The Clinical and Physiological Consequences of Blueberry Consumption in Older Adults

PROTOCOL

Version 1.3

March 17, 2022

Summary of Protocol Versions and Changes

Version 1.0

- Original version submitted

Version 1.1

- Incorporated edits from the IRB on 12/23/21 and 1/7/22

Version 1.2

- Removed cardiac ectopy as a secondary outcome and all procedures related to Holter monitoring

Version 1.3

- Added cardiac ectopy as a secondary outcome and all procedures related to including a 24-hour electrocardiograph monitor (Holter)

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List of Abbreviations / Glossary

ABPM	Ambulatory Blood Pressure Monitor
ALT	Alanine Aminotransferase
AOBP	Automated Office Blood Pressure
AST	Aspartate Aminotransferase
BIDMC	Beth Israel Deaconess Medical Center
BMI	Body Mass Index
BV	Baseline Visit
CDC	Centers for Disease Control & Prevention
CITI	Collaborative Institutional Training Initiative
CRC	Clinical Research Center
DBP	Diastolic Blood Pressure
DSQ	Dietary Screener Questionnaire
FV	Follow-up Visit
HDL-C	High-Density Lipoprotein Cholesterol
HIPAA	Health Insurance Portability and Accountability Act
HRQOL	Health-Related Quality of Life
HSL	Hebrew SeniorLife
INT	Intervention
NBoC	NewBridge on the Charles
NIA	National Institute on Aging
OHRP	Office for Human Research Protections
PCP	Primary Care Physician
PI	Principal Investigator
PSQI	Pittsburgh Sleep Quality Index
PSV	Pre-Screen Visit
QUID	Questionnaire for Urinary Incontinence Diagnosis
SBP	Systolic Blood Pressure
SPPB	Short Physical Performance Battery
STURDY	Study to Understand Fall Reduction and Vitamin D in You
SV	Screening Visit
USDA	United States Department of Agriculture
WO	Wash-Out

1. Abstract

The population of older Americans is growing rapidly. As these older adults continue to live longer, accruing greater health care costs, the health needs of this portion of the population are of truly paramount importance. Although much is already known about the benefits of a healthy diet for prevention of a wide range of chronic disease, including the particular health benefits of anthocyanin-rich foods, these effects have been examined with much less frequency in older adults, who tend to be excluded from formal feeding studies and, until recently, have represented a small proportion of ongoing cohort studies.

This parallel-design trial will evaluate the effects of whole blueberry consumption in older adults by leveraging a novel senior living campus. Participants will be randomly assigned to one of two fruits: whole frozen blueberries, equivalent to 1 cup per day, or a daily isocaloric serving of 2-3 dried dates. Dried dates are an attractive control because they contain negligible polyphenols, proportionately high caloric content, and a convenient form of storage. Participants will consume their assigned fruit for 12 weeks after enrollment. We anticipate to enroll approximately 70 participants over the length of the project.

The primary outcomes of this trial relate to blood pressure, including 24-hour ambulatory blood pressure monitoring and seated and standing office blood pressure measured at the baseline and final visits. Other outcomes include physical function, falls, cognition, sleep quality, incontinence, bone turnover, cardiovascular risk, cardiac ectopy, liver and kidney function, and glucose metabolism. The in-person follow-up visit will occur at Week 12, with weekly remote compliance checks starting at baseline. This trial aims to determine a wide range of health effects of whole blueberry intake in older adults in an innovative, cost-effective, and pragmatic manner.

2. Specific Aims

Blueberries and other anthocyanin-rich foods have been shown to prevent a wide range of chronic diseases, yet older adults are rarely included in feeding and cohort studies. Many of the known effects of blueberries could have particular importance for older adults, but because of their unique physiological needs, it is difficult to extrapolate the effects of diet from studies completed in younger populations. This randomized parallel-design trial will examine the clinical and physiological effects of 3-months of daily whole blueberry consumption in adults ages 70+, who are independent-living residents of the senior living facility NewBridge on the Charles in Dedham, Massachusetts.

Participants will be randomly assigned to consume either whole frozen blueberries or dried dates daily for 12 weeks. We anticipate enrolling approximately 70 adults (~60% female, ~40% male). In-person follow-up will occur at Week 12 post-randomization.

Primary Outcome: Blood pressure measured by 24-hour ambulatory blood pressure monitoring and seated and standing automated office blood pressure measuring at baseline and follow-up.

