September 27, 2017

The effects of E+shots on mood, cognitive function, heart rate, blood pressure, cortisol and fine motor task performance

Design: This is a double blinded cross-over experiment with repeated measures on treatment (25mg caffeine vs 100mg caffeine vs e+shots) and Time (Time 1 (pre-beverage), Time 2, Time 3, Time 4). Previous night's sleep and occupation may be used as a co-variate in the statistical analysis.

Procedure:

Screening day:

- Potential subjects will complete an initial online screening using questionnaires that will be posted on surveymonkey.com. The appendices listed below are the questionnaires that are posted in the survey.
- The survey may be accessed through the tiny URL, QR code, or through a link that will be provided by the researcher.
    - Appendix G- A demographic and medical history questionnaire
        - As seen in the exclusion criteria all subjects who use prescription or over the counter medications for chronic medical conditions will not be allowed to participate in the study, with the exception of people taking oral contraceptives.
        - Subjects who are taking a prescription or over the counter medication for an acute health condition may be asked to participate in the study after they are no longer taking the medication or they will be given the option to drop out of the study.
    - Appendix H- Profile of Mood Survey (POMS) brief form
        - We will calculate the energy score of the POMS.
        - If the energy score is >=13 then the potential subject will be eliminated from the study
    - Appendix I- Caffeine consumption questionnaire
        - Using the data from this survey subjects who consume >=200mg of caffeine per day will be eliminated from this study
    - Appendix J- Food frequency questionnaire
        - Using the data from this survey subjects who consume >=150 servings of polyphenols per month will be eliminated from the study
    - Appendix K- Mental and Physical State and Trait Energy and Fatigue Scales (see appendix R for approval to use questionnaire)
        - The objective of this scale is to control of cognitive bias by asking questions regarding use of prescription drugs and using a different energy and fatigue scale to measure energy levels.
        - A score of >10 on the energy scale will eliminate the potential subject
        - This scale also measures physical and mental activity levels which will be used in the statistical analysis as covariates
    - Appendix L- Pittsburgh sleep quality index
        - This survey will be used to measure sleep time and sleep quality of potential subjects.
        - Sleep will be used to determine eligibility on day of testing
        - Sleep quality will be used as a covariate in the statistical analysis
- After completion of survey, a research assistant will verify that the subject is eligible for the study and will contact them via the email that is provided by the subject.

Researchers will review the results of all of these questionnaires to confirm if a potential subject met the criteria or did not. Potential subjects who met the criteria will be asked via e-mail to schedule a mutually convenient date for "familiarization day" (see below) to confirm eligibility, perform the second informed consent, and begin participating in the study (appendix S). Potential subjects who did not meet the criteria will be notified and thanked for their time via e-mail (appendix T).

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Day 1: Familiarization day

To reduce experimental error that may occur due to learning effect, subjects will be asked to come in for approximately 40 minutes to familiarize themselves with the protocol. This time will also be used to confirm their eligibility by confirming the accuracy of the information entered in the initial screening questionnaires, obtain informed consent and begin the procedure. The following will be the procedure for the day:

- We will measure subjects' height and weight using a stadiometer and scale, as well as BP. We will eliminate subjects who have a BMI >30.0 and resting systolic BP >140mmHg or resting diastolic BP >90mmHg.
- Subjects will then go through a test battery of:
    - Computerized cognitive battery (see appendix V for description):
        - Serial subtraction of the number three — 2 minutes
        - Serial subtraction of the number seven — 2 minutes
        - Continuous performance test (CPT) — 5 minutes
        - Bakan rapid visual processing task — 16 minutes
    - Self-reported measures (see Appendix N, O, H)
        - Motivation to perform cognitive tasks — 1 minute
        - Visual analog scales of mental energy and fatigue — 1 minute
        - Profile of Mood State fatigue and vigor scales — 3 minutes
    - Physiological measures
        - Resting heart rate and blood pressure — 1 minute
    - Fine motor skills
        - 9-hole peg test — 5 minutes

    Total time — 36 minutes

Subjects who are excluded or choose to drop out on this date will not receive the $5 compensation.

Days 2-4

Testing days 2-4 will be similar except subjects will consume a different beverage daily (list the 3 different beverages). To reduce bias, the days that subjects will consume a certain beverage will be randomized using a randomizing software on randomizer.org. All beverages are labeled as products, A, B or C and are blinded for the researcher. Information regarding all beverages is listed in Table 1.

