Bioactive Compounds in Watermelon Modulating Oxidative Stress and Inflammation in Elders

NCT03626168

Study Protocol and Statistical Analysis Plan

February 16, 2016

Protocol

Study participants will be randomized to supplement their diets twice daily with either 100% watermelon juice or an isocaloric placebo for four weeks in a double-blind crossover design. Juice and placebo will be provided in two 12-ounce servings, and participants will be asked to consume one dose in the morning and another in the evening with a meal containing dietary lipid. Based on previous studies [62,87], two 12-ounce servings of 100% watermelon juice will provide approximately 1g of L-citrulline and 32 mg of lycopene. Pasteurized watermelon juice for this study will be supplied by Frey Farms (Keenes, IL) from Estrella variety melons. To ensure that all juice contains similar concentrations of the bioactive food compounds of interest, each batch will be tested in the Food and Nutrition Research Laboratory at the University of Alabama for lycopene, citrulline, and arginine content.

Two weeks prior to the intervention, during each intervention arm, and during the washout period, subjects will be asked to consume their typical diet of choice with the exception of foods high in lycopene. A list of lycopene-rich foods such as watermelon and tomato products will be provided, and participants will be asked to limit these foods to two servings per day for the duration of the study. Dietary intake will be closely monitored by 4-day food diaries (three weekdays, one weekend day) submitted during the 2-week run-in period and the 2-week washout period as well as by weekly unannounced 24-hour diet recalls during the treatment period.

Subjects will report to the University of Alabama campus for testing as follows:

Run-In	*4-Week Regimen of Juice or Placebo*		*2-Week Washout*	*4-Week Regimen of Juice or Placebo*	
Days 0-14	**Beginning of Week 1**	**End of Week 4**	**Weeks 5 & 6**	**Beginning of Week 7**	**End of Week 10**
4-day food diary	Fasting blood sample	Fasting blood sample	4-day food diary	Fasting blood sample	Fasting blood sample
	Anthropometrics	Anthropometrics		Anthropometrics	Anthropometrics
	FMD	FMD		FMD	FMD
	PWV	PWV		PWV	PWV

Weekly: Weight, Blood Pressure, Adherence

During weekly visits and phone calls with study personnel, subjects will be queried about adherence to the protocol and acceptability of the beverages. Adherence will be assessed by log forms with check-off boxes for each dose, return of all juice bottles at weekly visits, and random 24-hour diet recalls. Minimal adherence will be defined as 70% of recommended supplementation of two doses per day for four weeks.

Statistical Analysis

A biostatistician from the University of Alabama will be responsible for generating a block randomization with PROC PLAN in SAS (version 9.2). The randomization scheme will balance genotype in Groups 2 and 3 (those who receive genetic information at the beginning of the intervention). Differences between groups will be evaluated by one-way ANOVA with repeated measures with group as the between-subjects factor and time as the within-subjects factor.