**Official Title: Acute Effects of Cold Exposure on Cardiovascular System and Potential Mechanism in Healthy Young Adults: A Randomized Controlled Study**

**NCT Number:**

**Document Date: 10/14/2024**

# 1. Health outcomes related to cardiovascular system

## 1.1 Linear mixed-effects model

A linear mixed-effects (LME) model would be utilized to explore the effects of cold temperature on blood pressure and heart rate variability parameters. To control for the influence of baseline cardiovascular profile, 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), body mass index (BMI, $kg/m^2$), and environmental temperature(℃) before the trial, and relative humidity (%) before the trial. 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 cold exposure on serum CRP, APOA1, APOB, Cholesterol, Glucose, HDL, Hs-CRP, Insulin, LDL, LPa, and TG concentrations, based on post-exposure metrics comparing the intervention and control groups.

## 1.2 Analytical tools

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.

# 2. 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.