Dietary intervention targeting inflammation, motivation, and engagement in physical activity in sedentary, older adults with depressive symptoms aka Berries and Steps

Study Protocol
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CHAPTER 1. INTRODUCTION

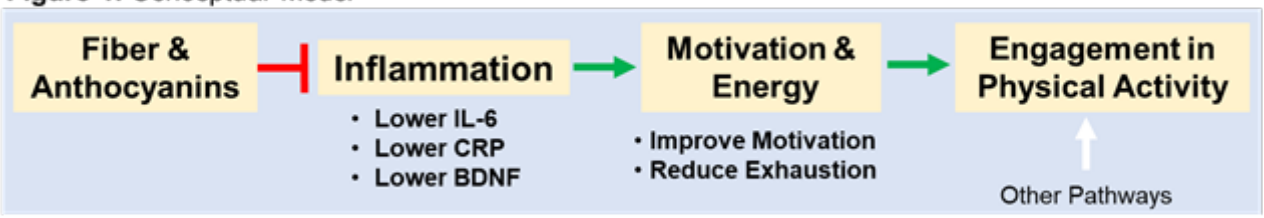
Older adults spend the majority of their day engaging in sedentary behavior, which increases risk of mortality by 22%. Despite the well-established health benefits of physical activity, a large portion of older adults remain sedentary. Recent evidence suggests that inflammation contributes to lack of motivation, which is a critical barrier to overcoming sedentary behavior in older adults. Given that inflammation is highly modifiable by diet, an anti-inflammatory dietary strategy may a viable way to improve inflammation-driven lack of motivation and ultimately increase physical activity in sedentary older adults. However, interventions targeting such a pathway are scarce. This proposal's objective is to determine the feasibility and preliminary efficacy of daily supplementation of freeze-dried blueberry which provides 2 anti-inflammatory nutrients (fiber and anthocyanins) to modulate inflammation-driven lack of motivation. We propose a single-site, randomized, double-blind, parallel pilot study in 40 sedentary, older adults with depressive symptoms. Individuals with depressive symptoms often have lack of motivation and increased levels of inflammatory cytokines, representing an ideal population for an anti-inflammatory intervention targeting to improve motivation. Participants will be randomized to consume either 48 g of freeze-dried blueberry powder (~600 mg of anthocyanins and ~8 g of fiber) or a nutritionally matched placebo powder (devoid of anthocyanins and fiber) each day for a total of 12 weeks. Identification of a dietary intervention to target the inflammatory pathways may offer a novel and feasible approach to increase motivation and increase the engagement of physical activity in older adults. If feasible and effective, such a strategy would help avoid the plethora of health consequences associated with sedentary behavior and physical inactivity.

CHAPTER 2. BACKGROUND

Sedentary behavior and physical inactivity are associated with serious health consequences.[1-8] A meta-analysis of 13 studies report that higher sedentary time is associated with a 22% higher risk of all-cause mortality.[1] Thus, sedentary behavior is a pressing public health concern that has direct impact on the well-being of the aging population. Although there are several determinants of sedentary behavior in older adults, lack of motivation is a significant contributor.[9] Thus, in order to increase physical activity in sedentary older adults, one must first modulate the motivation to engage in goal-directed behavior, such as physical activity; doing so may help **ameliorate the higher risk of mortality associated with sedentary behavior** in older adults.

Markers of inflammation have been linked with behavioral features of sedentary behavior,[10] such as physical fatigue and lack of motivation (**Figure 1**).[9-11] Inflammation has been shown to modulate dopamine activity, which results in lack of motiviation.[12] Targeting inflammation holds great promise to ameliorate the lack of motivation in sedentary older adults and improve the downstream effects on engagement in physical activity. Since inflammation is a key contributor to most age-related diseases, the results from this proposal could identify **new therapeutic strategies for other age-related diseases that feature inflammation as a central aspect of pathogenesis.**

Figure 1. Conceptual Model



Brain-derived neurotrophic factor, BDNF; C-reactive protein, CRP, Interleukin-6, IL-6;

It is well-known that inflammation is highly modifiable by diet. Anthocyanins are pigments found in fruits and vegetables that are responsible for the blue/purple colors.[13] Both anthocyanins and dietary fiber have anti-inflammatory properties.[14-21] However, older adults typically consume low quantities of both anthocyanins[22] and fiber.[19] Thus, such quantities may not be sufficient to harness the anti-inflammatory benefits. A plethora of

interventions supplementing berries/berry products (a concentrated source of fiber and/or anthocyanins) reduced markers of inflammation in various populations (age range: 20-70 y, including healthy individuals and those with diabetes, obesity, osteoarthritis etc.).[23-32] Older adults with depressive symptoms, who typically have high levels of inflammatory cytokines,[33] may be particularly responsive to an anti-inflammatory dietary intervention. Since fruits and vegetables are a rich source, fiber and anthocyanins can be easily obtained at grocery stores and are widely accessible to a large portion of older adults.

Despite consistent evidence that diet modulates inflammation and inflammation is linked to lack of motivation, to our knowledge no dietary interventions have supplemented anti-inflammatory nutrients to target motivation and physical activity. Fiber and anthocyanins are known for their anti-inflammatory capacity, and may be a viable supplemental strategy to reduce inflammation and improve motivation. The findings from this proposal will yield preliminary data on variation of change in motivation and aspects of physical activity needed to design a larger, more robust subsequent trials designed to establish definitive efficacy of a fiber and anthocyanin intervention to increase physical activity in sedentary older adults.

CHAPTER 3: RESEARCH DESIGN

3.1 Study Objectives and Aims

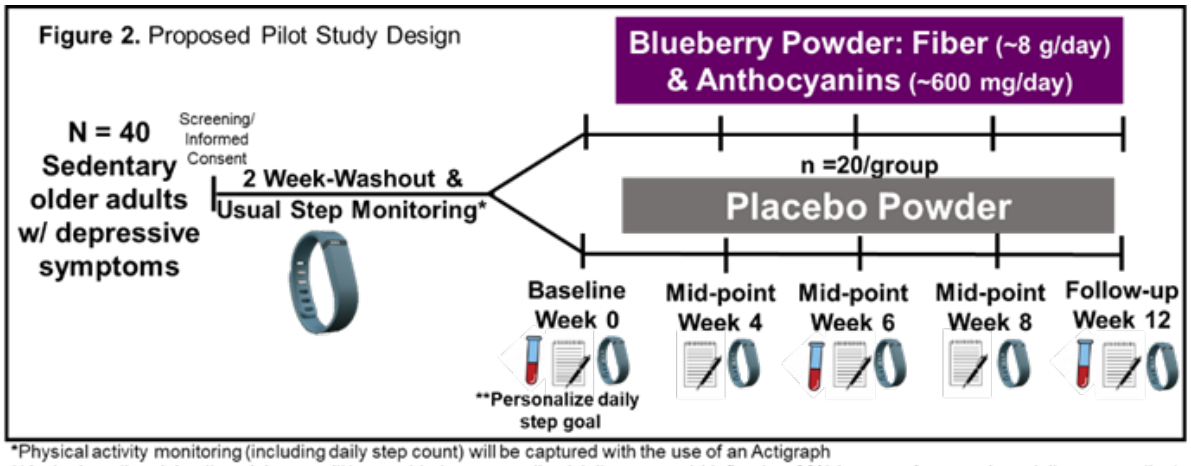
The overall objective of this study is to gather preliminary evidence on the feasibility and effectiveness of an intervention supplementing fiber and anthocyanins to usual diet compared to control (placebo). We will conduct a 3-month, double-blind randomized, parallel-arm, pilot study in 40 sedentary, older adults with depressive symptoms that is designed to improve inflammation, motivation, and consequent engagement in physical activity. Our specific aims are:

