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. However, these beneficial effects may largely be dependent on the type of exercise protocol conducted. While research has characterized responses based on exercise intensity, many beneficial effects of exercise are transient in nature, and therefore exercise frequency may play an important, yet currently under-appreciated, role in improving health.

2.0 OUTCOMES AND OBJECTIVES

2.1 Primary Objectives

This study aims to examine the efficacy of high-frequency endurance (END) and low-frequency sprint (SIT) training protocols, performed as per general practice, with respect to improving cardiometabolic health. Primary outcomes include 1) cardiorespiratory fitness (VO_2 peak); and 2) glycemic regulation, which includes a) free-living glycemic regulation assessed by continuous glucose monitoring devices; and b) 2-hour postprandial glucose responses assessed by oral glucose tolerance test.

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), non-HDL cholesterol, and cholesterol/HDL ratio.
- Fasting glycosylated hemoglobin (HbA1C)
- Post-prandial blood lipid responses
    - TAG and FFA following the consumption of an oral lipid beverage
- Blood pressure (systolic blood pressure, diastolic blood pressure, calculated mean arterial pressure)
- Body composition
    - Body weight, body mass index (BMI), total and regional body fat and lean mass percentages
- Arterial stiffness (central carotid-femoral, via pulse wave velocity)
- Vascular endothelial function (brachial artery, via flow mediated dilation)

Secondary outcomes during the first week of exercise training (free-living glycemic regulation, acute post-exercise arterial stiffness and vascular function) will also be assessed.

3.0 STUDY DESIGN

This is a parallel design, randomized clinical trial determining the efficacy of two different 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 Study Protocol document. The target sample size was 24 individuals, as

explained in SAP Section 4.1. **The actual sample size was 23 participants.** 24 participants were recruited, however one individual (END group) discontinued the study (week 3) due to unrelated personal reasons.

Recruited participants will be overweight/obese and sedentary male individuals, aged 18-70 years (full details in Study Protocol document). All participant characteristics will be assessed at baseline and following the intervention period. Acute exercise-mediated effects will be assessed during week 1 of the intervention period. The temporal sequence of procedures performed throughout the study are depicted below in *Table 1: Schedule of Procedures.*

Table 1: Schedule of Procedures

Parameter	Baseline	Week 1	Week 6	Post-Intervention
Blood pressure	x	x	x	x
Body composition (BMI, body fat, lean mass)	x			x
VO_2 peak	x			x
Free-living glycemic regulation	x	x	x	x
Pulse wave velocity	x	x		x
Flow mediated dilation	x	x		x
Post-prandial glucose tolerance (OGTT)	x			x
Fasting venous blood lipid profile (and HbA1C)	x			x
Post-prandial lipid tolerance (OFTT)	x			x
Daily sedentary/active time	x	x	x	x

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 primary endpoints are cardiorespiratory fitness (VO_2 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 (1-5), the standard deviation of **VO_2 peak** scores are approximately ~3.5 mL/kg/min, and the difference in effect between pre- and post-exercise time points is ~4.0 mL/kg/min. Therefore, based on a VO_2 peak endpoint, a minimum sample size per group was calculated as 12 individuals. With respect to **glucose AUC measures** during a glucose tolerance test, the approximate standard deviation from previous literature (6) was ~92mmol/L/hr, while the difference in effect between intervention and baseline timepoints is ~78mmol/L/hr, resulting in a sample size calculation per group of ~8 individuals.

The sample size calculation will be based upon the largest individual sample size parameter, and as a result this study will require 12 participants per intervention group. As the study will involve two parallel intervention groups, a total of 24 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 and standard deviations between groups. Should statistical significance be reached prior to recruitment of 24 participants (i.e 12 each intervention group), the study will conclude.

4.2 Randomization
Participants will be randomized to one of two possible intervention groups. This will occur based on pre-exercise VO_2 peak, BMI, 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 whether they fully completed the intervention protocol. 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. **Upon study completion, all participants (n=23) who finished the study successfully completed the 6-week intervention.** One participant (END group) discontinued the study (week 3) due to personal reasons, and as a result there was no final data on this individual, therefore this individual was not included in analysis.

4.4 Treatment Exposure and Compliance
Compliance to exercise protocols will be assessed during supervised exercise training sessions 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 workouts. **Upon study completion, adherence was 94-100% for all participants (n=23) who conducted the full study.**

5.0 STATISTICAL ANALYSIS
Statistical significance will be based upon an alpha error probability of 5%. The data will be assessed as continuous. Variables will be summarized with one- or two-tailed paired Student's *t*-tests comparing the effects of exercise within each group, with full details listed below.

