

## Supplementary Online Content

Svatikova A, Covassin N, Somers KR, et al. A randomized trial of cardiovascular responses to energy drink consumption in healthy adults. *JAMA*. doi:10.1001/jama.2015.13744

### **Trial protocol**

This supplementary material has been provided by the authors to give readers additional information about their work.

## **1. Trial protocol.**



## IRB Minimal Risk Protocol Template

**Note: If this study establishes a human specimen repository (biobank) for research purposes, do not use this template. Use the Mayo Clinic Human Specimen Repository Protocol Template found on the IRB home page under Forms and Procedures at <http://intranet.mayo.edu/charlie/irb/>**

**First-time Use:** Use this template to describe your study for a new IRB submission.

1. Complete the questions that apply to your study.
2. Save an electronic copy of this protocol for future revisions.
3. When completing your IRBe application, you will be asked to upload this template to the protocol section.

**Modification:** To modify this template after your study has been approved:

1. Open your study in IRBe. Click on the study 'Documents' tab and select the most recent version of the protocol. Save it to your files.
2. Open the saved document and activate "Track Changes".
3. Revise the protocol template to reflect the modification points, save the template to your files
4. Create an IRBe Modification for the study and upload the revised protocol template.

## General Study Information

Principal Investigator: Virend K. Somers, MD, PhD

Study Title: Cardiovascular Effects of Energy Drinks in Healthy Adults

Protocol version number and date: IRB 13-001918 May 10, 2013

## Purpose

### Hypothesis:

Increasing number of healthy adults consume energy drinks to enhance their physical and mental performance. Energy drinks contain caffeine and multiple other "natural" stimulants and their combined effects on the heart and heart function in healthy individuals are unclear. We hypothesize that energy drink consumption compared to a control drink in healthy adults alters the cardiovascular system, predisposing even healthy adults to new, otherwise unexpected cardiovascular event.

**Aims, purpose, or objectives:**

- 1) Determine the hemodynamic changes (magnitude of heart rate and blood pressure response) in healthy adults after consumption of an energy drink and compare these responses to those after a control drink
- 2) Determine ECG changes and prevalence of arrhythmias after consumption of an energy drink as compared to baseline ECG
- 3) Determine if consumption of energy drinks is associated with altered neurohormonal and cardiovascular activation, assessed by measurements of cardiovascular biomarkers, such as catecholamines before and after energy/control drink intake
- 4) Determine if taking energy drinks induces orthostatic changes and unmask potential cardiac associations
- 5) Assess baroreflex sensitivity (pace breathing) changes after energy drink intake
- 6) Determine the effect of energy drink on hemodynamics during stress conditions mental (arrhythmics), physical (hand grip) and pain (cold stress) responses as compared to measurements at rest
- 7) Determine the prevalence of energy drink usage among healthy adults and their subjective response to standard energy drink questionnaire.

**Background** (*Include relevant experience, gaps in current knowledge, preliminary data, etc.*):

Energy drink market is strikingly expanding. First noted to be highly used by competitive athletes, energy drinks are now widely popular among students, and even with Air Force personnel.<sup>1-3</sup> In 2012, for the first time, energy drinks have outsold bottled water. Energy drinks sales increased by almost 20% over the previous year, while bottled water saw <4% increase in sales, according to Beverage Industry 2012 State of Industry Report. There has been more than 70 % increase in the amount of caffeine intake for caffeine-consuming children and adolescents from 1977 to 2009. These ready-to-drink beverages are promoted to reverse fatigue and consequently increase performance, endurance and alertness within 60 minutes. Although energy drinks are regulated by the US Food and Drug Administration, the ingredients may be harmful. Caffeine concentration in an energy drink is greater than the amount in a standard cup of coffee. Other common ingredients in energy drinks are sugars, guarana (plant with seeds that contain caffeine), amino acid taurine, cocoa, B vitamins, herbs (such as ginseng, licorice, kola nut).<sup>4</sup> The physiological and particularly cardiovascular effects of energy drinks are not fully elucidated.

Latest media coverage speculates about possible temporal relationship between caffeinated energy drink intake and occurrence of acute cardiac events, including death. Thus the growing market of energy drinks raises questions regarding their safety even for otherwise healthy young adults. Furthermore cardiovascular effect of energy drinks is unknown in people with preexisting heart disease.