Primary Aims:

- Physical function, measured by grip strength and the Short Physical Performance Battery of balance/walk/chair stands
- Falls, defined as any fall, slip, or trip in which participant loses balance and lands on the floor, ground, or at a lower level, as measured by the Study To Understand Fall Reduction and Vitamin D in You (STURDY) screening protocol
- Cognition, measured by the Cogstate Brief Battery of four core cognitive domains including processing speed, attention, visual learning, and working memory

Secondary Aims:

- Sleep, measured by the Pittsburgh Sleep Quality Index (PSQI)
- Incontinence, measured by the Questionnaire for Urinary Incontinence Diagnosis (QUID)
- Bone turnover, measured by C-terminal telopeptide of type 1 collagen
- Cardiovascular risk, measured by troponin I, total and HDL cholesterol
- Cardiac ectopy, measured by atrial premature beats detected by Holter monitor
- Liver function, measured by AST, ALT, albumin, total and direct bilirubin
- Kidney function, measured by creatinine and albuminuria
- Glucose metabolism, measuring by fasting glucose and insulin, fructosamine, and free fatty acids
- Inflammation, measured by C-reactive protein and white blood cell count

We hypothesize that improved vascular function could ameliorate the high rates of hypertension while simultaneously also improving orthostatic hypotension in older adults. Orthostatic hypotension is the phenomenon of a drop in blood pressure upon standing that can lead to falls. Other clinical domains mentioned above, such as cardiac arrhythmias, falls, and physical function, are also critically important to older adults and require greater attention.

3. Significance and Scientific Background

Background

The population of older Americans is growing rapidly. The number of American adults aged ≥65 years will double between 2012 and 2050, reaching 84 million adults and a full 21% of the population. As these older adults continue to live longer, accruing greater health care costs, the health needs of this portion of the population are of truly paramount importance.

Although much is already known about the benefits of a healthy diet for prevention of a wide range of chronic disease,(1, 2) including the particular health benefits of anthocyanin-rich foods,(3-6) these effects have been examined with much less frequency in older adults, who tend to be excluded from formal feeding studies and, until recently, have represented a small proportion of ongoing cohort studies. Because older adults have unique physiological needs and are susceptible to specific geriatric syndromes like frailty or cognitive impairment, it is difficult to extrapolate the full health effects of diet on older adults from studies of younger populations. In addition, there may be potentially paradoxical effects of some lifestyle interventions, such as weight loss, on functional status in older women,(7) even though formal studies of those interventions have not clearly demonstrated different effects in younger and older participants.(8)

Many of the known effects of anthocyanin-rich foods like blueberries could have particular importance for older adults. For example, a recent comprehensive review of research on the health effects of blueberries by Kalt and colleagues identified potential benefits on lipids, vascular endothelial function, cognition, and (in many studies) glucose metabolism.(9) Glucose metabolism in particular remains associated with adverse events, even late in life.(10) Blueberries have also been associated with improved bone density.(11) Intriguingly, a few studies of blueberries (largely in the form of supplemented flavonoids or powder) did include older adults and suggested a possible benefit on cognitive function in as little as 3 months.(12-15) Other studies have demonstrated activation of specific brain regions with blueberry or anthocyanin intake, supporting the possibility of improved brain function with supplementation.(16)

Important limitations exist in the current body of evidence regarding blueberries in older adults. First, these studies have, to date, largely focused on blueberry extracts, rather than whole berries. Extracts have important advantages in reproducibility, blinding, and packaging, ensuring that studies have maximum internal validity. However, their acceptability to older adults is likely to be limited, reducing their generalizability and utility for longer-term studies. In our view, pragmatic designs that test real-world foods have the potential for the greatest impact on public policy and clinical practice, as they can be immediately translated into discrete behavioral changes, especially after preliminary studies using extracts or supplements have established plausible health effects. That is, studies using whole foods can be directly translated into clinical and public health guidelines that have profound societal impact and can be incorporated into specific diets and dietary patterns. While pragmatic designs can introduce more variability, studies conducted in well-controlled settings can overcome this limitation.

Second, truly older adults – in the fastest growing age groups – have not been included in representative numbers. For example, participants in the trial conducted by Miller and colleagues of 'older adults' had an average age of 67 years;(17) whether the provocative benefits they identified can be extrapolated to adults in their seventies and eighties remains unknown.

Third, many important clinical domains have yet to be fully tested in these studies. In particular, our group has substantial expertise in the evaluation of blood pressure in older adults, including 24-hour blood pressure monitoring.(18) Blood pressure in older adults poses enormous challenges because older adults have extremely high rates of hypertension yet also high rates of orthostatic hypotension, the phenomenon of a drop in blood pressure upon standing that can lead to falls.(19-21) Medications to decrease resting hypertension can potentially exacerbate standing hypotension, posing a specific risk of pharmacological antihypertensive therapy in this age group. We hypothesize that improved vascular function, as previously demonstrated with blueberry extracts,(22) could ameliorate both abnormalities, as expected from their associations with endothelial health,(23, 24) but this has never been tested. Other clinical domains that require greater attention because of their specific importance to adults late in life include cardiac arrhythmias (particularly paroxysmal atrial fibrillation), falls, and physical function.

