals) assumption for Peak VO₂. Therefore, the analysis will be performed on the log scale and the contrast estimates will represent relative change from baseline. We will estimate percentage change from baseline within EE, RE, and Control groups. An initial test of the global hypothesis that these changes are all equal will be conducted, followed by pairwise comparisons between EE, RE, and Control groups to evaluate the intervention effects.

Supporting analyses: Peak VO₂ will also be analyzed accounting for body weight. Relative Peak VO₂ normalized to body weight (ml/kg/min) is a ratio and use of ratios as dependent variables in regression analysis can lead to misleading inferences (Kronmal 1993). Therefore, we will also include baseline and follow-up weight in cLMM as a time-varying covariate to adjust for body weight. Additionally, another analysis will utilize both the 1st and the 2nd 30-second VO₂ measurements, instead of the average, to allow more flexible modeling of Peak VO₂ and potentially provide more power for testing hypotheses involving pre-post comparisons. Linear mixed effect models (LMM) will be used to incorporate all 4 VO₂ measures (2 baseline and 2 follow-up) while accounting for correlations of the repeated measures. This approach will allow us to estimate within-visit and between-visit Peak VO₂ correlations. It will also allow us to estimate change in VO₂ from the 1st to the 2nd 30-second measurement as well as all the group

differences between EE, RE, and Control.

Subgroup analyses: Pre-specified subgroup comparisons include sex and age categories. We will report tests of interactions to assess the consistency of differences between intervention groups for each of these subgroups.

4.2 Primary Outcome: Isometric Knee Peak Torque

The primary analysis for peak isometric knee strength will use the peak torque (in Newton meters) of 3 valid trials with CoV $\leq 10\%$ in the isometric knee extension test. Like the approach described in Section 4.1, we will employ cLMM to estimate (absolute) change from baseline within EE, RE, and Control groups. An initial test of the global hypothesis that these changes are all equal will be conducted, followed by pairwise comparisons between EE, RE, and Control groups to evaluate the intervention effects. We will conduct model diagnostics and use log transformation for analysis if warranted.

Supporting analyses: Like the approach described in Section 4.1, we will include baseline and follow-up weight in cLMM as a time-varying covariate to adjust for body weight. Average peak torque will also be analyzed.

Subgroup analyses: Pre-specified subgroup comparisons include sex and age categories. We will report tests of interactions to assess the consistency of differences between intervention groups for each of these subgroups.

4.3 Secondary Outcomes

The secondary outcomes include HDL-C, LDL-C, triglyceride, and HbA1c. Like the approach described in Section 4.1, we will employ cLMM to estimate (absolute) change from baseline within EE, RE, and Control groups. An initial test of the global hypothesis that these changes are all equal will be conducted, followed by pairwise comparisons between EE, RE, and Control groups to evaluate the intervention effects. We will conduct model diagnostics and use log transformation for analysis if warranted.

4.4 Exploratory Outcomes

The following CPET and physical measures will be examined as exploratory outcomes.

- Peak Ventilation (L/min)
- Peak VO₂ pulse (ml/beat)
- Peak RER
- Peak workload
- Peak heart rate
- Resting SBP
- Resting DBP
- Peak hand grip strength

5 References

National Research Council (2010). The prevention and treatment of missing data in clinical trials. National Academies Press.

Liu GF, Lu K, Mogg R, Mallick M, Mehrotra DV. Should baseline be a covariate or dependent variable in analyses of change from baseline in clinical trials? *Statist. Med.* 2009; 28:2509–2530.

Coffman CJ, Edelman D, Woolson RF. To condition or not condition? Analysing 'change' in longitudinal randomised controlled trials. *BMJ Open*. 2016 Dec 30;6(12):e013096. doi: 10.1136/bmjopen-2016-013096.

Kronmal RA. Spurious correlation and the fallacy of the ratio standard revisited. *J. R. Statist. Soc. A* 1993;156(3):379-392.

Log of changes to SAP:

2/27/25

Section 2.2 → During development of the code to perform the pre-specified analyses, and prior to the final participant visit and final database closure, the MoTrPAC Coordinating Center identified that the available multiple comparisons procedures for Tukey's approach could not accommodate the contrasts of interest from the constrained mixed models. Accordingly, on 2/27/25 a decision was made to use Bonferroni's multiple comparison procedure to adjust for multiple comparisons between randomized groups for primary, secondary and exploratory outcomes. Changes have been made in Section 2.2 to replace the use of Tukey's method with Bonferroni's method and to clarify that the Bonferroni method would be used for pairwise comparisons of secondary and exploratory outcomes between randomized groups.

4/8/26

Section 3.1 → Minor grammatical clarification was made in Section 3.3 that analyses including age and weight as covariates would be considered sensitivity analysis.

Section 3.4 → An optional sensitivity analysis involving the use of pattern mixture models to account for missing outcomes was removed.

Section 4.4 → SBP and DBP at termination of the CPET was initially identified as an exploratory outcome when this outcome was supposed to be resting SPB and DBP. The terminology has been changed to resting.