> **Aim 1**: Determine the feasibility of an intervention supplementing usual diet with fiber (8 g/day) and anthocyanins (600 mg/day). We hypothesize at least 80% of the participants enrolled will complete the 3-month intervention.
> **Aim 2**: Gather preliminary evidence on the impact of fiber and anthocyanin supplementation on relevant inflammatory markers. We hypothesize markers relevant to inflammation (interleukin-6, C-reactive protein, and brain-derived neurotrophic factor) tend to reduce after 3 months of fiber and anthocyanin supplementation compared to control.
> **Aim 3**: Obtain preliminary evidence on the efficacy of fiber and anthocyanin supplementation to improve the engagement in physical activity, and further determine if this effect is mediated by change in motivation (via Motives for Physical Activities Measure-Revised questionnaire). We hypothesize the physical activity (i.e., average daily step count via accelerometer) tends to increase after 3 months of fiber and anthocyanin supplementation compared to control, and that a substantial portion of this effect is mediated via an increase in motivation.

Our overall hypothesis is that a 3-month dietary intervention supplementing fiber and anthocyanins in sedentary, older adults with depressive symptoms will be feasible and demonstrate preliminary efficacy.

3.2 Overview

This will be an individual-level, double-blind, randomized, parallel pilot study in 40 sedentary, older adults with depressive symptoms. Screened and eligible participants will perform baseline assessments and provide a blood sample. Next they will be randomized to consume either blueberry powder (which provides ~8 g/day of fiber and ~600 mg/day of anthocyanins) or placebo powder (nutritionally matched powder without anthocyanins or fiber) for 3 months (**Figure 2**).



*Physical activity monitoring (including daily step count) will be captured with the use of an Actigraph
**At the baseline visit, all participants will be provided a personalized daily step goal (defined as 20% increase from washout daily step median).

3.3 Inclusion and Exclusion Criteria

Our target population is sedentary older adults, with minor depressive symptoms. Individuals expressing an interest in participating after recruitment out-reach will be screened in-person (e.g., at HRC or at participant's home). Participants will complete several assessments to confirm that they meet the following inclusion criteria.

Inclusion Criteria

- Men and women aged ≥65 years
- Self-reporting ≥ 8 hours of sitting per/day (e.g., sedentary behavior)
- Depressive symptoms (defined as ≥4 and <16 points on the center for epidemiological studies depression-scale)[34]

Exclusion criteria have been selected to ensure safety and optimize compliance, while minimizing confounds due to overt disease or conditions that may significantly influence study outcomes. Given that this is pilot a study, select criteria will be per discretion of the principal investigator. Exclusions may be during the telephone or in-person screening as described below:

Exclusion Criteria

- Unwilling to follow the study protocol
- A median daily step count >7,500 steps per day (as measured by the ActiGraph), or per discretion of the PI
- Cognitive impairment (defined as Montreal Cognitive Assessment,[37] MoCA <22 points)
- Self-reporting a history of inflammatory bowel disease/syndrome, major depression, bipolar, schizophrenia, or other psychotic disorders, or per discretion of the PI
- Self-reporting type 1 or type 2 diabetes
- Allergic to intervention or control products
- Recent use (within the last 3 months) of antibiotics or pro-biotics, or per discretion of the PI
- Current substance use disorder (Drug Abuse Screening Test,[38,39] DAST-10>2 points)
- Current alcohol use disorder (Alcohol Use Disorders Identification Test - Consumption,[40,41] AUDIT-C≥4 points)
- Unstable anti-depressant use (e.g., change in medication within last 3-6 months), or per discretion of the PI
- Current homicidal or suicidal ideation (assessed via the P4 Suicidality Screener[42])
- Current psychosis (via the Psychosis and Hallucinations Questionnaire,[43] PHQ>12 points)
- Manic symptoms (assessed by the Mood Disorder Questionnaire,[44] MDQ >5 points)

3.4 Number of Subjects and Study Duration

We aim to recruit a total of 40 individuals (both men and women), in proportion to the gender and racial distribution of the greater Boston population, through local newspaper and internet advertisements, physician referrals, our registry of research volunteers, Hebrew SeniorLife (HSL) senior housing sites, and patient registries (e.g., HRC or Beth Israel Deaconess Medical Center). Participants will remain in the study for a total of 14 weeks.

3.5 Study Endpoints

Primary Outcomes – The primary outcome for this study will be engagement in physical activity.

1. **Engagement in Physical Activity**: Engagement in physical activity will be evaluated by the median # of steps per/day (via ActiGraph) of the previous two weeks before each visit (e.g., 2 weeks before baseline or 2 weeks before follow-up). We will also evaluate median sedentary minutes/day of the previous two weeks before visits.

Secondary Outcomes – We will evaluate several secondary outcomes including:

1. **Feasibility** : Feasibility will be defined as participant retention rate (i.e., number of participants that complete the intervention/total randomized).
2. **Inflammatory Biomarkers**: Serum markers relevant to inflammation, (serum brain-derived neurotrophic factor, BDNF; C-reactive protein, CRP; interleukin-6, IL-6) at baseline and follow-up will be measured by Quest Diagnostics or our collaborators at the University of Connecticut Metabolic Phenotyping Lab.
3. **Motivation**: Motivation to engage in physical activity (our proposed mediator between inflammation and physical inactivity) will be assessed by the self-report questionnaire Motives for Physical Activities Measure-Revised that has been used in middle-aged and older adults.[38]

3.6 Study Intervention Products

Given that 100% purified anthocyanins are not commercially available, we will utilize a dietary source of anthocyanins. Blueberries are one of the richest sources of dietary anthocyanins and also conveniently concentrated with fiber. To allow for blinding, we will use freeze-dried powdered blueberries as our intervention product. The powder will be provided by the U.S. Blueberry Council. The placebo powder will also be provided by the U.S. Blueberry Council, which is a nutritionally matched powder except it contains minimal amounts of anthocyanins and fiber. The placebo powder will be labeled with either a letter or number that is different from the intervention powder. This is a double-blind intervention, so neither the participants nor the study staff will know which powder is the placebo and/or which is the blueberry powder. See **§**5.3 for further information on dietary intervention.

CHAPTER 4 RECRUITMENT AND DATA COLLECTION

4.1 Recruitment Overview

Participants will be recruited from the Boston area community, including senior housing facilities in urban/suburban areas and research recruitment repositories. We will utilize a multi-pronged approach to meet our recruitment goals:

- We will recruit from the research repository that resides at HRC
- We will connect with social workers in and outside the HRC
- We will perform medical record reviews to identify potentially eligible individuals at the Hebrew SeniorLife (HSL) geriatric medicine practices.
- We will advertise through direct mailings to all residents of HRC's seven supportive housing facilities (over 3,000 residents).
- We will give presentations at each Hebrew SeniorLife (HSL) facility.
- We will use the Harvard Catalyst (CTSA) Shared Health Research Information Network (SHRINE) to identify volunteers from Harvard-affiliated hospitals and clinics.
- We will advertise our study within numerous local media outlets, on HRC's Hinda and Arthur Marcus Institute for Aging Research and other websites (*e.g.*, Craig's List), and at www.clinicaltrials.gov.