Our research group has particular expertise in both clinical trials of diet and in assessment of older adults. We have conducted clinical trials of specific macronutrients (e.g., marine fatty acids), dietary elements (e.g., black tea), and whole diets.(25-29) We recently completed a full-diet replacement study at Jack Satter House, a HUD-subsidized congregate living facility for low-income elders in Revere, MA, in which we randomized 20 participants to receive a standard or low-sodium diet for two weeks and measured their effects on blood pressure, urine sodium excretion, functional status, and other outcomes.(30) In addition to epidemiological work studying the health effects of anthocyanins and other polyphenols,(5, 6) our group has also had major roles in ongoing cohort studies of older adults like the Cardiovascular Health Study and the Study to Understand Fall Reduction and Vitamin D in You (STURDY).(31, 32) Within these studies, we have examined all of the outcomes relevant to older adults that we propose in this trial, ensuring that we have the requisite experience to measure the health effects of blueberries in older adults with precision and accuracy.

To produce a useful contrast with blueberries, a control food would ideally have several characteristics. These include: 1) low-to-no polyphenol content; 2) sugar content at least as high as blueberries (to enable ready caloric equilibration); 3) availability as a discrete item (to enable easier distribution and simplify instructions on consumption; 4) limited content of other biologically-active factors (e.g., fiber, minerals); 5) appeal to older adults; and 6) long shelf-life. Based on these criteria, we propose to study dates, which have considerable appeal to health-conscious elders, moderate fiber and potassium content (similar to blueberries), long shelf-life (in their dried form), and very low polyphenol content.(33) Conveniently, their high sugar density enables an isocaloric contrast with ~2 dates per day, maximizing ease and adherence among participants.

Significance

Each of our primary and secondary aims has important implications for the health of older adults, can be measured effectively with resources available through the Clinical Research Center (CRC), and has the potential to change in a 12-week period of observation. Moreover, our primary domains have already been linked directly or indirectly to polyphenol intake. This trial takes advantage of a unique partnership between a Harvard-affiliated teaching hospital, Beth Israel Deaconess Medical Center, a Harvard-affiliated geriatric research center, Hebrew SeniorLife, and a congregate living facility, NewBridge on the Charles, to determine a wide range of health effects of whole blueberry intake in older adults in an innovative, cost-efficient, and pragmatic manner.

4. Design

Principal research objective

To conduct a single-center, randomized, parallel-design experimental trial comparing whole frozen blueberries (equivalent to 1 cup of fresh blueberries) to an isocaloric control, with single-blind assessment of outcomes among approximately 46 women and 24 men. Participants must be aged 70 years or older and independent-living residents of NewBridge on the Charles for at least 3 months at randomization. For feasibility, the study uses a pragmatic intervention, comparing blueberries to 2 dried dates daily for 12 weeks. Our specific objectives are to determine the clinical and physiological effects of 12 weeks of daily blueberry consumption primarily on blood pressure, physical function, falls, and cognition in older adults.

5. Study Population and Eligibility

The study population will consist of approximately 70 adults, aged 70 and older, who are independent-living residents of NewBridge on the Charles (NBoC) for at least 3 months at baseline. To enhance the generalizability of this trial, we have a few exclusion criteria. Eligibility will be determined over a series of contacts, including telephone contacts and in-person visits. Table 1 lists eligibility criteria.

Table 1. Eligibility Criteria.

Inclusion Criteria
• Age 70 and older • Independent-living • Resident of NewBridge on the Charles for at least 3 months • Willing to accept randomization to each fruit after tasting both options
Exclusion Criteria
• Myocardial infarction, stroke, or revascularization procedures ≤3 months prior to randomization • AHA Class III-IV heart failure • Active titration of blood pressure medication or titration within the last 3 months • Intolerance or allergy to blueberries or dates; allergy to tree nuts, peanuts, soy, or milk products • History of gastric bypass surgery • Planned hospitalization in the ensuing 4 months • Planned vacation lasting >1 week in the ensuing 4 months • Any current cancer treatment • End-stage renal disease • Any organ transplant • Uncontrolled diabetes mellitus with hemoglobin A1c >9% • Inability to provide personal informed consent (e.g., cognitive impairment) • Seated systolic blood pressure > 200 mm Hg at Screening Visit • Investigator concern

6. Recruitment

Field Center

The trial will take place at a single center facility, NewBridge on the Charles, in Dedham, MA. NBoC is a senior living campus operated by Hebrew SeniorLife, a Harvard-affiliated, non-profit care organization. All recruitment and clinical visits, aside from phlebotomy, will be conducted in private space on the NBoC campus. Conducting the study visits at NBoC will be extremely convenient for participants and thus increase retention. Phlebotomy appointments will be conducted at the CRC in Boston, MA. Reimbursement for transportation (i.e. Lyft, Uber) or parking will be provided. All residents have freezers in their kitchens for blueberry storage. There will also be a freezer in the central kitchen dedicated to blueberry storage for later distribution.

