Impacts of a combined berry extract supplement on vascular function and oxygen utility capacity in young adults: full study protocol and statistical analysis plan

NCT#04312022

10-04-2018

Study protocol

Participants

Healthy adults (n = 18, age 19-35, 9 males and 9 females) who were recreationally active volunteered to participate in this study. Exclusion criteria included 1) any cardiovascular, neurological, metabolic, respiratory, or renal diseases, 2) any musculoskeletal conditions or injuries, 3) presence or history of stomach ulcers, 4) prescribed medications or over-the-counter medications, 5) pregnant, trying to become pregnant, or breast-feeding women, 6) any history of smoking/current smoker, and 7) any allergies to fruits or vegetables. The procedures used in this study were approved by the Institutional Review Board at the University of Nebraska Medical Center and carried out in accordance with the Declaration of Helsinki. All participants gave their written informed consent prior to study enrollment, during which the experimental procedures, potential risks, and potential benefits were explained. This study was registered with https://clinicaltrials.gov/ (NCT04312022).

Study design

A randomized, double-blind, placebo-controlled, crossover study design with a 2-week washout period was used. After study enrollment, participants were randomly assigned to either the BE supplement group or the placebo control group. All data collections were performed at the same time of day (± 1h) after an overnight fast, and participants were asked to abstain from caffeine, alcohol, high intake of antioxidant-rich foods or supplements, and excessive exercise for at least 48 hours prior to their visits. Participants were also informed to not change their dietary habits during the study period. Descriptive measurements of height, weight, BMI, body composition, and hand grip strength were taken at both BE and placebo visits. Baseline measurements of RHR, BP, blood sampling for TAC, endothelial function, skeletal muscle oxygen utility capacity during exercise, and muscular fatigue index were assessed. Participants then consumed either the BE or placebo, and all baseline measures were repeated as experimental measures 1 hour after BE or placebo intake. A 1-hour digestion period was chosen because anthocyanins have been shown to reach peak levels in the blood within 1-hour post consumption[1,2]. After 2 weeks each participant returned for a second visit and the same protocol was repeated with the intervention (BE or placebo) they did not receive in the first visit. All women (n = 9) were tested during the early-to-mid follicular (days 1 – 10) and late-luteal phases (> day 19) of the menstrual cycle in order to avoid confounding effects of endogenous estrogen on autonomic function[3].

Supplementation

The antioxidant supplement used in this study contained 3 natural plant-based ingredients including 1) hawthorn berry extract, derived from the hawthorn berry (Crataegus spp.), 2) tart cherry powder (Prunus cerasus), a rich source of anthocyanins, and 3) bromelain, a mixture of proteases obtained from the stems and immature fruits of pineapples. The BE supplement consisted of 2 capsules with a total of 465mg, 480mg, and 400mg of hawthorn extract, tart cherry powder, and bromelain, respectively (CardioEffects, Fitfully LLC, Omaha, NE, USA). This is similar to other doses used in previous studies and has been shown to have no adverse side effects[4,5]. The anthocyanin content for this supplement was provided by the company, in which two capsules contain ~68 mg of anthocyanins. The separate

components include ~67 mg from tart cherry and ~2000 ng from hawthorn berry, which is consistent with previous studies[4,6]. The tart cherry anthocyanin content is similar to ~31 mL of tart cherry juice[4] while the hawthorn berry anthocyanin content is similar to ~1g of dried hawthorn berries[6]. Bromelain is primarily extracted from pineapple stems[7], and therefore bromelain is more often given in supplement form than by natural consumption. All capsules used in the present study came from the same batch from the manufacturer. The placebo consisted of 2 tapioca powder capsules that were identical in size and appearance to the BE supplement and did not possess any antioxidant properties. The capsules were given to the participant by a lab member not directly involved in the study measurements or analyses.

Anthropometrics

A standard stadiometer was used to measure height to the nearest 0.1 cm. Body mass was measured to the nearest 0.1 kg using a standard scale. Body mass index (BMI) was calculated as the body mass divided by the square of height (kg/m2). Body fat percentage was quantified using handheld bioelectrical impedance analysis (BIA) (Omron, model HBF-306C, Omron Healthcare, Inc., Lake Forest, IL). Body fat percentage was measured in duplicate, and the average of the 2 was recorded as the body fat percentage.