4.2 Recruitment Timeline

Our trial proposes to enroll 40 persons over approximately 6-months. We will recruit for two waves of our intervention. For the first wave of the intervention, we aim to recruit 16 individuals over ~3 months. The 16 individuals will then be randomized to the intervention or control group, and participate in the study for 3 months. During the 3 months the first wave of participants are on the study intervention, we will be recruiting for the second wave (n=24) of the intervention. Once the second cohort of 24 individuals is recruited, they will begin the study intervention as soon as possible. To stay on target, we need to recruit ~7 participants/month. Thus, based on our known research participation rates, and the expected prevalence of depressive symptoms in older adults, we will readily be able to meet our recruitment goal of 40 participants.

There will be two waves of the intervention (n=16 for the first wave, and n=24 for the second wave). For each wave, half of the participants will be randomized to the intervention group and the other half will be in the control group. In an attempt to account for the impact of seasonal variation on physical activity levels, we will employ block (blocks of 4) randomization. For every block of 4 participants, 2 will be randomized to consume the placebo powder and the remaining 2 will be randomized to the blueberry powder.

4.3 Informed Consent

All interested individuals will be asked to provide verbal consent to complete an initial eligibility screen during a phone conversation with study personnel. Potentially eligible participants will then schedule an in-person screening visit. Potential participants may be sent by email or conventional post (per request, and according to their preference) a copy of the informed consent form for them to review at their own pace prior to the in-person screening. Written informed consent will be obtained by study personnel at the beginning of the in-person screening visit.

4.4 Participant Withdrawal

Any participant who expresses a desire to discontinue participation in the study will be withdrawn at their request immediately. All data collected prior to withdrawal will be maintained in the study data set.

Additionally, a subject may be withdrawn from the study prior to completing all of the study related procedures due to the following conditions:

- Subject safety issues
- Failure of subject to adhere to protocol requirements (including low compliance with the intervention)
- Disease progression
- Subject decision to withdraw from the study (withdrawal of consent)

Withdrawn subjects may not reenter the study unless there are extenuating circumstances (e.g. family emergency or required travel out of town) that interfere with the start of the study before any medications are administered. In this case, they may be scheduled to start over again. If new medical conditions arise or are exacerbated during the study intervention, the withdrawal of a participant will be evaluated by the PI, SO, and/or study psychiatrist.

4.5 Methods to Protect Participant Privacy

The following are the planned procedures for effectively protecting against and minimizing loss of participant privacy:

1. Phone screening will be conducted in a private office space.
2. Study visits will be conducted in private rooms.
3. Each participant will be given a unique study identification number and data will not include any of the participant's PHI.
4. All participant-identifying information will be stored and managed on a secured database server. The information will be password protected.
5. Participant confidentiality will be maintained in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations.

6. Only the PI, study personnel, and laboratory personnel approved by the IRB and authorized to view PHI will have access to the information.
7. PHI will not be used during discussion, presentation or publication of any research data.
8. Files containing PHI data collected for recruitment and screening purposes will be kept in locked, secured filing cabinets accessible only to designated study personnel (research assistants and investigators)

4.6 Minimization of Bias

This study is designed as a double-blind intervention so neither staff nor participants are aware of their assigned intervention arm. Additionally, to minimize analyst bias, biomarkers will be de-identified and analyzed by technicians unfamiliar with the participants or study phase.

4.7 Maximizing Compliance and Minimizing Attrition

At the start of an individual's study participation, he/she will be given a schedule of their study visits. Visits will be scheduled at a time of day that the participant determines is most convenient for them, and will repeated at the same time for each visit. Transportation will be provided for each visit as needed, snacks will be available, and stipends will be provided for each study milestone. If necessary, reminder calls will be made to participants on approximately 2 days prior to study visits.

Participants will be tracked throughout their enrollment. Each study visit will be documented. Some study visits will be followed with a brief telephone check-in to ask the participant questions about medication compliance, adverse effects, and their experience during the most recent visit. All calls to the participant and their feedback will be carefully tracked. Notes that may facilitate compliance, such as "call before 10 am," etc., will be kept in participant files.

We will employ specific strategies to maximize participation and compliance:

- ***Positive Framing about Benefits:*** Information will be presented in terms of the possible gains rather than the avoidance of losses as this is a more effective motivational approach.
- ***Feedback and Recognition of Progress:*** Participants will be acknowledged throughout their participation with thank you notes, and will be recognized for their contributions to the study through regular brief flyers/newsletters such as "Partners in Progress – Mobility and Falls updates". We will remain in close contact with individuals throughout their participation with follow-up calls each month.
- ***Incentives and Rewards:*** Participants will receive snacks at each visit, cards for achieving milestones, such as birthdays, holidays, etc.; and certificates of completion.

CHAPTER 5. RESEARCH METHODS

5.1 Participant Visit Schedule

All study visits will take place at the Clinical Research Laboratory at HRC, Roslindale, MA, an HRC-affiliated housing site, or at the participants home (if possible).

Participant eligibility will be determined during an in-person screening. If eligible and interested, the participant will be asked to continue with 5 additional visits. The assessments/activities for each study visit is outlined below.

Visits	Assessments at Each Visit
Telephone Screening	We will assess: 1. Depressive Symptoms 2. Physical Activity/Behavior 3. Gastrointestinal Conditions 4. Mental Health Conditions 5. Other Medical Conditions/exclusion criteria

In-person Screening	We will perform/assess: 1. Informed Consent 2. Medical History and Health Behaviors 3. Depression Level 4. Cognitive Status 5. Substance/Alcohol Use 6. Psychiatric Symptoms 7. Suicidal Ideation 8. Wear an ActiGraph physical activity monitor 9. Avoid consumption of certain foods (e.g., blueberries that are rich in fiber and anthocyanins) If eligible, participants will be asked to: 10. Fill out a 3-day dietary record in the week prior to baseline visit
Baseline Visit, Week 0:	Assessments/Activities: 1. Turn in Diet Records 2. Sync ActiGraph Data 3. Vitals 4. Height 5. Weight 6. Depression Level 7. Suicidal Ideation 8. Blood Draw to Assess Serum Inflammatory Markers 9. Motivation 10. Social Network 11. Symptoms Participants will be asked to: 1. Continue to wear an ActiGraph physical activity monitor 2. Avoid consumption of certain foods (e.g., blueberries that are rich in fiber and anthocyanins
Visit 2, Week 4*:	Assessments/Activities 1. Sync ActiGraph Data 2. Depression Level 3. Suicidal Ideation 4. Motivation 5. Symptoms 6. Compliance Participants will be asked to: 1. Continue to wear an ActiGraph physical activity monitor 2. Avoid consumption of certain foods (e.g., blueberries that are rich in fiber and anthocyanins 3. Fill out a 3-day dietary record in the week prior to next visit
Visit 3, Week 6:	Assessments/Activities 1. Turn in Diet Records 2. Sync ActiGraph Data 3. Depression Level 4. Suicidal Ideation 5. Vitals 6. Weight 7. Blood Draw to Assess Serum Inflammatory Markers 8. Motivation 9. Social Network 10. Symptoms 11. Compliance Participants will be asked to: 1. Continue to wear an ActiGraph physical activity monitor 2. Avoid consumption of certain foods (e.g., blueberries that are rich in fiber and anthocyanins
Visit 4, Week 8*:	Assessments/Activities 1. Sync ActiGraph Data 2. Depression Level 3. Suicidal Ideation

	4. Motivation 5. Symptoms 6. Compliance Participants will be asked to: 1. Continue to wear an ActiGraph physical activity monitor 2. Avoid consumption of certain foods (e.g., blueberries that are rich in fiber and anthocyanins 3. Fill out a 3-day dietary record in the week prior to next visit
Visit 5, Week 12:	Assessments/Activities: 1. Turn in Diet Records 2. Sync ActiGraph Data 3. Medical History and Health Behaviors 4. Vitals 5. Weight 6. Depression Level 7. Suicidal Ideation 8. Blood Draw to Assess Serum Inflammatory Markers 9. Motivation 10. Social Network 11. Symptoms 12. Compliance 13. Cognitive Status

*If the participant prefers to complete the visit 3 or 5 via phone, then they will not be required to synch their ActiGraph since this requires a study computer.

