STATISTICAL ANALYSIS PLAN (SAP)

The Influence of Cannabis Inhalation During Exercise on Cardiovascular Health and Function: Exploring the Optimal Balance of Cannabinoids and Mode of Administration to Decrease Risk and Maximize Benefits

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1. STATISTICAL CONSIDERATIONS .

1. STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):
  1. Pulse Wave Velocity will be increased following exercise, smoking high THC cannabis, and vaporizing high THC cannabis relative to resting baseline; effects of cannabis consumption and exercise are likely to be additive. Vaporizing high CBD cannabis will not increase pulse wave velocity.
  2. Percent flow mediated dilation will be decreased following exercise and smoking high THC cannabis; effects are likely to be additive. Vaporizing both high THC and high CBD cannabis will not decrease percent flow mediated dilation.
  3. Ejection fraction will be decreased following smoking and vaporizing high THC cannabis. Exercise and vaporization of high CBD cannabis will not be associated with reductions in ejection fraction.
  4. E/A Ratio will be decreased following high THC cannabis smoking only.
  5. Myocardial strain will be decreased following high THC cannabis smoking and vaporizing only.
  6. Myocardial twist will be decreased following high THC cannabis smoking and vaporizing only.
  7. Burst Frequency will be increased following high THC cannabis only.
  8. Burst Incidence will be increased following high THC cannabis only.

- Secondary Efficacy Endpoint(s):
  1. Systolic Blood Pressure will be increased following cannabis consumption and will be decreased following all bouts of exercise.
  2. Diastolic blood pressure will be unchanged under all conditions
  3. Heart rate will be increased following cannabis consumption and exercise.
  4. Stroke volume will be increased with exercise, and will be reduced following cannabis consumptions. These competing effects will result in an attenuation of increases in stroke volume when exercise is preceded by cannabis consumption.
  5. Cardiac output will be unchanged under all conditions
  6. Work completed will be reduced when preceded by cannabis consumption of all forms
  7. Average power output will be reduced when preceded by cannabis consumption of all forms
  8. Oxygen consumption will be reduced when exercise is preceded by consumption of high THC cannabis
  9. Rating of Perceived Exertion will be increased following consumption of high THC cannabis
  10. Rate-pressure product will be increased during exercise, when the bout is preceded by cannabis consumption.

2. SAMPLE SIZE DETERMINATION

In accordance with our power calculation, 15 participants are required to have a power of at least 0.8 with an alpha of 0.05, for expected changes in measures of endothelial function (pilot work, change in FMD in response to exercise pre = 4.8 ± 2.5 %, post = 2.7 ± 1.7%) (FMD in rats in response to cannabis smoke, pre = 7.5 ± 2.5%, post = 2.4 ± 1.4 %)3, artery stiffness (pilot

work, change in PWV pre = 7.4 ± 1.3 m/s, post (at rest) = 6.0 ± 1.3 m/s) and cardiac function (pilot work, peak longitudinal strain: pre = -18.3 ± 1.2 %, post = -17.0 ± 1.9 %), therefore we will recruit 20 participants in each phase of the study to account for potential dropout.

3. POPULATIONS FOR ANALYSES

Only participants who complete all study procedures will be included for analysis of efficacy endpoints. All participants who consume cannabis at any time will be included in the analysis of safety endpoints.

4. STATISTICAL ANALYSES

1. GENERAL APPROACH

Our study will be divided into two phases each of which will be analyzed independently. All tests of significance will be two-tailed, and statistical significance will be based upon an alpha error probability of 5% and a power for 80%. The data will be evaluated using both parametric and non-parametric tests, as applicable. If the data are found to be not normally distributed, the raw data will be transformed using an appropriate transformation model in order to perform the parametric assessments. Non-parametric tests will be performed on raw data. The data will be assessed as continuous and will be presented as means with standard deviations. A one-way analysis of covariance will be used to assess the treatment effects (Phase I: exercise, vaporizing THC, smoking THC, vaporizing + exercise THC, smoking THC + Exercise, Phase II: exercise, vaporizing THC, vaporizing CBD, vaporizing + exercise THC, Vaporizing CBD + Exercise) on the primary endpoints. Covariates will be introduced into the models in order to assess the impact of certain potential factors on outcome, including fitness, blood pressure, resting heart rate, and any other factors that are observed during the study that appear to potentially influence the outcome.

Our secondary endpoints will be assessed using a repeated measures one-way ANOVA and paired t-tests. If there are significant interactions differences between pairs of means will be determined with a Bonferroni post-hoc test.

2. ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

All primary endpoints will be measured as continuous variables and will be repeated after each intervention performed on a given study visit. A one-way ANCOVA will be used to assess treatment effects. Covariates will include basic cardiovascular and haemodynamic variables that may confound primary endpoints. These covariates include heart rate, blood pressure, and pulse wave velocity. Results will be presented as adjusted means with standard deviations. Primary endpoints will only be analyzed for participants who complete the entirety of at least one phase of the proposed study. Missing data will be imputed using mean imputation from all other measurements collected from the given participant. Outliers will be included and excluded in independent analysis, both of which will be reported.

3. ANALYSIS OF THE SECONDARY ENDPOINT(S)

Analysis of secondary endpoints will be independent of analysis of primary endpoints. All secondary endpoints will be measured as continuous variables and will be reported as adjusted

means with standard deviations. Repeated measures one-way ANOVA and paired Student's *t*-tests will be used for analyses. If there are significant interactions differences between pairs of means will be determined with a Bonferroni *post-hoc* test. Missing data will be imputed using mean imputation from all other measurements collected from the given participant. Outliers will be included and excluded in independent analysis, both of which will be reported.

4. SAFETY ANALYSES

Safety analysis will be performed using summary statistics during treatment interventions. Adverse events will be coded according to the Medical Dictionary for Regulatory Activities and will have severity, and relationship of adverse event to study intervention presented by System Organ Class and preferred term groupings. Each instance of an adverse event will be presented as a discrete event. Duration of events, severity, relationship to intervention, expectation, and outcome will be presented. Adverse events that lead to premature discontinuation will be presented in a table, along with all treatment-emergent serious adverse events.

5. BASELINE DESCRIPTIVE STATISTICS

Baseline characteristics of participants will be presented in a table. No descriptive statistics other than mean and standard deviation will be presented as the proposed study uses a within-subjects design.

6. PLANNED INTERIM ANALYSES

No interim analysis is planned for the proposed study. *Phase I* of this study will be completed in advance of *phase II*, and as such efficacy and safety analyses for *phase I* may occur before the completion of *phase II*. The results of *phase I* will not be used to modify the desired sample size or planned analyses of *phase II*.

7. SUB-GROUP ANALYSES

Sub-analysis of each primary and secondary endpoint will be performed based upon sex. This analysis will be performed identically to all efficacy and safety analyses with the only adjustment being the exclusion of the sex not of interest.

8. TABULATION OF INDIVIDUAL PARTICIPANT DATA.

Data collected from each participant will be recorded by measure and study visit. Each participant will be assigned a study ID.

9. EXPLORATORY ANALYSES

There are no planned exploratory analyses in the current study.