Recently published International Society of Sports Nutrition position statement on energy drinks confirms their important physiological impact and points to the current gaps in knowledge.<sup>5</sup> As noted in this report, "Indiscriminant use of energy drinks or energy stimulants, especially if more than one serving per day is consumed, may lead to adverse events and harmful side effects."<sup>5</sup> This Document further highlights the need for studying the effects of caffeinated energy drinks on the cardiovascular system and their cardiovascular outcome.

Careful review of the research literature shows that currently there are several small studies addressing this important issue, however results are conflicting.<sup>6</sup> The caffeine related adverse effects of energy drinks were



recently discussed in JAMA 2013 Viewpoint as caffeinated drinks are linked to unexpected deaths even in healthy people.<sup>7</sup> In August 2012 at a webcast conference at the European Society of Cardiology Congress, Cameli et al reported that consuming energy drinks can exert acute positive benefits on myocardial performance. In their small study of 35 healthy subjects, one hour post energy drink intake, neither heart rate nor systolic blood pressure changed significantly, rather there was a significant increase in diastolic blood pressure, and improvement in left and right ventricular function. A case report recently published by our colleagues at Mayo, suggests that highly caffeinated energy drinks may unmask a long QT syndrome, an uncommon genetic disease.<sup>8</sup> This suggests a proarrhythmogenic effect of energy drinks. Physiologically, an energy drink may simulate an epinephrine stress test. The predicted health risks include increased and/or irregular heart rate, elevated blood pressure, sleep disturbances, diuresis, and hyperglycemia due to high sugar content of the energy drinks. Cardiovascular effects of the variety of ingredients in an energy drink that act as stimulants are unclear but may lead to a potentially lethal proarrhythmogenic outcome. The focus of this study is to elucidate the physiological/cardiovascular response to an energy drink consumption as compared to a control drink both at rest and during stressful conditions in healthy adults.

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**Subject Information** – charts, records, images, or specimens are considered ‘subjects’

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Target accrual: 25 healthy adults will be recruited for this study.

Subject population: Healthy adults

Inclusion Criteria:

- 1) Adults 18 years of age and older
- 2) Healthy subjects without known cardiovascular disease and thyroid disease
- 3) Subjects who are on no medications (except oral contraceptive pill)
- 4) Nonsmokers
- 5) No prior history of caffeine sensitivity or allergy

Exclusion Criteria:

- 1) Subjects with known cardiovascular or thyroid disease
- 2) Subjects currently taking medications other than oral contraceptive pill
- 3) Smokers
- 4) Prior history of caffeine sensitivity or allergy
- 5) Pregnancy

Will a Certificate of Confidentiality be obtained? No



## Study Design

Methods: *Describe, in detail, the research activities that will be conducted under this protocol:*

Energy drink consumption in general population is drastically increasing and its cardiovascular effects are not well understood. This is a pilot study to investigate the cardiovascular and metabolic response to energy drink consumption in healthy adults. Subjects will be studied using a randomized, double-blind, placebo-controlled, crossover design with two separate experimental sessions: control and energy drink, studies performed on 2 separate study days.

Energy drink used: commercially available in US stores, 16 ounce can of Rockstar Punched Energy Drink

Control drink used: HyVee fruit punch – 16 ounces

Nutritional details:

- 16 ounces of the HyVee fruit punch contains: 240 Calories, 0 grams fat, 62 grams carbohydrate and 0 gram protein
- 16 ounces of the Rockstar Punched contains: 260 Calories, 0 grams fat, 62 grams carbohydrate and 2 gram protein

Control drink and Rockstar Punched Energy Drink are similar in taste, texture, and color, except that control drink does not contain caffeine/energy blend. Control drink is carefully selected by our team of CRU Nutritionists to match in-stores available energy drink. Both preparations will be given in identical cups so that participants and study investigators are not aware of which preparation is being administered. Subjects will remain unaware of the nature of the drink administered on each study day throughout the study. Each experimental session (control and energy drink) will be conducted using the same protocol, in a random order.