5.2 Study Visits and Assessments

A summary of study visits and assessments is provided in the table below. Given that a primary goal of this pilot study is feasibility, we will allow for flexibility for the administration of some assessments at visits, as long as these changes do not impede the scientific interpretation.

Table 1. Assessments by Visit

	Telephone Pre-Screening	In-person Screening	Baseline	4 Weeks	6 Weeks	8 Weeks	12 Weeks
Depressive Symptoms (via CESD-R)	X						
Medical History/Health Behaviors	X	X					X
Cognitive Status (via MoCA)		X					X
Substance/Alcohol Use Questionnaires[1]		X					
Psychiatric Symptoms Questionnaires[2]		X					
Suicidal Ideation (via P4SS)		X	X	X	X	X	X
Depression Severity (via PHQ-9)		X	X	X	X	X	X
Social Network Questionnaire			X		X		X
ActiGraph Monitoring of Activity		X	X	X	X	X	X
Relevant Symptoms			X	X	X	X	X
Vitals			X		X		X
Height			X				
Weight			X		X		X
Serum Inflammatory Markers			X		X		X
Motivation (via MPAM-R)			X	X	X	X	X
3-Day Diet Record			X		X		X
Compliance				X	X	X	X

[1] via Drug Abuse Screening Test (DAST-10) and Alcohol Use Disorders Identification Test - Consumption (AUDIT-C)

[2] via Psychosis and Hallucinations Questionnaire (PsycHQ) and Mood Disorder Questionnaire (MDQ)

Center for Epidemiological Studies-Depression scale Revised, CESD-R; Motives for Physical Activities Measure-Revised, MPAM-R; Montreal Cognitive Assessment, MoCA; P4 Suicidality Screener, P4SS; PHQ-9, Patient Health Questionnaire-9.

This study consists of up to 6 study visits: a screening visit, a baseline visit, 3 mid-point visits, and a final follow-up visit. Detailed information of the study procedures at each visit is outlined below.

Telephone Pre-screen (~30 minutes): Volunteers will be asked about mood/behavior, depressive symptoms (via CESD-R), physical activity and behavior, any past/present gastrointestinal disorders, and any past/present mental health conditions and/or treatments, as well as other exclusionary criteria.

Center for Epidemiological Studies Depression Scale Revised (CESD-R): Depressive symptoms will be assessed by the CESD-R,[34] which is a validated questionnaire of 20 questions regarding feelings of depression, worthlessness, loneliness, energy level, and fear. The CESD-R has high internal consistency (r=0.90) and a test-retest reliability of 0.51.[34] Individuals with scores as ≥4 and <16 points, will be eligible to continue with the study.

Screening Visit (~60 minutes): At screening individuals deemed potentially eligible via the phone screen will complete an in-person screen. All screening assessments will be administered by trained research assistants. Eligible and interested participants will read and sign an informed consent form approved by HRC's IRB. A medical history and health behaviors questionnaire will be completed which will ask about current/past conditions, medications, etc. The depression level of participants will be monitored throughout the intervention to ensure the participants are not progressing into more severe categories of depression (e.g., moderate or major depression). The cognitive status of participants will also be evaluated via MoCA. Validated questionnaires that asses substance and alcohol use will be administered. Psychiatric symptoms will also be assessed to determine eligibility

Informed Consent: In order to participate in this study, all interested and eligible participants will be required to provide informed consent. They will be given ample time to ask any questions about the study. A trained research staff will answer any questions and if the individual is interested in participating in the study, they will be offered to sign the informed consent form. When the staff is confident that the participant is completely familiar with the document and understands all the aspects of the informed consent form, it should be signed by the participant in the presence of the staff member, and should then be signed by the staff member. All consent forms will be double checked to make sure they are properly signed and dated. Copies of completed consent forms will be given to the participant and the original signed document will be kept on file at the Hinda and Arthur Marcus Institute for Aging Research. As a part of the informed consent process, potential participants will be clearly informed that this intervention is not a treatment option for depressive symptoms or depression, but rather studying feasibility of a dietary strategy for health. If seeking a treatment, they will be directed to their primary care.

Medical History/Health Behaviors: Additional measures to characterize the participants will include existing or previous medical conditions, smoking status, etc.

Patient Health Questionnaire-9 (PHQ-9) is a 9-item, self-report questionnaire about feelings or problems that may affect feelings, similar to the CESD-R. The PHQ-9 has the added benefit over the CESD-R that it can be used to categorize participants into a depression level category (normal, mild, moderate, moderately severe, or severe). As a safety precaution, we will monitor the participant's depression level via the PHQ-9 which has relatively high sensitivity and specificity (ranging between 68-95% based on the cut-point used) compared to clinical diagnoses of depression.[36]

Montreal Cognitive Assessment (MoCA, Full 30-point version) will be administered by a trained research assistant. Individuals with a score <22 points, which indicates cognitive impairment,[37] will be excluded.

Drug Abuse Screening Test (DAST) will evaluate drug use. DAST will be administered to participants and individuals scores >2 points, will be excluded.[38,39]

Alcohol Use Disorders Identification Test (AUDIT) will be administered to participants to evaluate alcohol use. For this study, we will administer a shortened version (AUDIT-Consumption) by asking only the first three questions from the full questionnaire. Individuals scoring ≥4 points will be excluded.[40,41]

Psychosis and Hallucinations Questionnaire (PsycH-Q) will evaluate symptoms of psychosis and hallucinations. It is a 20-item self-report assessment that has been validated in older adults with Parkinson Disease.[43] We will administer the first section, which is a validated screening tool of 13 items that identifies core hallucinatory symptomatology (visual, audition, touch, olfaction, and gustation), as described in the National Institutes of Health diagnostic criteria.[45] Participants will rate questions asking about hallucinatory symptoms on a 5 point Likert scale and rate the frequency "0-4" ranging from "never", "<1 time per week", "weekly", "most days a week" and "daily." Participants scoring > 12 will be excluded from the study.

Mood Disorder Questionnaire (MDQ) will evaluate manic symptoms. For this study, we will administer a shortened version by asking only the first full question from the full questionnaire. It is a screener derived from the DSM-IV criteria used to identify mania or hypomania in adults (mean age 46 years),[46] and was recently used as a screener in a study for older adults (age 60-90 years) in Poland.[44] The first section of the MDQ is a validated self-report inventory of 13 yes/no items that asks about symptoms or behaviors that commonly occur during mania. A threshold of a score >7 points has been used to screen individuals for manic symptoms.[44,45] However, we have opted to apply a more stringent threshold of 5 points. A follow-up question asks the degree to which these symptoms cause a problem in carrying out daily life. An individuals can respond, "no problem," "minor problem," "moderate problem," or "serious problem."