Resting heart rate and blood pressure

RHR and BP were assessed before and after BE and placebo intake. Participants rested in a seated position in a quiet room for 5 minutes and were informed not to talk or move during this time. RHR, systolic BP, and diastolic BP were measured using an automated sphygmomanometer (Omrom Blood Pressure Monitor BP786N, Omron Healthcare, Inc., Lake Forest, IL) in duplicate. The two measurements of the RHR and BP were averaged and recorded as the resting values.

Blood sampling

Blood samples were collected from an antecubital vein by a trained phlebotomist using EDTA tubes before and after BE and placebo intake. Samples were centrifuged at 3500 rpm for 10 minutes at 4°C. Plasma samples were stored at -30°C for later analysis of total antioxidant capacity (TAC). TAC was assessed using a commercially available Total Antioxidant Capacity Assay Kit (CAT#: ab65329, Abcam, Cambridge, UK) according to the manufacturer's instructions. After incubating samples at room temperature (23°C), absorbances were measured at 570nm using a microplate reader. The average intra-assay and inter-assay coefficient of variation for TAC were 6.3% and 2.9%, respectively.

Endothelial function

Flow-mediated dilation (FMD) of the brachial artery was used to assess endothelial function before and after BE and placebo intake. FMD is an endothelium-dependent assessment that facilitates brachial artery relaxation in response to an increase in shear stress. FMD was assessed using a Terason uSmart 3300 Doppler ultrasound system (Terason Division Teratech Corporation, Burlington, MA) and EKG trigger

monitor (7700 Series Trigger Monitor, IvyBiomedical Systems, Inc., Branford, CT). The ultrasound probe was used to locate the brachial artery on the participant's right arm, and a rapid-inflation cuff (E20 Rapid Cuff, D.E. Hokanson, Bellevue, WA) was placed on the forearm distal to the ultrasound probe. A baseline resting brachial artery diameter was recorded for 5 minutes using an image capturing system (Vascular Imager, Vascular Research Tools 6, Medical Imaging Applications, Coralville, IA). The cuff was then inflated to 250 mmHg for 5 minutes. The cuff was released, and the reactive hyperemic response of the artery was recorded for 5 minutes using the ultrasound and image capturing system. The baseline resting diameter and post-hyperemic stimulus were analyzed using automated edge detection software (Brachial Analyzer, Vascular Research Tools 6, Medical Imaging Applications, Coralville, IA). The most stable 30-60 seconds of the baseline artery diameter measurement, including at least 10 cardiac cycles, was averaged as the resting diameter [8].

Skeletal muscle oxygen utility capacity

Skeletal muscle oxygen utility capacity during single leg extension exercise was assessed before and after BE and placebo intake. Oxygenation utility capacity of the vastus lateralis was measured during leg extension exercise with a commercially available NIRS system (Artinis PortaMon, Einsteinweg, The Netherlands). The PortaMon emits near-infrared wavelengths of 850 and 764 nm and has a detection probe to measure returning signals, and data were recorded continuously at 10 Hz to quantify tissue saturation index (StO2, %) and concentrations of both oxygenated hemoglobin ([O2Hb], a.u.) and deoxygenated hemoglobin ([HHb], a.u.).

To determine single leg extension strength, a 1-repetition maximum (1RM) test was performed using the participant's dominant leg. Participants were familiarized with the leg extension technique before the 1RM measurement, which was achieved within 3 attempts. The 1RM was considered the highest weight that could be lifted in good form through a full range of motion. The PortaMon was secured with a commercially available, double-sided adhesive at one- third of the distance from the lateral femoral epicondyle and the greater trochanter, and the device was adjusted to be on the belly of the vastus lateralis muscle[9]. The device was wrapped in black, light-absorbing cloth to reduce extraneous light that may affect the signals. Participants were then asked to perform 15 repetitions at 60% of their 1RM while NIRS data were recorded continuously throughout the exercise protocol.

Fatigability index

Fatigability index was quantified during Isokinetic contraction of the participant's dominant leg before and after BE and placebo intake using HUMAC NORM Isokinetic Dynamometer (CSMi Solutions, Stoughton, MA). The participants were seated upright with the axis of rotation of the dynamometer arm oriented with the axis of rotation of the participant's dominant knee. Belts and straps were used to secure the participant to the dynamometer, and participants were instructed to fully extend and flex their knee and to work to their maximal capacity during the leg extension exercise. Before the test, there were 4 familiarization repetitions at the testing resistance. The testing consisted of a mild resistance at 240°/sec to induce and measure muscular fatigue. The software (HUMAC 2015, v.15.000.0103) reported the data of fatigue index, which was calculated using the percentage peak torque differences that were

found on the first and final 5 repetitions of the exercise bout (fatigue index = [initial peak torque – final peak torque]/initial peak torque * 100). The data obtained from the analysis software allowed for the assessment of changes in numbers relative to the participant baseline measurements.

