STATISTICAL ANALYSIS PLAN (SAP)
The Relationship Between Exercise Frequency, Intensity, and Restoration of Cardiometabolic Health
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1.0 INTRODUCTION
Involvement in regular physical activity is known to elicit systemic adaptations and reduce the risk of cardiometabolic diseases, including hypertension, obesity, dyslipidemia, and hyperglycemia. As many of these beneficial effects are transient in nature, exercise-mediated adaptations are largely dependent on the type of exercise protocol conducted. However, the ideal exercise recommendations in clinical populations remain elusive.

2.0 OUTCOMES AND OBJECTIVES
2.1 Primary Objectives
This study aims to examine the efficacy of endurance (END), sprint (SIT), or combined endurance-sprint (COMB) training protocols in respect to improving cardiorespiratory fitness (VO\textsubscript{2} peak) and 24-hour blood glucose area under the curve (AUC) as assessed by continuous glucose monitoring devices.

2.2 Secondary Objectives
Several other parameters indicative of cardiometabolic health will be assessed, including:
- Fasting blood lipids
  - High-density lipoprotein (HDL), low-density lipoprotein (LDL), high-sensitivity C-reactive protein (hs-CRP), total cholesterol, triglycerides (TAG), free fatty acids (FFA)
- Post-prandial blood lipids
  - TAG and FFA following the consumption of an oral lipid beverage
- Blood pressure
- Body composition
  - Body weight, body mass index (BMI), total body fat and lean mass percentages
- Arterial stiffness (central carotid-femoral, via pulse wave velocity)
- Vascular function (brachial artery, via flow mediated dilation)
- Daily sedentary time

3.0 STUDY DESIGN
This is a parallel design, randomized clinical trial comparing the efficacy of three varying exercise training protocols. The study period will be a total of 7 weeks in duration, in which the first week will involve baseline testing followed by a 6-week training protocol. Full details of these procedures are provided in the attached Study Protocol document. The target sample size will be 45 individuals, as explained in SAP Section 4.1.

Recruited participants will be pre-diabetic, overweight, and sedentary male individuals, aged 45-70 years (full details in Study Protocol document, attached). All participant characteristics will be assessed at baseline and following the intervention period. Acute exercise-mediated effects on health outcomes will be assessed during both week 1 and week 6 of the intervention period, and will be compared to pre-exercising values. The temporal sequence of procedures performed throughout the study are depicted below in Table 1: Schedule of Procedures.
Table 1: Schedule of Procedures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 6</th>
<th>Post-Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Body composition (BMI, body fat, lean mass)</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VO₂ peak</td>
<td>x</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Pulse wave velocity</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>Flow mediated dilation</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Post-prandial glucose</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Fasting blood lipid profile</td>
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<td></td>
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<tr>
<td>Post-prandial lipid profile</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily sedentary time</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

4.0 STATISTICAL METHODOLOGY

4.1 Sample Size Calculations

The sample size for this study was calculated using an alpha of 0.05 and a power of 0.80. The two primary endpoints (used to determine improvements in fitness in response to exercise training) are cardiorespiratory fitness (VO₂ peak) and 2-hour blood glucose area under curve (AUC) following an OGTT. Based on previous literature involving chronic exercise intervention trials in similar populations, the standard deviation of VO₂ peak scores are approximately 3.5 mL/kg/min, and the difference in effect between pre- and post-exercise time points is approximately 4.0 mL/kg/min. Therefore, based on a VO₂ peak endpoint, a minimum sample size per group was calculated as 13 individuals. In respect to glucose AUC measures using CGM devices, the approximate standard deviation from previous literature was 61mmol/L/hr, while the difference in effect between intervention and baseline situations is approximately 63mmol/L/hr, resulting in a sample size calculation per group of 15 individuals.

This study will also investigate several novel parameters, such as arterial stiffness, between intervention groups. In a recent study assessing arterial stiffness between END and interval training, the standard deviation was 0.39m/s, and the difference in effect between END and HIIT training was 0.51m/s (more specifically, END improved arterial stiffness while HIIT did not). Therefore, based on the PWV endpoint, a minimum sample size per group was calculated as 10 individuals.

The sample size calculation will be based upon the largest individual sample size parameter, and as a result this study will require 15 participants per intervention group. As the study will involve three parallel intervention groups, a total of 45 participants will be required. Recruitment for this study will occur on a rolling basis, in which statistical significance will be assessed throughout based on study-specific effective sizes, standard deviations between groups, and power calculations. Should statistical significance be reached prior to recruitment of 45 participants (i.e. 15 each intervention group), the study will conclude.
4.2 Randomization
Participants will be randomized to one of three possible intervention groups. This will occur via stratification based on pre-exercise VO₂ peak and age.