Recruitment Strategies

Participants will be recruited on-site at NewBridge on the Charles by study staff. Because residents live on the campus, we will undertake numerous precautions to educate them regarding the voluntary nature and potential specific importance of our protocol. We will conduct several site-wide introductory presentations about the study to garner interest and awareness. Study staff will be available to answer questions for those interested.

Staff will also provide informational flyers and advertise with posters around the site. Residents will receive a recruitment letter and/or postcard from the study with general information. NBoC has several display monitors throughout campus that are used to share information, activities, and events on campus that the study will use to advertise. In addition, study information will be included in the weekly flyer that is distributed to residents by NBoC. Given the demographic characteristics of individuals at NBoC, we anticipate that women will make up approximately 65% of our study population.

7. Data Collection and Measurements

Data Collection Contact Schedule

Eligibility, baseline, and follow-up data will be collected by phone and at in-person meetings. In-person data collection will be primarily conducted at NBoC in Dedham, MA by the Research Coordinator. However, we will have participants complete blood draws and spot urine collections at the Clinical Research Center in Boston, MA. In some situations and with participant consent, we may have to collect data in other locations (e.g., the participant's apartment on-site), if they cannot get to our clinical location. This would be due to illness or immobility. In general, we will try to be as flexible as possible to meet the needs of our participants. For example, we may divide or bundle data collection, as needed. See Table 2 for proposed data collection items by contact. The primary data collection points for participant-level data are as follows:

Pre-Screen Visit (PSV): A brief medical history questionnaire that asks about conditions noted in the exclusion criteria will be administered by telephone or in person to identify potentially eligible participants quickly and efficiently. Contact information will be recorded for potential participants.

Screening Visit (SV): This in-person visit will be conducted at NBoC. It will include written informed consent, questions about demographics and confirming eligibility, a taste test of the frozen blueberries and dried dates, and a seated blood pressure measure to determine eligibility.

Wash-Out (WO): The Wash-Out will be the 1-2 week period leading up to the Baseline Visit. Participants will be asked to avoid all anthocyanin-rich foods for at minimum 1 week before baseline.

Baseline Visit (BV): After confirming eligibility and signed consent form and orally re-affirming consent, participants will complete fasting blood draw, spot urine collection, questionnaires, physical assessments, and set-up with a 24-hour ambulatory blood pressure monitor and Holter monitor. After successful completion of all BV data collection, participant will be randomized using REDCap. Participants will be given at least a 1-month supply of assigned fruit. They will also receive various handouts for compliance and adherence, tracking intake and falls, reporting unexpected events, and fruit pick-up.

Intervention (INT): Participants will receive a weekly email check-in from BV to FV to measure self-reported compliance with consuming study fruit and avoiding other anthocyanin-rich foods. The questionnaire will also include a section on reporting falls, as defined by the protocol. The purpose of the compliance questionnaire is to maintain rapport and improve adherence. If a participant does report a fall, study staff will call the participant within 3 days to administer the Fall Follow-Up Questionnaire. Additional fruit pick-ups will occur as needed.

Follow-Up Visit (FV): Participants will have an in-person visit at Week 12 after BV to re-do all BV assessments including phlebotomy, questionnaires, and physical function testing. Study staff will debrief with participants and provide some individualized results from the trial.

Table 2. Data collection schedule.

Study Visit Timeline	Visit 1 Screening (Week -2+)	Visit 2 Baseline (Week 0)	Week 4	Week 8	Visit 3 Week 12
Consent	X				
Demographic and medical questionnaires	X				
Vital signs	X				
Taste test	X				
24-hour electrocardiographic monitoring		X			X
24-hour ambulatory blood pressure monitoring		X			X
Fasting blood draw and spot urine		X			X
Randomization		X			
Fruit pick-up		X	X	X	
Compliance and STURDY questionnaires			X	X	X
Seated and standing blood pressure measures and anthropometrics		X			X
Physical function		X			X
Cognitive function		X			X
Sleep, quality of life, incontinence, and dietary questionnaires		X			X

Debrief					X

Measurements
The following sections describe the specific measurements to be collected from participants, in accordance with the above Data Collection Schedule. We have used all of the surveys and questionnaires proposed below in other studies, and all are suitable for older adults. Surveys include the PSQI, (34) the Cogstate Brief Battery, (35) the QUID, (36) the NHANES 2009-10 Dietary Screener Questionnaire (DSQ), (37) and the Health-Related Quality of Life (38). Functional measures include the SPPB and grip strength (39).

For the primary outcomes:
Blood pressure. In this trial, blood pressure will be assessed in several ways. At the BV and the FV, participants will be fitted with and instructed on a 24-hour Ambulatory Blood Pressure Monitor (ABPM). Blood pressure will be continuously monitored and recorded; average wake-time systolic blood pressure will be the primary outcome. Participants will return the ABPM for data upload the following day. At the BV and the FV, participants will undergo seated and supine-to-standing orthostatic blood pressure measurements. A&D Medical ABPMs and software will be used. Omron 907 AOBP machines will be used to measure seated and standing blood pressure.

