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PROTOCOL: The Diurnal Rhythm in Natriuretic Peptide Levels

Study Design, Setting, and Location

The prospective, single-center, mechanistic clinical trial was conducted at the Clinical Research Unit (CRU) at the University of Alabama at Birmingham (UAB). The trial was approved by the institutional review board (IRB No: 300004115), and participants provided written and informed consent. Healthy participants were recruited from the Birmingham metropolitan area and the UAB campus.

Study Protocol

After obtaining informed consent, we completed a medical history and physical examination during the screening visit and determined protocol eligibility based on the inclusion and exclusion criteria. Eligible subjects met with a registered dietician, who explained the five days of standardized diet to be consumed before the 24-hour inpatient visit and reviewed instructions for complying with the diet. All the female participants completed the study protocol during the follicular phase of the menstrual cycle.

Each eligible participant was provided five days of a standardized diet and the SpaceLabs 90227 monitor device, which is a 24-hour ambulatory blood pressure monitoring (ABPM) device(31), along with detailed operative instructions. Each day the participants consumed three meals and snacks, which were provided based on the participant preferences and in accordance with macronutrient RDAs, as per the 2005 USDA Guidelines. The standardized study diet was given to each participant comprised of 51% carbohydrate, 31% fats, 18% proteins, ~4.5 gm sodium, and ~2.5 gm potassium. Additionally, the participants were instructed to drink only water and refrain from consuming alcohol or caffeinated beverages. The participants were instructed to consume only the food provided by the CRU metabolic kitchen during the study period.

The five-day diet was followed by an inpatient visit for a 24-hour assessment of NP levels and BP. The patients wore a 24-hour ABPM device starting on day 5, i.e., one day before the inpatient visit. The participants also collected urine for 24-hours before the inpatient visit to assess the adequacy of standardizing the dietary sodium intake. During the inpatient visit, blood was drawn using an intravenous catheter, a total of 5 times during wakefulness (7 am to 10 pm), and 5 times during sleep (10 pm to 7 am). A flexible intravenous catheter was placed in the antecubital space of the arm to avoid repeated need-stick of the patients. The participants were asked to stay supine 30 minutes before each blood draw to rule out any plausible postural influences on the RAAS, BP, and NP system. The light intensity was ensured to be normal day-light during daytime and dim light (<10 lux) for night blood draws. Participants were provided small standardized meals after each blood draw during wakefulness (i.e., 8 am, 11 am, 2 pm, 5 pm, and 8 pm). Subjects were provided distilled water to drink (50-85 ounces), and the amount of water consumed was recorded. The participants also collected urine stratified in the daytime 7 am to 10 pm) and nighttime (10 pm to 7 am) periods during the visit.

24-Hour Ambulatory BP

The 24-hour ABPM was obtained using a standard protocol every 20 minutes between 6 am and 10 pm and every 30 minutes from 10 pm to 6 am using SpaceLabs 90227, starting a day

prior to the 24-hour inpatient visit to allow the participants to become accustomed to the device. The maximum number of analyzable measurements were 48 daytime and 16 nighttime (Total=64).

Statistical Analyses

All statistical analyses were conducted using SAS 9.4 (Cary, NC) and R (R Core Team, Version 4.0.1). The baseline characteristics were compared using descriptive statistics. Continuous variables were summarized as the median and interquartile range (IQR:25^{th} centile, 75^{th} centile). Continuous data were compared using the Wilcoxon rank-sum test. Pearson χ^2 test was used to compare the categorical variables. The range was computed as the difference between the minimum and maximum value of the analyte over the 24-hour period. The range spread (%) was computed as (range/minimum value)*100. The cosinor analysis of the normalized analyte values (normalized to the 24-hour mean value) was conducted using the cosinor2 package in R Statistical Software (R Core Team, Version 4.0.1). This package uses linear regression to identify the cosine curve that best fits the data and tests the overall significance of the cosinor model using an F-ratio.) The 24-hour data was considered rhythmic if it fit the cosinor function i.e., $f(t) = M+AMP*Cos[(2\pi t)/24 + \phi]+ \epsilon_t$, where M refers to the Mesor (rhythm-adjusted mean), AMP refers to the amplitude (difference between peak and M), and ϕ refers to the phase (time of peak). Data was considered rhythmic if the p-value of the R^2 of the cosine function was <0.05. The rhythm parameters are based on the grouped analysis of the lean and obese population groups. The cosinor parameters of interest while comparing the normalized NP rhythms between lean and obese individuals were the amplitude and acrophase. The reported cosinor parameter of interest while comparing the normalized NP and BP rhythms was acrophase. The amplitude and acrophase are defined based on the fitted cosinor curve. Linear regression models were used to assess the relative percentage difference with a 95% confidence interval (adjusted for age, sex, race) for the 24-hour, daytime, and nighttime mean MRproANP, BNP, NTproBNP, renin, and aldosterone levels between lean and obese individuals. This was calculated using the following formula: $(e^{\beta} – 1) \times 100$ (β coefficient from linear regression). A two-sided type-I error of <0.05 was considered statistically significant for all analyses.