On each day subjects will complete the following procedure:

Subjects will complete a pre-screening questionnaire on each day. The questionnaire will contain the following surveys

- Appendix M- 24 hour drug/medicine/supplement recall
    - This survey asks for a 24-hour prescription medication, over the counter medication, and supplement recall. Because of the potential for cognitive bias (as described above) we are asking the subjects for the names of any medications they may be taking. In our past studies there were a few subjects that initially reported that they were not taking any prescription or over the counter medications (either for a chronic or acute health condition) however, during the daily testing procedures when we asked these subjects specifically what medications they were taking they listed certain medications. By asking specifically what medications the subjects are taking we are hoping to reduce the risk of cognitive bias and appropriately eliminate subjects who are taking prescription or over the counter medications while they are in the study.
        - The survey asks if subjects are taking an oral contraceptive and specifically what the name of the oral contraceptive is. The information on the specific type of oral contraceptive is needed as certain oral contraceptives have an impact on mood while others do not (Kahn & Halbriech, 2005).
    - We are also asking for caffeine intake and time of intake. To control for cognitive bias we are asking for time of intake.
- Appendix N- Sleep questionnaire
    - This survey will be used to determine the amount of sleep the subject had the night before.

    - This survey will serve two purposes
        - If subjects had > or < ±2 hours of sleep from their normal sleep then they will be rescheduled. Their normal sleep will be determined using Appendix L. Their normal sleep will be given to the researcher in charge of collecting data prior to subject's arrival.
        - If the subject is eligible for the study, the sleep may be used as a covariate in the data analysis.
- Appendix Q- What I ate today
    - This survey will be used to determine what the subject ate the day of the testing
    - The information may be used during data analysis to estimate methyxanthine intake

After subjects have been pre-screened for the day, the research assistant will collect saliva using the drool down method. To collect data using the drool down method subjects must collect saliva in their mouth and let it drool down through a straw into a 15mL saliva collection tube. After ~2mL of saliva has been collected, saliva will be stored in a chest filled with ice, prior to transportation to a freezer that is set at -80F.
After saliva collection, subjects will go through the following mental energy test battery (see table 2). Consumption of beverages and subsequent mental energy test batteries will be administered as outlined in table 2.
Dependent Variables

1) Computerized cognitive battery:
    a. Serial subtraction of the number three — 2 minutes
    b. Serial subtraction of the number seven — 2 minutes
    c. Continuous performance test (CPT) — 5 minutes
    d. Bakan rapid visual processing task — 16 minutes
2) Self-reported measures
    a. Motivation to perform cognitive tasks (Appendix N) — 1 minute
    b. Visual analog scales of mental energy and fatigue (Appendix O) — 1 minute
    c. POMS (Appendix H) — 3 minutes
3) Physiological measures
    a. Resting heart rate and blood pressure — 1 minute
4) Fine motor skills
    a. 9-hole peg test — 5 minutes

Total time — 36 minutes

The 9-hole peg test is used to measure finger dexterity. The test is administered by asking the client to take the pegs from a container, one by one, and place them into the holes on the board, as quickly as possible. Participants must then remove the pegs from the holes, one by one, and replace them back into the container.

After completion of the mental test batteries and procedure outlined in Table 2, subjects saliva will be collected again using the drool down method.

Table 1

Ingredient Name	**Current E-shot**	**Placebo w/ Caffeine**	**Placebo w/o Caffeine**
Water	4oz	4oz	4oz
LERA Herbal Extract Complex	Proprietary	0	0
Green Tea (Camellia sinensis) Leaf Extract (50% Caffeine)	Proprietary	0	0

Yerba Mate Extract (25% Caffeine)	Proprietary	0	0
D-Ribose	25.000 mg	0	0
Synthetic Caffeine	0.000	100.000mg	0
Apple Juice Concentrate 70 Brix	Trace amounts	Trace amounts	Trace amounts
Glycerin (vegetable source)	Trace amounts	Trace amounts	Trace amounts
Stevia (Stevia rebaudiana) Leaf Extract, 97%	Trace amounts	Trace amounts	Trace amounts
Pomegranate Juice Concentrate ME-3605	Trace amounts	Trace amounts	Trace amounts
Masking Flavor Nat ME-1748	Trace amounts	Trace amounts	Trace amounts
Pomegranate-Berry Flavor Nat ME-2569	Trace amounts	Trace amounts	Trace amounts
Mint Flavor Nat WONF ME-2759	Trace amounts	Trace amounts	Trace amounts
Sodium Citrate	Trace amounts	Trace amounts	Trace amounts
Malic Acid	Trace amounts	Trace amounts	Trace amounts
Potassium Sorbate	Trace amounts	Trace amounts	Trace amounts