Physical function. We will assess physical function in various ways. Participants will undergo a grip strength assessment using the average of two measurements in the dominant hand with a Jamar digital handheld dynamometer at the BV and the FV. We will measure functional limitations using the Short Physical Performance Battery (SPPB), an objective assessment developed at the NIA. The SPPB includes timed tests for usual gait speed, balance, and the ability to rise from a chair. A usual-paced 4-meter walk is timed to assess gait speed. For balance, participants are asked to maintain their feet in side-by-side, semi-tandem, and tandem positions for 10 seconds each. Finally, participants are asked to stand up and sit down five times as quickly as possible. Each test is scored from 0 to 4 using cut points from a large population-based study. The final SPPB score, which will serve as the primary functional outcome, is calculated as the sum of the three tests with a range between 0 and 12, with higher scores reflecting better physical performance. Individual components will be secondary outcomes of the trial.

Falls. We will follow the STURDY screening protocol to identify falls in this study. According to STURDY, a fall is defined as any fall, slip, or trip, in which the participant loses his or her balance and lands on the floor, ground, or at a lower level. Following the BV, we will ask participants to document any incidence of a fall on their compliance calendar at the end of each day or the morning of each subsequent day. Participants will be instructed to contact the research coordinator after any fall (after seeking medical attention, if needed).

A standardized follow-up interview will be administered to obtain details about when the fall occurred, the circumstances of the fall, and any resulting injuries and treatment. If a previously

unreported fall is reported during a follow-up interview, a second fall follow-up interview will be administered.

Cognition. Cognitive performance will be measured using the standard Cogstate Brief Battery. The Cogstate Brief Battery measures four core cognitive domains including processing speed, attention, visual learning, and working memory. Participants will complete the Brief Battery at the BV and the FV. The assessment will be administered on a standard PC laptop in a quiet, private room.

The Cogstate Brief Battery has been used extensively in research as a rapid and reliable screening assessment in a range of clinical indications. It has also been widely used in the context of measuring cognitive dysfunction relative to healthy groups in adult populations. Each of the tests maintains high reliability across repeated testing and cross-sectional research designs.

Other data collected:

Medical history. A brief prescreening questionnaire will be administered to collect general medical history information and specifically information related to the inclusion and exclusion criteria. This will be administered by telephone or in-person. Information will be confirmed at the SV.

Contact information. Detailed contact information for the participant and an emergency contact will be obtained at the PSV. Contact information will be updated at subsequent visits, if necessary.

Demographics. At the SV, detailed demographic information will be collected.

Vital Signs. Seated blood pressure measurement and heart rate will be recorded at the SV. This is to partially determine eligibility. A systolic blood pressure >200 mm Hg will exclude participation.

Taste test. Participants will be asked to taste a sample of each study fruit at the SV to ensure palatability. Responses will be recorded to document participant agreement to comply with either assignment at randomization.

Anthropometrics. Height will be measured at the BV using a floor stadiometer. Weight will be obtained at the BV and the FV using a digital calibrated scale.

Medications. Participants will provide a list of their current medications at the BV, which will be updated at the FV, if necessary.

Sleep. The Pittsburgh Sleep Quality Index will be administered at the BV and the FV. Participants can complete this questionnaire on their own, but it will be reviewed for

completeness and entered by study staff. The PSQI is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval. Measures include subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The sum of scores yields one global score. It has been shown to be consistent, valid, sensitive, and specific in distinguishing good and poor sleepers in research settings.

Incontinence. The Questionnaire for Urinary Incontinence Diagnosis will be given at the BV and the FV. QUID is a self-administered, 6-item questionnaire used to distinguish between stress urinary incontinence and urge urinary incontinence. It is a reliable and valid diagnosing tool for incontinence when compared to standard clinical evaluation in an outpatient setting.

Dietary Intake. The NHANES 2009-10 Dietary Screener Questionnaire (DSQ) will be given at the BV and the FV. It is a self-administered questionnaire, which asks about the frequency of consumption in the past month of selected foods and drinks. The DSQ captures intakes of fruits and vegetables, dairy/calcium, added sugars, whole grains/fiber, red meat, and processed meat.

Quality of Life. The CDC Health-Related Quality of Life Measures will be administered to participants at the BV and the FV. The HRQOL includes 4 self-reported questions related to overall health, physical health, mental health, and their effects on day-to-day living.

Cardiac ectopy. Atrial and ventricular premature beats will be detected by a 24-hour Holter monitor. Holter monitors will be fitted to participants at the BV and the FV and worn for at least 24-hours before returning them for data upload.