P4 Suicidality Screener is a validated 4-item questionnaire that will be used to assess suicidal ideation.[42] It has been used in several trials as an assessment in adults between the ages of 55-73 years.[47-49] This is a questionnaire that will be administered to participants by a trained staff member. Responses that indicate a potential suicidal risk (e.g., specific responses on the CESD-R or the P4 Suicidality Screener) will trigger further assessment of imminent risk of the participant. Any participant that presents as a potential suicide risk will be referred to a suicide risk hotline (e.g., **The Samaritans of Boston: 617-247-0220).** Study staff will be trained to call 911 for immediate assistance if a participant indicating he/she has a plan to commit suicide and is perceived as a serious and/or dangerous situation, The project director(s), PI, and SO will be notified.

Objective Measure of Sedentary Behavior will be measured as a final assessment of eligibility. All participants that pass the in-person screen up to this point will be provided an accelerometer (ActiGraph, GT9X Link), which is a research-grade activity tracker that monitors several aspects of activity, including step counts. They will be instructed to wear the device during the week to use as an objective assessment of their sedentary behavior, specifically their usual daily step count. After a week of wearing the activity monitor, the participant's ActiGraph will be synched in order to calculate their median daily step count over the past week. Individuals that have a median step count >7,500 steps per day will be excluded. All eligible participants will be asked to continue to wear the ActiGraph for the remainder of the study.

To ensure participants have a stable level of dietary influence on inflammation, all participants will be asked to avoid consumption of certain foods (e.g., fiber-rich and anthocyanin-rich foods; i.e., washout period). Additionally, the week before baseline participants will be asked to fill a 3-day diet record to estimate nutrient intake.

Baseline Visit (~60 minutes): At the baseline visit, participants will be asked to turn in their 3-day diet records and sync their ActiGraph devices to transfer their activity data and evaluate engagement in physical activity. Height, weight, vitals, suicidal ideation (P4SS), depressive level (PHQ-9), social network, motivation, and relevant symptoms will be evaluated. Additionally, up to 10 mL of blood will be taken to measure inflammation-related markers in serum. Finally, a personalized daily step goal will be calculated for each participant. Utilizing the step count data assessed during the 2-week washout, the 20% increase of his/her median usual daily steps measured will be calculated and be defined as a personalized step-goal. Study staff will share this personalized step goal with the participant and say, "this is the recommended number of steps you should try to meet each day during the study." Participants will be given their allotment of powder to consume.

Social Network: Subjective perception of social support and connectedness will be evaluated using a validated questionnaire.

3-Day Diet Records: Diet records (consisting of 2 weekdays and 1 weekend day) will be reviewed by research staff for accuracy and completeness. Records will be entered into a dietary analysis program (e.g., Nutrition Data System for Research) to estimate dietary intake of nutrients.

Height/Weight will be measured at the baseline visit.

1. Height will be measured using a stadiometer.
2. Weight will be measured using a digital Health-o-meter scale.

Vital Signs (e.g., body temperature, pulse, and seated blood pressure) will be measured at the baseline visit. After 3-5 minutes of rest, seated blood pressure will be measured twice with an automated cuff.

Motives for Physical Activities Measure-Revised (MPAM-R): Motivation to engage in physical activity will be assessed with the MPAM-R,[51] which is a self-report questionnaire has been used in middle-aged and older adults.[52]

Blood (up to 10 mL) will be collected by a trained phlebotomist using sterile procedures. Blood will be processed and stored at -80 degree C for future analyses. Batch analyses of relevant inflammatory markers (e.g., C-reactive protein, interleukin-6, and brain-derived neurotrophic factor) will be measured by a reputable lab (e.g., Quest or collaborators at the University of Connecticut).

Relevant Symptoms: Information on relevant symptoms including gastrointestinal distress, appetite, pain etc., will be collected by self-report.

Compliance: Compliance with our dietary intervention will be evaluated throughout the study. Participants will be asked to log consumption of their powder and keep all of their used powder packets. At the study visit, participants will return all unused and used powder packets to estimate number of intended doses that were consumed.

Week 4 Mid-point Visit (~45 minutes): The Week 4 Mid-point Visit will include an evaluation of motivation, depression level (PHQ-9), suicidal ideation, relevant symptoms and compliance. Participants will again be asked to continue to wear the ActiGraph and provided with new 3-day diet records to fill out the week prior to their next visit.

Week 6 Mid-point Visit (~45 minutes): The Week-6 Mid-point Visit will be the same as Week 4 Mid-point Visit, but it will also include turning in diet records, a blood draw of ~10 mL, as well as an evaluation of vitals and social network.

Week 8 Mid-point Visit (~45 minutes): The Week-8 Mid-point Visit will be the same as Week 4 Mid-point Visit.

Week 12 Final Follow-up Visit (~60 minutes): At the final follow-up visit, participants will be asked to turn in their 3-day diet records and synchronize their ActiGraph devices to transfer their activity data and evaluate engagement in physical activity. Medical history/health behaviors, weight, suicidal ideation (P4SS), social network, depression level (PHQ-9), motivation, vitals, cognitive status (MoCA) and relevant symptoms will be evaluated. Additionally, up to 10 mL of blood will be taken to measure inflammation-related markers in serum. Dietary compliance will also be estimated as previously described.

5.3 Dietary Intervention

The duration of this study will be a total of 14 weeks. The first two weeks will require participants to avoid eating certain foods (e.g., fiber- and anthocyanin-rich foods) while also monitoring their usual physical activity with the ActiGraph just prior to the intervention period.

Individuals will then be randomized to either the intervention or the control group. This is a double-blinded study, so the study staff nor the participant will know what powder the participants are assigned to. Those randomized to the intervention group will be asked to consume approximately 48 g of freeze-dried blueberry powder as a source of ~8 g of fiber and 600 mg of anthocyanins. The proposed dose of 48 g of freeze-dried blueberry powder is equivalent to approximately 2 cups of whole blueberries. Individuals randomized to the control group will be asked to consume approximately 48 g of a nutritionally matched placebo powder that is devoid of anthocyanins and fiber.

The powders will be individually packaged in 24 g amounts. Participants will be instructed to consume 2 packets of their respective powder each day for 12 weeks. Participants will be provided with suggestions/recipes on how to consume the powder (for example, mixed in 12 fl oz of water or mixed n 12 fl oz of almond milk). At the beginning of the study the participants will be given a sample of materials (e.g., mixing apparatus) or ingredients (e.g., almond milk) that participants will be able to use to make the suggested recipes they were provided with. It is recommended that participants consume their powder all at once. Given that intervention and control products are regularly consumed, we do not anticipate any toxicities.

5.4 Outcome Measures

Our primary endpoint is engagement in physical activity after 12 weeks of the dietary intervention. Secondary outcomes include feasibility, inflammatory markers, and motivation; however, we will also explore alternative outcomes.

Name	Type	Timeframe	Brief description
Feasibility	Secondary	Throughout the entire study	Participant retention (e.g., the number of participants that complete the intervention out of all participants randomized to an intervention arm)
Inflammatory Markers	Secondary	Baseline, Week 6, and Final Follow-up (Week 12)	C-reactive protein, interleukin-6, and brain derived neurotrophic factor will be measures in blood collected from participants.
Motivation	Secondary	Baseline, Week 4, Week 6, Week 8, and Final Follow-up (Week 12)	Motivation to engage in physical activity will be assessed via self-report questionnaire, Motives for Physical Activities Measure-Revised,
Engagement in Physical Activity	Primary	Baseline, Week 4, Week 6, Week 8, and Final Follow-up (Week 12)	Median daily-step count over the 2 previous weeks.