Table 2

Time (minutes)	Event	Test Phase
0 – 36	Test battery	Trial 1
36-66	Beverage then seated rest	
66-102	Test battery	Trial 2
102-112	Rest	
112-148	Test battery	Trial 3
148-158	Rest	
158-194	Test battery	Trial 4

Analysis of outcomes:

Hypothesis will be tested using a two factor (3 beverages x 5 times) repeated measures ANCOVA on change scores (Times 4, 3 and 2 minus Time 1 are the 5 Times). The primary interest will be whether there is a statistically significant main effect for time or interaction. Focused contrasts between specific time points as follow up tests.

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- Informed consent for online screening

Clarkson University
Documentation of Informed Consent to Participate in Research

I agree to answer online questions about my demographics, medical and physical activity history, caffeine consumption, and recent dietary patterns including alcohol consumption truthfully. It takes about 15 minutes to answer the questions.

Purpose. The purpose of me providing this information is to determine whether or not I am eligible to participate in a research study titled **"Effect of Isagenix e-shot energy beverage on mental energy"**.

Project Title: **Effects of Isagenix e-shot energy beverage on mental energy**
Researcher(s): **Ali Boolani, Costel Darie**
Institutional Review Board (IRB) approval number: 16-34.1 **Approval valid until**: 9/26/2018

You have been asked to be a part of the research described here. Participation is voluntary.

The purpose of this study: The purpose of this study is to determine the impact of natural and synthetic caffeine on mood and cognitive function.

What to expect:
I will complete a series of questionnaires that will determine my eligibility for this study. The surveys that I will be completing will include a list of demographic, medical and physical activity history, mood, caffeine consumption and recent dietary patterns including alcohol consumption. If I feel tired at any time I may stop taking the surveys. I cannot exit the surveys and then re-take them but I can stop and start them at any time. If I feel uncomfortable taking any of these surveys I may stop the surveys and drop out of this study at any point. If you have any questions about this research you may contact
Ali Boolani at aboolani@clarkson.edu

Risks and discomforts to you if you take part in this study:
It is possible that you may experience some discomfort and fatigue from filling out the survey. You may stop the survey at any time. Your information will be kept private on a password protected computer. If you feel uncomfortable with any of the information that is asked during this study you may drop out at any time.

The benefits to you if you take part in this study:
There are no direct benefits to you from completing this online survey.

What will you receive for taking part in this study:
You will receive no compensation for completing this survey. However, if you qualify for the study you may be eligible to receive up to $175.

What will happen to the information collected in this study: The information collected will be kept confidential as much as is permitted by law. Please note that Internet communications are insecure and there is a limit to the confidentiality that can be guaranteed due to the technology itself. If you are not comfortable with the level of confidentiality provided by the Internet, please feel free to print out a copy of the survey, fill it out by hand, and mail it to me at the address given below, with no return address on the envelope. Ali Boolani, Department of

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Physical Therapy, Box 5880, 8 Clarkson Avenue, Potsdam, NY, 13699. You should include your name and a valid email address in the survey for us to contact you

What rights do you have when you take part in this study: Participation in this research is voluntary. Deciding not to take part, or to stop being a part of this research will result in no penalty, fine or loss of benefits that you otherwise have a right to. If you have questions about your rights as a research subject or if you wish to report any harm, injury, risk or other concern, please contact Dr. Johndan Johnson-Eilola, Chair of the Clarkson University Institutional Review Board (IRB) for human subjects research: (315) 268-6488 or johndan@clarkson.edu

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Conflict of Interest: This study is sponsored by the Isagenix corporation

Informed Consent: Please acknowledge reading this consent form by providing your name and email in the text box below. By entering your name and email you are consenting to the use of your information for the purposes of this study. Since there is no simple way to sign an online form you are being asked to enter your information and click submit. By providing your name and clicking submit you are attesting to the fact that you are between the ages of 18-45:

Name:
Contact email:

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In-person Informed consent

Clarkson University
Documentation of Informed Consent to Participate in Research

Project Title: **Effects of Isagenix e-shot energy beverage on mental energy**
Researcher: **Ali Boolani**
Institutional Review Board (IRB) approval number: 16.34.1 **Approval valid until**: 09/26/2018

You have been asked to be a part of the research described here. Participation is voluntary.