Specimen collection. Participants will undergo 8-hour fasting blood draws and spot urine collections prior to their BV and their FV. Collections will occur at the CRC by a registered nurse. A total of 45 mL of blood will be taken at both the BV and the FV. Approximately 30 mL of urine will also be collected at each visit. The CRC will process and aliquot serum, plasma, whole blood and spot urine samples. Samples will be stored at -80°C in BIDMC freezers. Following the conclusion of the trial, all samples from BV and FV will be sent to Quest Diagnostics, a CLIA-certified laboratory for analysis. For each participant's BV and FV samples, laboratory testing will include C-terminal telopeptide of type I collagen, troponin I, total and HDL-cholesterol, AST, ALT, albumin, total and direct bilirubin, creatinine, albuminuria, fasting glucose and insulin, fructosamine, free fatty acids, C-reactive protein, and white blood cell count.

For immune diversity measurement, 3-5 mL of freshly spun specimens will be transported from the CRC at baseline and at the end of 12 weeks of intervention to the laboratory of collaborator Ramy Arnaout, MD, PhD. In addition to conducting high-throughput AIRR-seq at low cost, his laboratory has developed new computational methods to assess the overall diversity of the 40,000+ sequences measured in a given blood sample, along with cutting-edge methods for measuring the overlap between repertoires. His laboratory will perform AIRR-seq as previously described, sequencing every measureable component of the immune repertoire (~40,000

sequences for each participant at each time point – approximately 3.7 million sequences) and will provide multiple calculated measures of diversity on each individual at each time point, including species richness and entropy.

Adherence. Study staff will remind participants about the importance of adherence at the BV and any in-person or phone contacts. Various tools will be used to measure and promote adherence. Participants will receive various tracking tools, instructions, and ideas for consuming their assigned fruit. Participants will complete a short questionnaire on a weekly basis to self-report intake of assigned fruit, intake of any of the anthocyanin-rich foods to avoid, and incidence of falls. If participants are unable to complete the questionnaire remotely, study staff will administer by telephone. If participants missed >2 servings or consumed >1 serving of a restricted food, study staff will provide a follow-up counseling telephone call, text, or email, at participant discretion.

Participants will also be asked to return their containers of fruit at subsequent food pick-ups or at the FV. This will be used to measure compliance based on the weight/amount of leftover fruit since the last distribution.

Symptoms and adverse events. Study staff will collect information on symptoms and adverse events when reported by participants during the study and at the FV. Adverse events will be recorded. Serious adverse events will be recorded and reported to the PI and the BIDMC CCI.

8. Quality Assurance and Quality Control

The investigative team understands the critical importance of collecting complete, high-quality data and developing procedures to accomplish this important objective. Core activities include:

- Standardization - maintaining common study documents (protocol, MOP, case report forms) with special efforts to minimize version control issues.
- Training – developing training procedures led by experienced investigators and license holders, developing and implementing certification procedures and performance metrics, and conducting annual training.
- Robust data systems – implementing web-based data entry system through REDCap; using off-site data storage with automated back-up systems; checking logic and consistency of data between forms and over time.
- Performance monitoring with feedback – tracking enrollment and follow-up (observed/expected, overall and by key subgroups); monitoring missed visits, data completeness, protocol deviations, and data entry errors; distributing feedback through routine trial monitoring reports for the investigators, safety monitor, and IRB as needed. These reports, together with constructive feedback, have an important role in identifying and resolving issues expeditiously.

9. Randomization and Masking

Randomization

Random treatment assignment will be generated via a web-based system available to the research staff. Allocation uses centrally-allocated 1:1 permuted block randomization in blocks of 2 and 4. Eligibility and baseline data will be collected and entered into the database. The system will confirm eligibility and assign the study fruit.

Masking

Because this is a whole foods study, it is not feasible for treatment assignments to be masked to the participants or the research coordinator. Clinicians conducting ABPM and Holter readings and laboratory technicians will be blinded to study groups.

10. Intervention and Control Arms

This trial uses a pragmatic design to maximize generalizability and adherence. Participants randomized to the blueberry arm consume the equivalent of 1 cup of fresh blueberries daily in the form of frozen whole blueberries for 12 weeks. Blueberries are provided in a single-batch from the U.S. Highbush Blueberry Council. All participants have freezers in their apartments to store a supply of blueberries. Surplus storage will be kept in a freezer in the main kitchen for subsequent pick-ups at week 4 and week 8 after randomization.

Those in the control arm are asked to consume no blueberries and to consume an isocaloric portion of dried dates, equivalent to 2 dates daily for 12 weeks. All dates will be pitted and purchased from the same supplier. Dates are an ideal control because they have similar amounts of fiber, potassium, and calories compared to blueberries. The USDA estimates that dates contain approximately 1.6 mg of total polyphenols per 100 gm of edible content (~3 dates). By contrast, blueberries contain approximately 100 mg or more. Therefore, we expect a substantial anthocyanin difference between arms.

Participants that share a residence will be randomized as a household. That is to say, the second participant from the same household enrolled in the study will not be randomized, but rather automatically assigned to the study fruit that their spouse was assigned. This approach is intended to prevent cross-contamination and promote adherence and statistical plans will account for clustering if multiple household members are enrolled.