Study will be performed at St Mary's CRU on two separate days minimum 24 hours and maximum 1 month apart. Participants will be consented upon arrival to the CRU and will be consented by the Research Staff. The start time of the studies will be the same. Participants will be fasting 4 hours prior to the study and will be asked to abstain from caffeine and alcohol for at least 24 hours prior to initiation of this study. When presenting to the CRU on the first day of the study, participant's height and weight will be obtained by the CRU staff. On each study day, CRU personnel will perform urine pregnancy test if needed. If positive pregnancy test, participant will not be able to participate in the study. The research staff will obtain waist and hip and bioimpedance measurements (described below); research staff will fill out the Copyrighted Cardiovascular Research Data Sheet Questionnaire, Copyrighted Sleep Breakfast Questionnaire and Caffeine Intake Questionnaires (all attached with application). Subsequently, the participants will take part in research studies as outlined below. All studies constitute minimal risk. Baseline measurements will be obtained during and after 10 minutes of rest. After the end of baseline recordings, the subjects will be given up to 500 ml (1 can, approximately 16 fluid ounces) of caffeinated energy drink or a control drink. They will have 30 minutes to drink it. The second set of measurements will be obtained after an energy drink or control drink intake, using exactly the same protocol as at baseline.

We expect to enroll approximately 25 healthy subjects.



## **PROCEDURES:**

During this study, the participants will take part in the following studies:

1. Medical history interview and completion of questionnaires: This involves answering questions about past and present medical, family history, sleep habits, and social history including intake of caffeine and other stimulants. Questionnaires used are: Caffeine Intake Questionnaire, Copyrighted Cardiovascular Data Sheet Questionnaire and Copyrighted Sleep Breakfast Questionnaire.
2. Baseline body composition measurements: This involves measurements of height, weight, waist and hip circumferences and bioimpedance measurement. Bioimpedance measures the resistance of the skin on the foot and hand using a bioimpedance device. It involves attaching 2 pairs of electrodes – one on the foot and one on the hand.
3. Blood pressure and heart rate measurements: This involves having a blood pressure cuff inflated around the arm at periodic intervals in standing and lying positions. Heart rate will be measured at the time of blood pressure measurement. Measurements will be taken at regular intervals.
4. 12-lead ECG recording: A set of electrodes will be attached to the skin on the chest and ECG tracing will be obtained in lying position throughout the study.
5. Blood samples: A peripheral IV catheter will be inserted into patient's forearm to obtain blood samples and will be kept until the end of the day study. Samples will be obtained at baseline and after energy drink/control drink administration during supine rest. In total about 1/2 cup of blood will be drawn to measure levels of cardiovascular markers including plasma catecholamines (epinephrine and norepinephrine), in addition to measurements of caffeine and glucose level. Blood samples will be stored safely for future studies of serum markers at Mayo and will be given a code. This code will allow the sample to be used without identifying the origin of the sample. The samples will not be sent outside of the institution.
6. Paced Breathing: This involves measurements of heart rate variability and baroreflex sensitivity to enable better characterization of cardiometabolic changes done before and after energy drink intake. Subjects will undergo detailed training to perform spontaneous and controlled breathing tests in the supine position. Blood pressure will be monitored during this test using a very small finger blood pressure cuff. Breathing will be measured by a loose strap around the chest.
7. Stress response: The effects of energy/control drink cardiovascular/circulatory response to stress stimuli will be also studied. Stress tests (sustained handgrip, mental stress, and the cold pressor test) will be conducted in a randomized fashion and continuous blood pressure and heart rate measurements will be done. Mental stress will be conducted by asking the subjects to complete serial mathematical tasks as fast as possible for two minutes. Physical stress, isometric hand grip performed with a dynamometer and by asking the subjects to sustain a handgrip of one-third of their maximum voluntary hand grip contraction maintained for 2 minutes. The cold pressor test requires subjects to place one hand into an ice water for two minutes up to the level of



the wrist. The cold pressor test will be performed always last because of sustained effects of the test. Throughout all tests, measurements of blood pressure, heart rate and ECG will be obtained.

**Resources:** *Describe the available resources to conduct the research (personnel, time, facilities, mentor commitment, etc.):*

Research fellows of Dr. Somers' laboratory, named in the IRB application, will be the primary individuals conducting this research study. Dr. Somers is the principal investigator in this project. Studies will be conducted in the Clinical Research Unit at St Mary's Hospital which is equipped for measurement of cardiovascular and hemodynamic function.