CHAPTER 6. STATISTICAL DESIGN

6.1 Statistical Analysis

All analyses will be performed by intent to treat, and further evaluated per protocol. As a first step, we will assess distribution characteristics of the primary and secondary outcomes. Where appropriate, transformation of variables to combat skew or other irregularities will be employed. Participant characteristics will be summarized using means, medians, standard deviations, interquartile regions and ranges for continuous variables and sample counts and proportions generated for discrete characteristics. Comparability of treatment arms will be assessed on potentially confounding characteristics using tabular and graphical methods.

6.2 Outcome Variables

Feasibility is defined as participant retention (e.g., the number of participants that complete the intervention out of all participants randomized to an intervention arm), however others will be explored. Feasibility outcomes will be summarized using sample quantities and corresponding 80% confidence interval estimates. The intervention will be considered feasible if at least 80% of the participants randomized, complete the final follow-up.

Relevant inflammatory markers (C-reactive protein, interleukin-6, and brain derived neurotrophic factor) will be measured in blood collected from participants at baseline and final follow-up. Change in inflammatory markers will be calculated. The distribution of change in inflammatory markers will be summarized using sample quantities and kernel density estimates. We will develop 80% confidence interval estimates of inter- and intra-individual variation (i.e., standard deviation) of change in these measures.

Motivation to engage in physical activity will be assessed via self-report questionnaire, Motives for Physical Activities Measure-Revised. Change in motivation will be calculated between baseline and follow-up periods. The distribution of change in motivation scores will be summarized using sample quantities and kernel density estimates. We will develop 80% confidence interval estimates of inter- and intra- individual variation (i.e., standard deviation) of change in these measures.

Engagement in physical activity will be defined as the median daily-step count over 2 weeks prior to each mid-point and/or follow-up visit. We chose the median daily step count, because step counts varies from day to day. Change in median step count will calculated between the baseline and follow-up. The distribution of change in physical activity will be summarized using sample quantities and kernel density estimates. We will develop 80% confidence interval estimates of inter- and intra- individual variation (i.e., standard deviation) of change in these measures.

6.3 Statistical Methods

Formal inference and estimation of treatment effects will use a Student's t-test. The mean change (baseline-follow-up) in our outcome variables (inflammatory markers, motivation, step count) in the control group will be compared to the mean change (baseline-follow-up) of the intervention group overtime. A p-value less than 0.05 will be used to determine statistical significance.

The relationship between inflammatory markers, motivation, and engagement in physical activity will be assessed. Given that consistent evidence in populations (including older adults) have shown that supplementation with berries (a source of fiber and anthocyanins) successfully reduces inflammatory markers, we are confident our intervention will successfully lower inflammatory markers. Thus, we will evaluate the relationship between of change in inflammatory markers with change of physical activity utilizing a linear regression.

To determine if change in motivation is on the pathway between change in inflammation and engagement in physical activity, we will use linear regression with and without the addition of change in motivation to the model. If adjusting for change in motivation changes the estimate by >10%, then motivation will be considered a part of the causal pathway. A p-value less than 0.05 will be used to determine statistical significance.

6.4 Statistical Power

The sample size for our pilot study has been chosen to provide a practical basis for developing variance measures of motivation and engagement in physical activity that will be needed to design a subsequent larger randomized controlled trial. The daily step count was the outcome that this study power calculation was based off of. Since studies have not yet evaluated change in step count in response to an anti-inflammatory strategy in our specific population (older, sedentary adults with depressive symptoms), the variations of change that are gathered from this study will be used to estimate power for large scale efficacy trials. Regardless, we have estimated the standard deviation of change in steps utilizing the variation of steps in older, sedentary adults without depressive symptoms previously published.[53] Assuming SD of change=1045, 80% power, and an alpha=0.05, a sample size of 18/group is required to detect a change of 1000 steps/day. An increase in 1000 steps per day was associated with a 16% reduced risk in all-cause mortality.[53] Thus, to account for attrition, we propose a sample size of 20/group. Additionally with a sample size of 20/group, we will also have 73% power (assuming SD of change=41) to detect a change of 30 min/day of sedentary time. Such a change is noteworthy epidemiological evidence suggests that 30 min/day of sedentary time is associated with 17% increased risk of all-cause mortality in sedentary older adults.

CHAPTER 7 DATA MANAGEMNT AND QUALITY

7.1 Data Management

All data collected for analysis will be de-identified and assigned a unique study number. Any data collected on paper forms will be kept in a locked file cabinet at HRC. Data collected on paper forms will be entered and stored on a password-protected secure server at HRC. When possible, data will be collected directly via our electronic data capture system (e.g., REDCap).

The Institute for Aging Research primarily employs the REDCap system to facilitate data management operations. REDCap is a full-featured clinical trials data management system (DMS) accessible to data entry and data analysis workstations using secure Web technologies. The REDCap product is developed and maintained by Vanderbilt University in cooperation with REDCap Consortium members, including HRC. HSL hosts and maintains a dedicated instance of REDCap for use across our research enterprise. Each research study is provided separate project workspace in which all of the study data are stored in a MySQL relational database on the private corporate network behind several firewalls and located physically within the HSL data center.

7.2 Participant Tracking

Each recruited participant will be tracked closely throughout study enrollment. If desired, a study events calendar will be created for each participant. Any outstanding or incomplete visits will be accessible in real time to the project director and study team. The study team will maintain regular communications with each study participant throughout enrollment, through regularly scheduled follow up calls, and established retention strategies will be used as discussed in **§**4.7.

CHAPTER 8 DATA SAFETY MONITORING PLAN

8.1 Participant Risks

Participation in this study may be associated with minor risks or safety concerns. The potential risks of this study fall into 5 categories: 1) those related to research participation; 2) those related to testing procedures; 3) those related to the intervention or control products; 4) those related to depressive symptoms; 5) those related to physical activity. The risks are outlined for each category below:

Minor Risk of Participation in Research: With any study, risk of breach of confidentiality is possible since we are collecting personal health information.

Minor Risk of Testing Procedures: The potential risks of the testing procedures are minor since the majority will be questionnaire based assessments. It is possible that participants may find the questionnaires tedious or may be uncomfortable being asked about sensitive topics like suicidality. The participants may also experience pain or bruising that results from the blood draw. With any puncture of the skin, there is an increased likelihood for infection, although this is minor.

Minor Risk Related to Intervention or Control Products: We do not anticipate any major risks for the participants with consumption of either the intervention (freeze dried blueberry powder) or control (placebo powder) products. Participants might grow weary and uninterested in consuming the products each day over the 12-week period. Additionally, the proposed intervention will provide an older adult with 30% of the recommended daily intake of dietary fiber. One may experience side effects that commonly occur with increased dietary fiber consumption if the individual typically consumes relatively low amounts of dietary fiber.

Risk Related to Depressive Symptoms: It is possible that individuals with depressive symptoms may progress in symptom severity during the study period. Although we do not anticipate a change to a more severe category of depression, we recognize that our proposed population of older adults with prevalent depressive symptoms are already predisposed to development of more severe depressive disorders. Importantly, our population of interest is also more likely to experience suicidal ideation.

Risk Related to Physical Activity: Given that our cohort includes older adults who primarily engage in sedentary behavior, a sudden increase in activity may result in musculoskeletal injury and/or exhaustion.