In both arms, participants are asked to avoid other anthocyanin-rich foods, including blackberry, cherry, concord grape, cranberry, currant, eggplant, plum, purple potato, red and black raspberry, red cabbage, red wine, rhubarb, and strawberry.(40) Participants will be provided a list of foods to avoid. They will also receive counseling and materials at the BV and throughout the study on ways to track and incorporate the assigned fruit. All participants will also be asked to maintain their ‘usual’ lifestyle throughout the 12-week period. This includes restricting changes to their exercise, diet, medication use, and other behaviors.

11. Safety

Safety monitoring

The study will actively monitor participant safety. One aspect of safety monitoring is to evaluate screenees to determine whether it is safe for them to participate. Key safety related eligibility criteria are the exclusion of persons with (a) an intolerance or allergy to blueberries or dates or (b) allergy to tree nuts, peanuts, soy, or milk products. The dried dates procured for the study are purchased from a supplier that also handles tree nuts, peanuts, soy, and milk products.

A second aspect is monitoring enrolled participants for safety issues potentially related to the study. Surveillance for serious adverse events and other relevant clinical events will occur by questionnaire and contact with participants. If a participant develops a medical problem, the safety of continuing or resuming the study will be ascertained by the PI and other clinical study team members.

In addition, blood pressure values at the SV, BV, and FV will be compared to the alert values based on average seated measurement. All participants with an elevated seated blood pressure measurement (SBP >180 or DBP >110 mm Hg) should be asked if they have the following symptoms: headache, vision change, chest pain, shortness of breath, or change in urine color. Study physician and/or PCP will be notified as necessary.

Table 3. Blood pressure alert values.

Issue	**Action**
SBP >200	Notify PI and advise participant to follow-up with PCP within 1 day (refer to emergency room if symptomatic)
SBP >180	Notify PI and advise participant to follow-up with PCP within 3 days (same day if symptomatic)
SBP >160, ≤180	Advise participant to follow-up with PCP within 1 month
DPB >120	Notify PI and advise participant to follow-up with PCP within 1 day (refer to emergency room if symptomatic)
DBP >110	Notify PI and advise participant to follow-up with PCP within 3 days (same day if symptomatic)
DBP >100, ≤110	Advise participant to follow-up with PCP within 1 month
Pulse >120 bpm	Do not have participant participate in the SPPB and advise them to follow-up with their PCP within 7 days
SBP <90	Notify PI and advise participant to follow-up with PCP within 7 days
DBP <40	Advise participant to follow-up with PCP within 1 month

Table 4. ABPM daytime average alert values.

Issue	Action
SBP <90	Notify PI and advise participant to follow-up with PCP within 2 weeks
DBP <50	Notify PI and advise participant to follow-up with PCP within 2 weeks
SPB 130-149	Notify PI and advise participant to follow-up with PCP within 2 months
DBP 80-89	Notify PI and advise participant to follow-up with PCP within 2 months
SBP 150-174	Notify PI and advise participant to follow-up with PCP within 1 month

DBP 90-104	Notify PI and advise participant to follow-up with PCP within 1 month
SBP 175-194	Notify PI and advise participant with follow-up with PCP within 1 week
DBP 105-114	Notify PI and advise participant to follow-up with PCP within 1 week
SBP ≥195	Notify PI and call participant immediately to advise them to follow-up with PCP within 1 day (go to emergency room if symptomatic)
DBP ≥115	Notify PI and call participant immediately to advise them to follow-up with PCP within 1 day (go to emergency room if symptomatic)

Participants that undergo the fasting blood draw will receive their results, along with standard ranges for interpretation, at the end of the study with two exceptions. A Comprehensive Metabolic Panel (CMP) and a Complete Blood Count (CBC) will be run in real-time and participants will receive those results once they are available. The PI will review all CMPs and CBCs at baseline and follow-up within 1 day as a safety check. Participants will be notified via phone of any results that require immediate medical attention.

This trial will also be advised by an independent safety monitor. Rahul Ganatra, MD, director of continuing medical education at the West Roxbury Veterans Affairs Medical Center, will serve as safety monitor. Recurring meetings between Dr. Ganatra, the study PI, and the study research coordinator will be held at least every 6 months to discuss any safety concerns and review all adverse events.