Dr. Somers serves as the Director of the Sleep Core and of the Cardiovascular Core of the Mayo Rochester Clinical Research Unit. Support for the patient-related and technical aspects of this study will be provided by the nursing, engineering and other staff of the Clinical Research Unit. This proposal therefore provides a unique and synergistic integration of skills and resources of the Cardiovascular Division, Sleep Medicine and the Mayo Rochester Clinical Research Unit.

**Check all that apply. If none apply, leave blank:**

- This is a multisite study involving Mayo Clinic and non-Mayo Clinic sites.  
When checked, describe the research procedures/activities being conducted **only** at Mayo Clinic:
- Mayo Clinic staff will be engaged in research activity at a non-Mayo Clinic site. *When checked, provide the location and a detailed description of the Mayo Clinic research staff involvement.*
- This study is to establish and/or maintain an ongoing database or registry for research purposes only.
- The research involves contact or interaction with subjects, for example, surveys, questionnaires, observation, blood draw.
- The study involves audiotaping or videotaping

### Blood Collection

If this study involves prospective blood collection by finger, heel, ear stick or venipuncture, complete the following:

- From healthy, non pregnant, adult subjects who weigh at least 110 pounds.** For a minimal risk application, the amount of blood drawn from these subjects may not exceed 550ml in an 8 week period and collection may not occur more frequently than 2 times per week.

Volume per blood draw: up to 100 ml



Frequency of blood draw (e.g. single draw, time(s) per week, per year, etc.): two draws per study session

**From other adults and children considering age, weight, and health of subject.** For a minimal risk application, the amount of blood drawn from these subjects may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period, and collection may not occur more frequently than 2 times per week.

Volume per blood draw: \_\_\_\_\_ ml

Frequency of blood draw (e.g. single draw, time(s) per week, per year, etc.) \_\_\_\_\_

**Review of Chart, Images, Specimens**

Provide the date range for collection of data and/or specimens that will be included in your research dataset. (Example: 01/01/2000 to 12/31/2012)

Date range: From 04/01/2013 to 04/01/2017

**Check all that apply:**

This study involves only data and/or specimens that exist at the time this application is submitted to the IRB (IRB submission date). No data or specimens will be collected beyond this date.

This study involves only data and/or specimens that will be collected after submission to the IRB.

The study involves data and/or specimens that exist at the time of submission to the IRB **and** data and/or specimens that will be collected after submission to the IRB, for example a study that includes collection of existing data and prospective collection of specimens.

Data and/or specimens used in this study are collected under another IRB protocol. *When checked, provide the IRB number(s) from which the research material will be obtained and check the box below to attest that subjects have provided consent for future use of their data and/or specimens, as described in this protocol.*

IRB Number(s):

Subjects have provided consent for use of their data and/or specimens, as described in this protocol.

Other data sources will be utilized in this study. When checked, provide all data sources:



### Data Confidentiality, HIPAA Subject Identifiers

Review the list of subject identifiers below and, if applicable, check the box next to each subject identifier being recorded at the time you are collecting/abstracting data/specimens for use in this study.

**Subject Identifiers:** Individually identifiable information, including demographic data, that identifies the individual or for which there is reasonable basis to believe it can be used to identify the individual. NOTE: Identifiers apply to subjects enrolled in your study and to the subject's relatives, household members, employers, etc.

**Internal** refers to subject identifiers that will be included in the dataset maintained by the study team.

**External** refers to subject identifiers that will be shared with persons outside of the immediate study team, for example, sent to an external collaborator or shared with a national registry.

SUBJECT IDENTIFIERS Check all that apply	INTERNAL IDENTIFIER	EXTERNAL IDENTIFIER
Name	x	
Social Security number		
Medical record/patient registration number, lab accession, specimen or radiologic image number	x	
Study number, subject ID, or any other unique identifying number, characteristic or code that can be used to link the identity of the subject to the data	x	
Dates: All elements of dates [month, day, and year] directly related to an individual. Their birth date, date of death, date of diagnosis, etc. <b>Note:</b> Recording a year only is not a unique identifier.	x	
Medical device identifiers and serial numbers		
Biometric identifiers, including finger and voice prints, full face photographic images and any comparable images		
Web Universal Resource Locators (URLs), Internet Protocol (IP) address numbers, email address		
Street address, city, county, precinct, zip code, and their equivalent geocodes	x	
Phone or fax numbers	x	
Account, member, certificate or professional license numbers, health beneficiary numbers		
Vehicle identifiers and serial numbers, including license plate numbers		
<b>If None of the above identifiers will be recorded or maintained in the dataset and/or sent outside of the study team, please check "None".</b>	<input type="checkbox"/> None	<input type="checkbox"/> None