Statistical analysis

A Shapiro-Wilk's test was used to determine normal distribution of the data. Independent t-tests were used for evaluating baseline characteristics at the BE and placebo visits. Dependent variables were assessed suing a 2 x 2 repeated measures analysis of variance (ANOVA) [group (BE and placebo) x time (before and after supplement intake)] to determine differences between pre- and post-BE and placebo intake. If a significant effect was noted, paired t-tests were used for post hoc comparisons. All statistical analyses were performed with SPSS 26.0 (IBM, Armonk, NY). Data are presented as Mean ± SD unless noted otherwise. Statistical significance was set to $p < 0.05$. It was calculated that a minimum of 16 total participants in a crossover design (16 each group, BE and placebo) would enable 80% power to observe a 3 to 5% change in FMD between the two groups[10]. An effect size analysis was performed using Cohen's d and interpreted 0.2, 0.5, and 0.8 as small, medium, and large effect sizes, respectively[11].

1. Matsumoto H, Takenami E, Iwasaki-Kurashige K, Osada T, Katsumura T, Hamaoka T. Effects of blackcurrant anthocyanin intake on peripheral muscle circulation during typing work in humans. Eur J Appl Physiol. 2005;94:36-45. doi: 10.1007/s00421-004-1279-y.

2. Stoner GD, Sardo C, Apseloff G, Mullet D, Wargo W, Pound V, Singh A, Sanders J, Aziz R, Casto B, et al. Pharmacokinetics of anthocyanins and ellagic acid in healthy volunteers fed freeze-dried black raspberries daily for 7 days. J Clin Pharmacol. 2005;45:1153-1164. doi: 10.1177/0091270005279636.

3. Saeki Y, Atogami F, Takahashi K, Yoshizawa T. Reflex control of autonomic function induced by posture change during the menstrual cycle. J Auton Nerv Syst. 1997;66:69-74. doi: 10.1016/s0165-1838(97)00067-2.

4. Levers K, Dalton R, Galvan E, O'Connor A, Goodenough C, Simbo S, Mertens-Talcott SU, Rasmussen C, Greenwood M, Riechman S, et al. Effects of powdered Montmorency tart cherry supplementation on acute endurance exercise performance in aerobically trained individuals. J Int Soc Sports Nutr. 2016;13:22. doi: 10.1186/s12970-016-0133-z.

5. Zick SM, Gillespie B, Aaronson KD. The effect of Crataegus oxycantha Special Extract WS 1442 on clinical progression in patients with mild to moderate symptoms of heart failure. Eur J Heart Fail. 2008;10:587-593. doi: 10.1016/j.ejheart.2008.04.008.

6. Rodrigues S, Calhelha RC, Barreira JCM, Dueñas M, Carvalho AM, Abreu RMV, Santos-Buelga C, Ferreira ICFR. Crataegus monogyna buds and fruits phenolic extracts: Growth inhibitory activity on human tumor cell lines and chemical characterization by HPLC–DAD–ESI/MS. Food Research International. 2012;49:516-523. doi: 10.1016/j.foodres.2012.07.046.

7. Saptarini NM, Rahayu D, Herawati IE. Antioxidant Activity of Crude Bromelain of Pineapple (Ananas comosus (L.) Merr) Crown from Subang District, Indonesia. J Pharm Bioallied Sci. 2019;11:S551-S555. doi: 10.4103/jpbs.JPBS_200_19.

8. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. Hypertension. 2010;55:1075-1085. doi: 10.1161/HYPERTENSIONAHA.110.150821.

9. McLean S, Kerherve H, Lovell GP, Gorman AD, Solomon C. The Effect of Recovery Duration on Vastus Lateralis Oxygenation, Heart Rate, Perceived Exertion and Time Motion Descriptors during Small Sided Football Games. PLoS One. 2016;11:e0150201. doi: 10.1371/journal.pone.0150201.

10. Figueroa A, Park SY, Seo DY, Sanchez-Gonzalez MA, Baek YH. Combined resistance and endurance exercise training improves arterial stiffness, blood pressure, and muscle strength in postmenopausal women. Menopause. 2011;18:980-984. doi: 10.1097/gme.0b013e3182135442.

11. Sullivan GM, Feinn R. Using Effect Size-or Why the P Value Is Not Enough. J Grad Med Educ. 2012;4:279-282. doi: 10.4300/JGME-D-12-00156.1.