5.1 Primary Outcomes
5.1.1 VO_2 Peak
Pre-training and post-training results of this test will be summarized by treatment group as a continuous variable, and pre-training vs. post-training values will be analyzed using a two-tailed paired Student's *t*-test within each group. Units of mL/kg/min will be used for all VO_2 peak aerobic capacity measures.

5.1.2 Free-living Glycemic Regulation
Interstitial glucose AUC measures will be calculated using the trapezoidal method and will be summarized by treatment group as a continuous variable. Units of mmol/L/hr will be used. Average and peak daily glucose levels will also be determined for each day in units of mmol/L. Pre-training vs. post-training values will be analyzed using a one-tailed paired Student's *t*-test within each group. Given the variable frequency of training, free-living glycemic parameters will also be compared on exercise vs. rest days of training within each individual and analyzed using a one-tailed paired Students *t*-test.

5.1.3 Oral Glucose Tolerance

Blood glucose AUC will be calculated using the trapezoidal method and will be summarized by treatment group as a continuous variable. Units of mmol/L/min will be used. Pre-training vs. post-training values will be analyzed using a one-tailed paired Student's *t*-test within each group.

5.2 Secondary Outcomes

5.2.1 Fasting Blood Lipids and HbA1C

- High-density lipoprotein (HDL), low-density lipoprotein (LDL), high-sensitivity C-reactive protein (hs-CRP), total cholesterol, triglycerides (TAG), free fatty acids (FFA), non-HDL cholesterol, cholesterol/HDL ratio, and HbA1C.

Pre-training and post-training results of the various parameters measured within blood samples will be summarized by treatment group as a continuous variable and compared using a one-tailed paired Student's *t*-test within each group. 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; mmol/L for FFA; mmol/LD for non-HDL cholesterol; and % for HbA1C.

5.2.2 Post-Prandial Blood Lipids

- Triglycerides (TAG) and free fatty acids (FFA) following the consumption of an oral lipid beverage

Pre-training and post-training results of 5-hour plasma TAG and FFA responses, calculated using the trapezoidal method for AUC, 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. Pre-training vs. post-training values will be analyzed using a one-tailed paired Student's *t*-test within each group.

5.2.3 Blood Pressure

Pre-training and post-training resting blood pressure values will be summarized by treatment group as a continuous variable and will be analyzed (between pre-training vs. post-training) using a two-tailed paired Student's *t*-test within each group. Units of mmHg will be used for all blood pressure measures.

5.2.4 Body Composition

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

Pre-training and post-training 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/m_2 will be used for BMI; kg for body weight; and both percentage and kilograms for body fat and lean mass composition. Pre-training vs. post-training values will be analyzed using a two-tailed paired Student's *t*-test within each group.

5.2.5 Arterial Stiffness

Pre-training and post-training arterial stiffness results will be summarized by treatment group as a continuous variable and compared using a two-tailed paired Student's *t*-test within each group. Units of m/s will be used for all central arterial stiffness measures via pulse wave velocity.

5.2.6 Vascular Function

Pre-training and post-training results of FMD will be summarized by treatment group as a continuous variable. Units of relative FMD (% change) or normalized FMD (% change divided by arterial shear rate) will be used. Pre-training vs. post-training values will be analyzed using a two-tailed paired Student's *t*-test within each group.

Data may also be compared between END and SIT groups based on the respective change in each parameter. In this instance, the change scores between END and SIT will be analyzed using two-tailed unpaired Student's *t*-tests. Individual participant responses following training will be characterized in comparison to the smallest robust change, determined as the pre-training standard deviation multiplied by 0.2 (7).

5.2.7 Acute Post-Exercise Secondary Outcomes (PWV, FMD, Glycemic Regulation)
Secondary outcomes during the first week of exercise training (free-living glycemic regulation, acute post-exercise arterial stiffness and vascular function) will be analyzed using the appropriate units/statistical conditions as explained above for each parameter. Data will be compared between pre-exercise and acutely post-exercise within each group using paired Student's *t*-tests.

5.3 Handling of Dropouts

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.

If a participant randomized to a treatment group discontinues the study at any time, the reason for the withdrawal will be recorded (one participant, END group, discontinued the study due to unrelated personal reasons). Participant data will remain on file. 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, if any.

7.0 REFERENCES

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