## Statistical Information

*Note: Power analyses and study endpoints are not needed for a pilot or feasibility studies.*

No statistical information. *If checked, please explain:*

### Statistical Considerations

Data Analysis Plan: Plan is to analyze data using two-way analysis of variance (ANOVA) for repeated-measures with time (before vs after drink intake) as the within factor and group (energy drink vs control drink) as the between factor. The key variable will be the group-by-time interaction. Differences in hemodynamics (blood pressure, heart rate) before and after consumption of a drink will be determined by repeated measures ANOVA. Student *t* test (paired or unpaired) will be used for between group comparisons with respect to change in given variable.  $P < 0.05$  will be considered statistically significant. Data will be expressed as mean  $\pm$  SEM. Importantly, blinding will be maintained until completion of the analysis of data.

Power Statement: This is a pilot study, no power analysis is necessary.

### Endpoints

Primary: Electrocardiographic and hemodynamic response to consumption of an energy drink

Secondary: Biochemical/metabolic response to consumption of an energy drink



## References:

1. Hoyte CO, Albert D, Heard KJ. The use of energy drinks, dietary supplements, and prescription medications by united states college students to enhance athletic performance. *J Community Health*. 2013
2. Park S, Onufrak S, Blanck HM, Sherry B. Characteristics associated with consumption of sports and energy drinks among us adults: National health interview survey, 2010. *J Acad Nutr Diet*. 2013;113:112-119
3. Malinauskas BM, Aeby VG, Overton RF, Carpenter-Aeby T, Barber-Heidal K. A survey of energy drink consumption patterns among college students. *Nutr J*. 2007;6:35
4. Torpy JM, Livingston EH. Jama patient page. Energy drinks. *Jama*. 2013;309:297
5. Campbell B, Wilborn C, La Bounty P, Taylor L, Nelson MT, Greenwood M, Ziegenfuss TN, Lopez HL, Hoffman JR, Stout JR, Schmitz S, Collins R, Kalman DS, Antonio J, Kreider RB. International society of sports nutrition position stand: Energy drinks. *J Int Soc Sports Nutr*. 2013;10:1
6. Burrows T, Pursey K, Neve M, Stanwell P. What are the health implications associated with the consumption of energy drinks? A systematic review. *Nutr Rev*. 2013;71:135-148
7. Sepkowitz KA. Energy drinks and caffeine-related adverse effects. *Jama*. 2013;309:243-244
8. Dufendach KA, Horner JM, Cannon BC, Ackerman MJ. Congenital type 1 long qt syndrome unmasked by a highly caffeinated energy drink. *Heart Rhythm*. 2012;9:285-288

## **2. Statistical analysis plan and calculation of sample size.**

Data Analysis Plan: Plan is to analyze data using two-way analysis of variance (ANOVA) for repeated-measures with time (before vs after drink intake) as the within factor and group (energy drink vs control drink) as the between factor. The key variable will be the group-by-time interaction. Differences in hemodynamics (blood pressure, heart rate) before and after consumption of a drink will be determined by repeated measures ANOVA. Student *t* test (paired or unpaired) will be used for between group comparisons with respect to change in given variable.  $P < 0.05$  will be considered statistically significant. Data will be expressed as mean  $\pm$  SEM. Importantly, blinding will be maintained until completion of the analysis of data.

Power Statement: With 25 patients, we have 80% power to detect a significant increase in the blood parameters (i.e. blood pressure, heart rate, etc.) for the energy drink as compared to the placebo drink, with a 2-sided significance level of 0.05. Specifically, we are powered to detect an effect size of 0.80, which is a difference in the means between the different types of drinks (energy vs. placebo) that is 80% of the standard deviation. For example, we are powered to detect an increase of 5% in the blood parameters for the energy drink vs. only a 1% increase for the placebo drink (4% difference), assuming a standard deviation of 5% (effect size = 0.80).