Potential risks

This study should not involve any major risks to screenees or participants and represents minimal risk above usual activities. Both dates and blueberries are widely consumed, readily commercially available, and (as whole fruits) considered healthy food choices. However, there are some potential risks associated with participation in this study, which are as follows:

- Participants may be uncomfortable with certain sensitive questions or questionnaires
- Bruising and a rare chance of local infection from venipuncture to collect blood samples could occur
- Participants may experience transient dizziness during measurement of orthostatic blood pressure
- Application of ABPM and ambulatory electrocardiographic monitoring may disrupt sleep
- There might be loss of confidentiality and privacy

Adverse event surveillance and reporting procedures

An adverse event is defined by the OHRP as "Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign,… symptoms, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research." The National Institute on Aging defines serious adverse events as any adverse event that:

- Results in death,
- Is life-threatening or places the participant at immediate risk of death from the event as it occurred,

- Requires or prolongs hospitalization,
- Causes persistent or significant disability or incapacity,
- Results in congenital anomalies or birth defects,
- Is another condition which investigators judge to represent significant hazards

Surveillance for SAEs and other relevant clinical events that may be associated with study participation will occur at in-person and telephone visits. In addition to the fixed time points, participants may report events in other settings, such as telephone contacts. The PI will review completed forms, will classify the event according to several dimensions (expectedness, relatedness to participation in the trial, and type), and take appropriate action.

Although the duration of the study is relatively short, the older study population increases the likelihood of medical events occurring unrelated to participation in this trial.

All serious adverse events and all unanticipated problems will be reported individually to Dr. Ganatra and the study PI within 7 days of when the research coordinator learned of the event.

In general, all other adverse events will be reported in aggregate form to the study PI and independent safety monitor at the time of regular data reports.

12. Analysis

Experimental design

We will conduct a single-center, randomized, parallel-design trial comparing whole frozen blueberries to an isocaloric serving of dried dates, with single-blind assessment of outcomes among 70 adults aged 70 years and older.

Analysis plan

The trial statistician will conduct all analysis of study data. Working with the PI, the statistician will develop an appropriate analysis plan. Our analyses will be conducted using the intention-to-treat principle, with linear regression models that adjust for baseline values as our primary statistical method.(41) If missing data exist (e.g., due to attrition), linear mixed models that incorporate both baseline and end-of-treatment values will be used. Because randomization in studies of this size can be imperfect, we will also adjust for any baseline characteristics that appear to be uneven (typically at $p<0.10$). Because of the strong underlying hypotheses and correlated nature of our outcomes, we do not intent to adjust for multiple comparisons, but will treat results that are not corroborated by related outcomes with caution. Methods appropriate for clustered data (e.g., generalized estimating equations) will be used if multiple household members are recruited.

Power and sample size

We estimate power for our continuous endpoints, contrasting end-of-treatment values between arms (using N=70). With $\alpha=0.05$ and power=80%, we can detect a 0.68 standardized effect size. This is considered a medium-to-large-sized effect, similar to that tested in many studies of blueberries. With power=50% (i.e., aiming for an exact p-value=0.05), we can detect a 0.48 standardized effect size (medium). Thus, we should detect most moderate-to-large and many medium-sized effects. Thus, we should detect most moderate-to-large and many medium-sized effects.

13. Data Management

Data will be collected from three main sources: 1) data collected by trial staff on case report forms for later entry into REDCap; 2) data entered by participants and/or trial staff directly into REDCap; and 3) data generated by ancillary facilities, such as laboratories, and sent to the trial analysis team directly. REDCap will only be accessible via the secure website and to authorized personnel. REDCap is a secure web application for building and managing online surveys and databases. It is secure behind the BIDMC firewall. All data collected on paper case report forms will be checked before data entry and maintained for later random audits. Any missing data will be marked as such, when possible. Data will be entered as soon as possible after it is collected.

Data confidentiality

All trial staff will be cognizant for strict controls to ensure data integrity. All staff with access to any portion of the study data will receive training on the importance of maintaining participant confidentiality, including appropriate BIDMC HIPAA training and CITI best research practices training. All data collected and stored electronically will be password protected and saved on secure servers. All study-related computers will be located behind BIDMC firewalls and maintain automated virus update mechanisms. Hard copies of data collection forms will be stored in secure areas (locked offices or cabinets). All staff will sign statements attesting to their understanding of and willingness to abide by policies designed to protect the integrity of the study data. General access to the data entry website is password protected with individual usernames and passwords. Data will be backed-up and checked periodically.

14. Timeline

The trial consists of three pain phases: planning, implementation (recruitment, intervention, and data collection), and data analysis. Planning for the trial commenced in early 2020 but was put on hold due to COVID-19. Planning for the trial resumed in June 2021. Recruitment will be rolling, commencing in late 2021, and should last approximately 10 months, with randomization beginning within 1-2 months after recruitment. Participants will be followed for 12 weeks after randomization. Remaining time will be devoted to data analysis, presentation, and publication.

Table 5. General Timeline of the Effects of Blueberries in Older Adults Trial.

Activity	**Month**
Safety officer approval of final MOP	M3
Recruitment efforts and presentations begin	M3-5
Recruitment of participants	M4-14
Follow-up of participants	M4-17
IRB progress reports due	M12, 24
USHBC progress reports due	M6, 12, 18, 24
Final participant close-out	M18
Data cleaning and management	M18-20
Data analysis	M19-22
Data presentation and publication	M21-24

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