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# Statistical Analytical Plan

# 1. Health outcomes related to cognitive function

## 1.1 Linear mixed-effects model

A linear mixed-effects (LME) model would be utilized explore the effects of high temperature on cognitive function. To control for the influence of baseline cognitive function, the changes in measures from pre-exposure to post-exposure periods would be firstly calculated, and the differences of changes in measures between the intervention and control groups would be assessed using LME model. To account for potential confounding effects, several confounders would be included in the LME model, including gender (male, female), age (years), and body mass index (BMI, $kg/m^2$). Additionally, random intercepts for each participant would be included to account for the repeated measurements. Similarly, LME model would be used to examine the effects of high temperature on fMRI metrics, based on post-exposure metrics comparing the intervention and control groups.

## 1.2 Analytical tools

MRI metrics were estimated using SPM12 (Statistical Parametric Mapping). All statistical analyses are carried out in R software version 4.1.1. Statistical tests are two-sided, and $p < 0.05$ or FDR < 0.1 are considered statistically significant.

# 2. Health outcomes related to cardiovascular system

## 2.1 Linear mixed-effects model

We mainly use a linear mixed-effects model (LME) to explore the effects of high temperature on cardiovascular health. To account for the impact of varied outcomes levels at baseline, the change in outcome level is calculated as the percentage changes of post-intervention from pre-intervention, comparing the change in health outcomes between the intervention and control groups after the intervention. We further adjusted for the following confounders as categorical variables: gender (male, female); and as continuous variables: age (years), body mass index (BMI, $kg/m^2$), environmental temperature(°C) before the trial, and relative humidity (%) before the trial. We included random intercepts for each participant to account for the repeated

measurements.

### 2.2 Analytical tools

Arterial stiffness indicators were estimated using HMS Client-Server software. All statistical analyses are carried out in R software version 4.1.1. Statistical tests are two-sided, and $p < 0.05$ is considered statistically significant.

## 3. Health outcomes related to respiratory system

### 3.1 Linear mixed-effects model

A linear mixed-effects (LME) model would be utilized explore the effects of high temperature on lung function. To control for the influence of baseline lung function, the changes in measures from pre-exposure to post-exposure periods would be firstly calculated, and the differences of changes in measures between the intervention and control groups would be assessed using LME model. To account for potential confounding effects, several confounders would be included in the LME model, including gender (male, female), age (years), and body mass index (BMI, $kg/m^2$). Additionally, random intercepts for each participant would be included to account for the repeated measurements. Similarly, LME model would be used to examine the effects of high temperature on airway biomarkers and the biomarkers in blood samples, based on post-exposure metrics comparing the intervention and control groups.

### 3.2 Analytical tools

The 16S rRNA sequences of respiratory microbiota from pharyngeal secretion samples were analyzed using the Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline for microbial community analysis. All statistical analyses are carried out in R software version 4.1.1. Statistical tests are two-sided, and $p < 0.05$ is considered statistically significant.

## 4. Omics analysis

All the omics analyses are performed in strict accordance with the manufacturer's

guidelines. All omic data were log-transformed before formal analyses. We mainly apply the linear mixed-effects model to analyze the percent changes of detected omics features associated with heat exposure. Differential analytes are further conducted based on the p values obtained from the LME models. For the identified differential features from the LME model, pathway enrichment is performed at the ingenuity pathway analysis platform (IPA, QIANGEN, Germany). The pathways with FDR of less than 0.05 are considered statistically significant.