8.2 Risk Minimization

We will attempt to minimize the identified risks as specified below:

Risk Minimization of Participation in Research: To minimize the risk of breach in confidentiality, all primary study data will be recorded with computer tablets on electronic case report forms (CRF) or as digital files generated from laboratory equipment. All data recording will be in accordance with procedures and guidelines outlined in the study's Manual of Procedures (MOP) authored by the study team. Participant confidentiality will be maintained by recording subject data using a unique subject identifier. Identifiable data, such as contact information and medical record numbers, will be recorded and stored separately from the clinical study data. Any paper-based study material and any identifiable data will be kept separate in a locked file cabinet accessible by authorized study staff only. Only the study staff directly responsible for the data collection and the safety of the participant will have access to identifiable information. All electronic CRF data will be stored securely in an electronic data capture and management system. Raw electronic instrumentation data will be organized and saved on a private network file dedicated to the research project. Only those listed on the approved IRB protocol will have access to subject data. Subject data will be coded and locked in a file cabinet in a locked office. Identifying information will not be used during discussion, presentation or research publication. All documents and electronic data will be archived for a minimum of three years, or as required by the IRB and federal regulations, after the completion of the clinical trial. The study will be registered at clinicaltrials.gov.

The Hinda and Arthur Marcus Institute for Aging Research employs the Research Electronic Data Capture (REDCap) system for data capture and data management operations. REDCap is a full-featured clinical trials data management system (DMS) accessible to data entry and data analysis workstations using secure Web technologies. While REDCap can be used to collect virtually any type of data (including 21 CFR Part 11, FISMA, and HIPAA-compliant environments), it is specifically geared to support online or offline data capture for research studies and operations. REDCap is developed and

maintained by Vanderbilt University in cooperation with REDCap Consortium members, including HRC. HSL hosts and maintains a dedicated instance of REDCap for use across our research enterprise. Each research project is provided separate workspace in which all of the study data are stored in a MySQL relational database on the private corporate network behind several firewalls and located physically within the HSL data center.

Risk Minimization of Testing Procedures: The majority of the testing procedures will be questionnaire based. Participants will be advised that they can refuse to answer any of the questions. Participants will be permitted to rest between studies to prevent fatigue. To minimize the risk of being uncomfortable during questionnaires on sensitive topics like suicidality, only trained research staff will administer the questionnaires. Research staff will be trained to administer them in a calm, welcoming demeanor and will reassure the participants that the assessment(s) can stop at any time.

To avoid any risk associated with blood draws, only individuals with trained phlebotomist skills will draw blood using standard, sterile safety procedures to minimize risk of infection. Unless performed at the participant's home/residence or other HSL housing facility, blood draws will be done at our study clinic that resides in Hebrew SeniorLife, which is a functioning hospital. Thus, in the rare case a participant needs additional care that the study team is not qualified to provide, the individual will be transferred immediately to the adjacent hospital.

Risk Minimization of Intervention Product: Individuals with allergies to intervention products will not be included in this study to avoid any adverse effects/allergic reactions. We anticipate that the blueberry and placebo powder will be well-tolerated by participants since they consist of dietary nutrients that are regularly consumed. Regardless, every 4 weeks we will ask participants about any complaints or adverse events that are directly related to the study intervention products. We plan to track diarrhea, gas, bloating, abdominal pain, constipation etc. Since the intervention arm will be increasing fiber consumption and sudden changes in fiber intake may cause gastrointestinal distress, participants will be counseled to consume their dietary intervention products alone (i.e. without other foods) to avoid consumption of large quantities of fiber at one time. Since our intervention provides only ~30% of the recommended intake of daily fiber, we do not anticipate this amount will result gastrointestinal distress, if consumed alone. Nevertheless, symptoms of gastrointestinal distress will be captured at each visit and appropriately addressed by the study team. Dr. Millar, who has a PhD in Nutrition, will consult with participants on their experience with the fiber and reconcile any gastrointestinal stress. If a participant develops a health problem or a potential health problem (in addition to the ones outlined below), the PI will be notified ASAP. If necessary, the SO will be contacted to help decide whether the participant should continue in the study, and/or what further steps regarding medical evaluation should be performed.

Risk Minimization of Depression Severity: Although we do not anticipate a change to a more severe form of depression, we recognize that our proposed population of older adults with prevalent depressive symptoms are already predisposed to development of more severe depressive disorders. The level of depression will be evaluated and monitored at all visits (except for the telephone screen) to proactively monitor participant safety. If at any time during the study an individual's assessments indicate they have progressed to a more severe forms of depression, then specific safety protocols will be followed (e.g., notifying the study psychiatrist and/or PI). Our population of interest is also more likely to experience suicidal ideation, which will also be monitored at every visit to identify any individuals, who may need psychiatric care outside this study. If the participant is deemed to be dangerous or at imminent risk of harm, study staff will contact emergency services (i.e., #911) for immediate medical assistance.

Risk Minimization Related to Physical Activity: Throughout the intervention, study staff will ask participants to report any symptoms, injuries, or adverse events. Any relevant symptoms or adverse events will be monitored throughout the study, and reported to regulatory agencies per their respective reporting guidelines. If participants report a musculoskeletal injury and/or exhaustion, they will be advised to reduce physical activity until the injury has resolved. If

necessary, participants will be recommended to seek medical attention. The PI may consult the SO, to determine what further steps regarding medical evaluation should be performed.

General Risk Minimization: The proposed protocol requires 5 visits over a total 14 weeks and therefore imposes a moderate amount of participant burden with respect to time and effort. Our institute has a strong track record of successful clinical research requiring similar participation, and retention has been high in these projects. The Clinical Research Laboratory at the Marcus Institute is located near a cafeteria and rest room, and is equipped with comfortable seating, a TV, movies, books, and magazines to keep individuals occupied during rest periods. Several additional strategies will be employed to minimize participant burden and maximize adherence to the protocol. We will:

- Develop a personal relationship between participants and members of the staff.
- Schedule appointments at convenient times with familiar staff.
- Explain to participants all aspects of their participation and follow up. We will demonstrate and practice study procedures before beginning data collection.
- Provide reminders of all appointments and follow-up phone calls.
- Include personal notes in the participant's data file to remember events in the life of the participant; these can be commented on at the next visit (e.g., birthday, birth of a grandchild).
- Provide snacks and lunch during all visits.
- Provide transportation for all visits, if required.
- Provide valet or dedicated on-site parking spaces.
- Compensate participants for visits.

8.3 Quality Assurance and Safety Monitoring

The Principal Investigator (PI) will assume primary responsible for ensuring participants' safety on a daily basis. Since this is a single-site, phase 1 pilot study, without high risk, our study will not require an official Data and Safety Monitoring Board (DSMB). However, to ensure and monitor participant safety, a study psychiatrist will oversee all possible adverse events and we will also have a Safety Officer (SO) designated for this study, who resides in the Boston area.

The PI, study psychiatrist, and SO will meet at least twice per year, either in-person or by teleconference call to review study progress, data quality, and participants safety. They will discuss any reported AE's of the participant. The SO will be provided a detailed Data Safety and Monitoring Report of study progress, data, and safety issues.

The Data and Safety Monitoring Report will include 1) recruitment and participant status, 2) reasons for screening failure, 3) any protocol deviations, 4) participant demographics, and 5) all reports of AE's and unanticipated problems. For each AE/unanticipated problem, report will be generated that includes the participants age, sex, medical history, related symptoms, and descriptions of the AE/unanticipated problem.

The responsibility of the SO includes (but is not limited to) data and safety monitoring, advising the NIA Program staff and the Principal Investigator (PI) regarding participant safety, study risks and benefits, scientific integrity, participant recruitment, and ethical conduct of a study. Prior to the intervention period, SO will review the entire IRB-approved study protocol regarding subject safety and analysis, the informed consent documents regarding applicability and readability, and participant recruitment and retention milestones.