The purpose of this study: The primary purpose of the research is to learn the extent to which synthetic and natural caffeine influence measures of mental energy.

What to expect: If you volunteer, your participation will involve a total time commitment of about 17.5 hours. You will be required to visit the laboratory in room 256 in Clarkson Hall 4 separate days. Testing on days 2-4 will take approximately 3 hours and 20 minutes each day. You will do the following:

1. You will go to the lab on testing day 1 and complete questionnaires and practice the measures of mental energy. This will take about 30 minutes.
2. On testing days 2-4 you will go to the lab at my agreed upon time and location. You will report on how you slept the night before and recreational and/or prescription drug use. You will provide saliva and complete a series of questionnaires, cognitive tests, fine motor tasks and my heart rate and blood pressure will be monitored. You will consume the beverage provided to you. You will rest for 30 minutes. Then over the next 140 minutes you will complete questionnaires, cognitive tests, fine motor tasks and my heart rate and blood pressure will be monitored four more times. Also, you will provide a two milliliters (about ½ of a teaspoon) of saliva, into a coded test tube (Your name will not be on the test tube) two times each day (at the beginning and end of testing). These coded test tubes will be frozen, analyzed in labs at Clarkson for cortisol and caffeine. Any remaining saliva will be thrown away.

Risks and discomforts to you if you take part in this study: It is possible that you will experience minor stomach discomfort and an elevated heart rate after consuming the beverage. The only minor risks include possible stomach discomfort and the risk is being reduced by excluding people with allergies or gastrointestinal problems. We are minimizing the risk by checking for heart rate every 30 minutes. If you feel uncomfortable answering any of the questions that are asked in this study you may drop out of the study at any time.

The benefits to you if you take part in this study: You will receive no direct benefit by participating. New knowledge about the influence of acute cocoa consumption on mental energy will be obtained and shared with the scientific community.

What will you receive for taking part in this study: You will receive monetary compensation in the amount of $5 for completing day 1, $10 for completing day 2, $10 for completing day 3 and $150 for completing day 4 for a total of $175.

What will happen to the information collected in this study: No individually-identifiable information about you, or provided by you during the research, will be shared with others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care), or if

required by policy of Clarkson University's Research Participant Payment Policy.

The two consent forms that contain my name and that you sign will be kept in a location separate from the information that will be obtained from you during this study. To safeguard your identity, you will give the researcher a 5-digit code that the researcher will use on all forms. This code will not appear on the consent forms. If selected to qualify, your 5-digit number will be given to you on a notecard to type and use for the rest of the protocol.

My identity will not be revealed in any publication of the results of this research. The individually-identifiable results of your participation will be confidential. A copy of your test results and the study results will be made available to you if you request it. You will need to remember and provide your 5-digit code. To receive the payment you will have to provide your name and address on a sheet of paper that will be given to the Department of Physical Therapy. You can participate without payment.

The Principal Investigator, Dr. Ali Boolani, or the research assistant will be happy to answer any further questions about the research, now or during the course of the project. Ali Boolani can be reached by telephone in his office (315-268-1663) or on his cell phone (504-319-5828) or by email (aboolani@clarkson.edu).

What rights do you have when you take part in this study: Participation in this research is voluntary. Deciding not to take part, or to stop being a part of this research will result in no penalty, fine or loss of benefits that you otherwise have a right to. If you have questions about your rights as a research subject or if you wish to report any harm, injury, risk or other concern, please contact Dr. Johndan Johnson-Eilola, Chair of the Clarkson University Institutional Review Board (IRB) for human subjects research: (315) 268-6488 or johndan@clarkson.edu

Conflict of Interest: The researchers have no financial interest in performing this study.

Informed Consent: Please sign here to show you have had the purpose of this research explained and you have been informed of what to expect and your rights. You should have all your questions answered to your satisfaction. Your signature shows that you agree to take part in this research. By signing below you also attest that you are

- Not taking any prescription for chronic conditions
- Are between the ages of 18-45.
- Not a woman who is pregnant, or is trying to become pregnant
- Not breastfeeding
- Not diagnosed with a heart conditions, high blood pressure, live disorder, bipolar disorder or iron deficiency.
- Not allergic or sensitive to caffeine.

If you have any of the preceding conditions you may be putting your health at risk by participating in this study.

You will be given a copy of this consent form to keep for your records.

Signature of volunteer: _____ **Date:** _____

Signature of researcher obtaining informed consent: _____ **Date:** _____

In addition to the Primary Investigator, listed above, the following people have been authorized to obtain Informed Consent: