January 20, 2021

PROTOCOL: Racial Differences in the Natriuretic Peptide Response to Glucose Challenge

Study Design, Setting, and Location

Our study was a single-center, prospective, physiological clinical trial approved by the institutional review board at the University of Alabama at Birmingham (UAB). Participants were recruited from the university campus and surrounding areas. All participants signed informed consent.

Sample Selection

Prospective participants were first contacted by phone to determine eligibility prior to scheduling a screening visit at the clinical research unit (CRU). Self-identified healthy black and white men and women were eligible to participate in the clinical trial based on the following criteria: between 18 and 40 years of age, blood pressure (BP) less than 140/90, estimated glomerular filtration rate >60 ml/min, and willingness to adhere to the study diet. Exclusion criteria included the following: history of hypertension, history of cardiovascular, renal, or liver disease, history of diabetes or glucose-lowering medications, use of vasoactive or diuretic medications, anemia, abnormal serum sodium or potassium, pregnancy, elevated liver function tests defined as >3x the upper limit of normal, women taking hormonal birth control, history of smoking, body mass index (BMI) <18.5 kg/m^2, or regular use of non-steroidal anti-inflammatory medications.

Study Protocol

For the screening visit, subjects were educated on the study protocol and directed to complete a questionnaire regarding medical and family history. The study investigators further evaluated subjects who agreed to the study and signed consent. Blood was drawn for a basic metabolic profile, liver enzymes, and complete blood count. Additional samples were collected if consent was provided for DNA extraction and gene expressions for NPs. The CRU nursing staff obtained height, weight, and BP (i.e., an average of three BP measurements) for a baseline. Female participants provided a urine sample to test for pregnancy. Before completion of the screening visit, subjects met with the registered dietitian to discuss the study diet and were provided with a 3-day meal log to assess dietary habits.

Upon review of screening labs, eligible subjects were enrolled in the study [stratified by race using permuted blocks for age and sex)] and invited back for food pick up and protocol (i.e., a high-carbohydrate challenge) visits. Within seven days after the screening visit, participants visited the CRU metabolic kitchen for food pick-up. Three days of standardized meals were provided to be consumed for the three days leading up to the protocol visit. Three meals per day and snacks were provided based on participant preferences and by following macronutrient RDAs from the USDA 2005 Guidelines. The study nutritionist calculated caloric needs. In brief, each study participant was provided on an average 1,993 kilocalories worth of meals comprising 4,667 mg of sodium per day for three days. The contribution of carbohydrate, fat, and protein in total caloric intake was 51%, 31%, and 18%, respectively. Additional instructions were given to drink only water, to refrain from alcohol and caffeine use, and to consume only

food provided by CRU metabolic kitchen for the remainder of the study. Participants were also advised to avoid strenuous activities for 24 hours prior to the protocol visit. For the protocol visit, participants fasted overnight for 10 hours, before reporting at 7:00 AM to the CRU. Upon arrival, participants were assessed for compliance with the study diet and were positioned in the sitting position with the back-rest at 45 degrees and legs elevated. Heart rate, oxygen saturation and BP (an average of three measurements using a Carescape V100 monitor) were obtained. Participants were given glucola, a 75mL, 75g glucose equivalent, and were provided only distilled water to drink as needed for the remainder of the protocol. Blood pressure was measured every 30 minutes, and heart rate and oxygen saturation were measured every 60 min. Blood was drawn in 60 min intervals for 8 hours for biomarker profiling. All the participants remained in fasting state (except distilled water) till the end of the study protocol.

Statistical Analyses

Our study was powered to detect the change in ANP levels in response to a high-carbohydrate challenge among black individuals. Based on a prior investigation among Whites, a high-carbohydrate challenge can reduce circulating plasma ANP levels up to 27%. With an estimated standard deviation of 0.2 (a high estimate) and utilizing prespecified sample size of 30 black individuals, our study was powered to detect a change as small as 11% in ANP levels after a high-carbohydrate challenge, with β=0.8 and α=0.05. We expected a similar effect size for change in ANP levels after a high-carbohydrate challenge among whites.

All analyses were conducted using STATA, version 14.2 MP (StataCorp LP), and LIMMA R package. Baseline characteristics of participants were compared between blacks and whites using Student's t-test (normal distribution) or Mann-Whitney test (non-normal distribution) for continuous variables and Pearson χ^2 test for categorical variables. The normality of continuous variables was assessed using histogram and Q-Q plots (visual assessment) as well as Shapiro Wilk test (statistical assessment). Plasma NP levels at baseline and after a high-carbohydrate challenge were found to have a non-normal distribution and were log-transformed for the analyses. Linear regression models were used to assess the racial differences in plasma NP levels. Choosing white race as a reference, a relative percentage difference in plasma NP levels with 95% confidence interval (CI) in blacks was calculated using the following formula: $(e^{\beta} - 1) \times 100$ (where β is the beta coefficient from linear regression). Linear mixed-effect models excluding missing data (<1%) from 72 individuals were used to assess the effect of a high-carbohydrate challenge over time on plasma NP levels. In these models, we assessed for repeated measurements of NP levels with fixed effects of race, time, and their multiplicative interaction term (race*time), accounting for the correlation among repeated measures in the same person. Participants were treated as random effects in these models. The percentage difference with 95% CI in plasma NP levels after 8 hours in overall as well as by race was calculated: $[(e^{\beta} - 1) \times 100$ multiplied by 8 hours (where β is the beta coefficient from linear regression)]. The models mentioned above were further assessed after adjusting for their baseline demographics [i.e., age, sex, body mass index (BMI), insulin].