8.4 Adverse Event Collection and Reporting

Any adverse or serious adverse events will be logged using forms either provided by or modeled after the forms that are provided by the NIA Clinical Research Toolbox .

Adverse Event Definition and Categorization

An adverse event is any untoward medical occurrence in a participant, whether or not it is causally related to the study. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the study. We have defined

specified thresholds of change in some of our assessments (e.g., change in depressive symptom severity or change in related symptom severity) that will qualify as an adverse event which is outlined in our manual of procedures. All adverse events will be recorded on the appropriate case report forms and source documents. The PI, study psychiatrist, and/or trained staff member will evaluate all adverse events as to their severity and relation to the test article. The severity of adverse events will be graded as follows:

Mild: Awareness of a sign or symptom but easily tolerated.
Moderate: Discomfort sufficient to cause interference with usual activity or to affect clinical status.
Severe: Incapacitating with inability to do usual activity or to significantly affect clinical status.
Life Threatening: The participant was at immediate risk of death from the adverse event as it occurred.

The Investigator will also assess the relationship of any adverse event to the study, based upon available information, using the following guidelines:

0 = Unlikely: No temporal association, or the cause of the event has been identified
1 = Possible: Temporal association, but other etiologies are likely to be the cause; however, involvement of the study procedures cannot be excluded.
2 = Probable: Temporal association, other etiologies are possible, but not likely.

To determine the attribution and temporal association of an adverse event we will consider the following:

1) Whether the participant reports they have experienced the same symptom prior to the study intervention.
2) Whether the symptom occurred and resolved within 24 hours of taking the study intervention.

The PI, study psychiatrist, and/or SO will consider the symptom according to the conditions stated above and determine temporality as per clinical judgment.

Definition of a Serious Adverse Event

A serious adverse event is any experience that results in any of the following outcomes:

- Death
- Is life-threatening
- Inpatient hospitalization or prolongation of hospitalization

A persistent or significant disability/incapacity. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. We do not anticipant any Serious Adverse Events with our intervention.

Adverse and Serious Adverse Event Reporting

There is a potential for adverse events and incidental findings during this study. A structured questionnaire asking about adverse events will be assessed during each visit of the intervention period. However, when any adverse has been identified, the study team will take appropriate action necessary to protect the study subject and then complete the Adverse Event form that will be modeled after the form provided by the NIA Clinical Research Toolbox. This form requires Principal Investigator review and signature. After review by the Principal Investigator any adverse event will be reported to the IRB as appropriate. Routine reporting of AEs will be monthly or quarterly as determined by the NIA PO and the Safety Officer.

AE's that 1) are unexpected in nature, severity, or frequency, 2) are possibly, probably, or definitely related, and 3) suggests that the research places participants at a greater risk of harm than previously known or recognized, will be reported to the IRB, NIA PO, SO, and OHRP within 2 weeks of the event.

If a serious event occurs, it will be brought immediately to the attention of the Principal Investigator and study psychiatrist. The study psychiatrist will contact the participant, decide if immediate treatment is necessary, initiate such treatment an appropriate hospital or urgent care setting, contact the primary care physician, and notify the IRB. A Serious Adverse Event form that is modeled after the one provided by the NIA Clinical Research Toolbox will be completed, which requires Principal Investigator review and signature. If an AE is defined as a SAE, the Principal Investigator will be notified as soon as the event is known about. Routine reporting of

expected SAEs will be monthly or quarterly as determined by the NIA PO and the Safety Officer. If the SAE is unexpected or unanticipated, the Principal Investigator will notify the NIA PO, the Safety Officer, and IRB within 48 hours of being notified.

Unanticipated problems or adverse events will be reported according to Advarra's IRB written guidelines for interventional studies. Unanticipated problems and serious adverse events that are probably, possibly, or definitely related to the study will be reported as soon as possible from the time of learning of the event, but reported within 10 days to Advarra's IRB per Advarra IRB guidelines. Advarra will be provided a written report submitted and a submission of the incident via the eIRB system. This form will record any adverse symptoms and/or study protocol deviations. Study staff will reference a Subject Safety Event Reporting Decision Chart provided and updated regularly by Advarra to determine whether an event needs to be reported to the Advarra IRB.

All other adverse events/study incidents will be logged on an Adverse Event log and reported to the IRB following the appropriate reporting times as defined by the Advarra IRB.

For less serious or incidental findings the Principal Investigator will speak with the participant about the finding. If necessary, the PI may suggest appropriate follow-up with the study psychiatrist and/or provide a letter describing the findings and need for follow-up. The study psychiatrist will also speak with the participant's primary care provider if the participant gives permission to do so.

Any adverse events that take place during testing will be reported to the PI and recorded in the database. The PI will have ultimate responsibility for monitoring participant safety in the trial. The investigators will be responsible for reviewing each adverse event in a timely fashion, and reporting all incidents to the appropriate regulatory agencies according to written guidelines.

8.5 Participant and Study Stopping Rules

Participant Stopping Rules: If a participant experiences any adverse event that is deemed "severe" as outlined in **§**8.4 (Adverse Events Collection and Reporting) their continuation in the study will be determined by the PI. If necessary, the PI will consult the study psychiatrist and/or SO to gain additional insight on participant continuation. Additionally, if a serious adverse event (SAE) occurs, it will be carefully reviewed by the SO. Any report of a serious adverse event (SAE) that is thought to be directly related to the study products or study procedures, will result in the participant's discontinuation from the study.

Study Stopping Rules: Similar to the participant stopping rules, all serious adverse events (SAE) will be carefully reviewed by the SO to determine if study termination is warranted.

8.6 Potential Benefits

Participants may not receive any significant health benefit from participation, although some may benefit from knowledge of their health status, as well as potential therapeutic effects freeze-dried blueberry powder. This will be a first of its kind trial that evaluates a dietary intervention in sedentary, older adults with depressive symptoms. Identification of a feasible dietary intervention to target inflammatory pathways relevant to lack of motivation may provide older adults with a safe, well-tolerated, cost effective alternative strategy to increase engagement in physical activity and avoid sedentary behavior. If our findings confirm that our intervention is feasible and demonstrates preliminary efficacy, subsequent trials will be appropriately designed using the variation in change of motivation and physical activity gathered from this study. Such a study will be adequately powered to determine definitive efficacy of a dietary intervention to target motivation and physical activity in sedentary, older adults with depressive symptoms.

8.7 Participant Compensation

Participants will be provided up to $300 stipend to compensate them for their time spent completing study procedures.

CHAPTER 9. TRAINING

A manual of operations will be created with standard participant instructions for each question and assessment. All research staff will review and sign the Site Signature Log – Delegation of Authority Log that is modeled after the log provided by the NIA Clinical Research Toolbox to confirm their responsibilities related to the study. During startup, staff will undergo intensive training, and all training sessions will be logged and signed accordingly. They will conduct all study procedures on 4-5 older adult volunteers (more if necessary) with oversight from the PI to ensure consistency of raters and equipment setup. Quality checks will be done every six months throughout the data collection period.

Training will be based on standardized materials developed for the study, and coordinated by the Project Director/Study Coordinator. Every six months, the staff will undergo training review and quality checks on all assessments and drug distribution protocols. Additionally, any time there is an amendment to the study protocol, the change will be logged on a Change in Protocol Log. All study staff will be provided a summary of the protocol modifications and under-go re-training for the new protocol. The date, duration, and certification of all training will be documented and signed by the Principal Investigator on the appropriate training logs.

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