4.3 Study Populations
Results will be analyzed on both intention-to-treat and per protocol basis. If these analyses do not differ, the findings will thereafter be reported as per protocol.

Intention-to-treat analysis is defined as a comparison between all subjects randomized to an allocated treatment group, irrespective of when they withdrew or discontinued the study. Subjects will be included in analysis based on the original treatment group they were randomized to.

Per-protocol analysis is defined as a comparison between treatment groups which only includes participants who successfully completed the original intervention.

4.4 Treatment Exposure and Compliance
Compliance to exercise protocols will be assessed during supervised exercise training session occurring at each laboratory visit. A minimum of 80% adherence will be required for full analysis on a per-protocol basis. This represents a maximum of three (3) missed SIT workouts, or a maximum of six (6) missed END or COMB workouts.

5.0 STATISTICAL ANALYSIS
Statistical significance will be based upon an alpha error probability of 5% and a power for 80%. The data will be assessed as continuous. Variables will be summarized with two-way repeated measure ANOVA with comparisons within and between group effects. Analysis of variance will be used to assess the effects of treatment interventions on the key outcomes in this study. If there are significant interactions, the differences between pairs of means will be determined with a Newman-Keuls post-hoc test.

5.1 Primary Outcomes
5.1.1 VO₂ Peak
The baseline and post-intervention results of this test will be summarized by treatment group as a continuous variable, and the change will be analyzed by a two-way repeated-measure ANOVA. Units of mL/kg/min will be used for all VO₂ peak aerobic capacity measures. The model will include intervention group (SIT vs. END vs. COMB) x time (pre- vs. post-intervention); i.e. 3 (group) x 2 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

5.1.2 Blood Glucose
The baseline, week 1, week 6, and post-intervention blood glucose AUC, calculated using the trapezoidal method, will be summarized by treatment group as a continuous variable. Units of mmol/L/min will be used for all blood glucose measures. The change between various time points will be analyzed using a two-way repeated-measure ANOVA. The model will include intervention group (SIT vs. END vs. COMB) x time (baseline vs. week 1 vs. week 6 vs. post-intervention); i.e. 3 (group) x 4 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

5.2 Secondary Outcomes
5.2.1 Fasting Blood Lipids
- High-density lipoprotein (HDL), low-density lipoprotein (LDL), high-sensitivity C-reactive protein (hs-CRP), total cholesterol, triglycerides (TAG), free fatty acids (FFA)

The baseline and post-intervention results of the various parameters measured within blood samples will be summarized by treatment group as a continuous variable using a two-way repeated-measure ANOVA. Each variable will be assessed independently. Units of mmol/L will be used for HDL; mmol/L for LDL; mg/L for hs-CRP; mmol/L for total cholesterol; mmol/L for TAG; and mmol/L for FFA. The model will include intervention group (SIT vs. END vs. COMB) x time (pre- vs. post-intervention); i.e. 3 (group) x 2 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

5.2.2 Post-Prandial Blood Lipids
- Triglycerides (TAG) and free fatty acids (FFA) following the consumption of an oral lipid beverage

The baseline and post-intervention results of the 6-hour plasma TAG and FFA AUC, using the trapezoidal method, will be summarized by treatment group as a continuous variable. Each variable will be assessed independently. Units of mmol/L will be used for both FFA and TAG assessments. The change between baseline and post-intervention will be analyzed using a two-way repeated-measure ANOVA. The model will include intervention group (SIT vs. END vs. COMB) x time (pre- vs. post-intervention); i.e. 3 (group) x 2 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

5.2.3 Blood Pressure
The resting blood pressure values from baseline, week 1, week 6, and post-intervention will be summarized by treatment group as a continuous variable and analyzed with a two-way repeated-measure ANOVA. Units of mmHg will be used for all blood pressure measures. The model will include intervention group (SIT vs. END vs. COMB) x time (baseline vs. week 1 vs. week 6 vs. post-intervention); i.e. 3 (group) x 4 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

On an acute timescale, blood pressure will be assessed pre-exercise, throughout the exercise bout, and up to 30-minutes post exercise. A two-way repeated-measure ANOVA model will summarize acute changes in blood pressure and will include exercise bout (SIT vs. END) x time. Mean values will be reported, expressed ± standard error, with a 95% confidence interval and p-value.

5.2.4 Body Composition
- Body weight, body mass index (BMI), total body fat and lean mass composition

The baseline and post-intervention results of the various parameters generated from the DXA scan will be summarized by treatment group as a continuous variable. Each variable will be assessed independently. Units of kg/m² will be used for BMI; kg for body weight; and both percentage and kilograms for body fat and lean mass composition. The change between baseline and post-intervention will be analyzed using two-way repeated-measure ANOVA. The model will include intervention group (SIT vs. END vs. COMB) x time (pre- vs. post-intervention); i.e. 3 (group) x 2 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.
5.2.5 Arterial Stiffness
Baseline and post-intervention arterial stiffness results will be summarized by treatment group as a continuous variable using a two-way repeated-measure ANOVA. Units of m/s will be used for all central arterial stiffness measures via pulse wave velocity. The model will include intervention group (SIT vs. END vs. COMB) x time (baseline vs. post-intervention); i.e. 3 (group) x 2 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

On an acute timescale, arterial stiffness will be assessed pre-exercise, immediately post-exercise, 15-minutes post-exercise, and 30-minutes post exercise. A two-way repeated-measure ANOVA model will summarize acute changes in arterial stiffness and will include exercise bout (SIT vs. END) x time (pre-exercise vs. post-exercise vs. 15-minutes post-exercise vs. 30-minutes post-exercise); i.e. 2 (group) x 4 (time) ANOVA. Mean values will be reported, expressed ± standard error, with a 95% confidence interval and p-value.

5.2.6 Vascular Function
The baseline and post-intervention results of this test will be summarized by treatment group as a continuous variable. Units of relative FMD (% change) will be used. The change between various time points will be analyzed using two-way repeated-measure ANOVA. The model will include intervention group (SIT vs. END vs. COMB) x time (baseline vs. post-intervention); i.e. 3 (group) x 2 (time) ANOVA. Statistical analysis will determine mean values per group, expressed ± standard error, and will be reported with a 95% confidence interval and p-value.

On an acute timescale, vascular function will be assessed pre-exercise, post-exercise, and 30-minutes post exercise. A two-way repeated-measure ANOVA model will summarize acute changes in vascular function and will include exercise bout (SIT vs. END) x time (pre-exercise vs. post-exercise vs. 30-minutes post-exercise); i.e. 2 (group) x 3 (time) ANOVA. Mean values will be reported, expressed ± standard error, with a 95% confidence interval and p-value.

5.2.7 Daily Sedentary Time
Baseline, week 1, week 6, and post-intervention daily sedentary time results will be summarized by treatment group as a continuous variable, using the units of hours/day. The change between various time points will be analyzed using two-way repeated-measure ANOVA. The model will include intervention group (SIT vs. END vs. COMB) x time (baseline vs. week 1 vs. week 6 vs. post-intervention); i.e. 3 (group) x 4 (time) ANOVA. Mean values will be reported, expressed ± standard error, with a 95% confidence interval and p-value.

5.3 Covariates
Covariates will be introduced into the models in order to assess the impact of certain potential confounding factors on outcome, including diet and any other variables that are observed during the study which may appear to potentially influence the outcome.

5.4 Handling of Dropouts and Missing Data
All participants will be administered a subject ID number during the baseline pre-screening visit. In the event a participant is not eligible for the study, the participant will be excluded from further analysis without randomization to a treatment group. All pre-screening data from these individuals will be destroyed.
Missing data will be handled with the single imputation method. This analysis is used commonly within clinical trials when missing data occurs completely at random, independent of the group allocation or study intervention\textsuperscript{6}. Briefly, in the event of a missing data point, group mean change scores will be imputed for the missing value in order to maintain aspects of the data distribution\textsuperscript{6}.

If a participant randomized to a treatment group discontinues the study at any time, the reason for the withdrawal will be recorded. Participant data will remain on file for intention-to-treat analysis following the completion of this study. Recruitment will occur on a rolling schedule and once statistical significance is achieved (stated in Section 4.1), recruitment for the study will conclude.

6.0 SAFETY ANALYSIS
6.1 Adverse Events
An adverse event is defined as any unfavorable and unintended medical occurrence during a clinical investigation, whether or not directly related to the intervention. The absolute number and percentages of adverse events (of any degree; ranging from mild to moderate to severe) will be tabulated in a general summary document.

7.0 REFERENCES