Tart Cherry Juice for Sleep in Older Adults with Insomnia: A Pilot Study of Feasibility and Comprehensive Mechanisms (CherryZZZ)

Study Protocol
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CHAPTER 1. INTRODUCTION

Preliminary studies have shown that drinking tart cherry juice daily can help increase sleep quantity in older adults with insomnia. This study aims to corroborate previous findings, determine study feasibility, and generate preliminary data on several putative corresponding mechanisms on how tart cherry juice may impact sleep. It is planned as a randomized, double-blind, placebo-controlled, cross-over intervention in 20 older adults with self-reported insomnia. After daily consumption of tart cherry juice and placebo (each for 4 weeks), change in sleep quantity/quality and relevant biomarkers (e.g., melatonin, cortisol, serotonin, and inflammatory makers) will be measured.

CHAPTER 2. BACKGROUND

An alarming 40-70% of older adults are estimated to have chronic sleep problems, which consequently increases risk of detrimental health outcomes, including physical ailments, mental health issues, and cognitive decline.[1] Although effective, pharmacological strategies are often paired with severe adverse effects, which dissuade older adults from seeking treatment and leave them suffering from poor sleep.[2] Thus, there is an urgent need to identify safe, non-pharmacological strategies that optimize sleep to maintain healthy aging in older adults and avoid the adverse outcomes often resulting from poor sleep.

Diet offers a safe, non-pharmacological strategy to modulate sleep. Two small pilot studies in older adults with insomnia (i.e., a condition marked by poor sleep) report that intake of tart cherry juice for 2 weeks increased sleep quantity (e.g., total sleep time), but not quality (e.g., patient-reported satisfaction with sleep).[3,4] Poor sleep quality is associated with mortality and other diseases of aging,[5-8] which *underscores the importance of improving both sleep quantity and quality*. Interventions targeting improved sleep quality typically have durations of at least 4 weeks as previously reviewed.[9] Given that the previous studies had 2-week durations of tart cherry juice intake, longer studies may be required to see increases in both sleep quantity and quality.

Despite 2 successful pilot studies that show tart cherry juice increases sleep quantity in older adults, the underlying mechanisms remain unclear. Tart cherry juice is made from a specific type of cherry variety that is uniquely concentrated with **melatonin,** an important compound involved with the initiation of sleep.[10] Yet, there are also other factors in tart cherries that may impact sleep,[11-15] including: **tryptophan**, a precursor to serotonin, a mood regulating hormone,[15-18] **magnesium**, a micronutrient that regulates the production of cortisol, a stress hormone,[19,20] and **flavonoids**, plant-based compounds that protect cells from damage and lower inflammation in the body (**Figure 1**).[11,13] Cherry-derived melatonin, tryptophan, magnesium, and

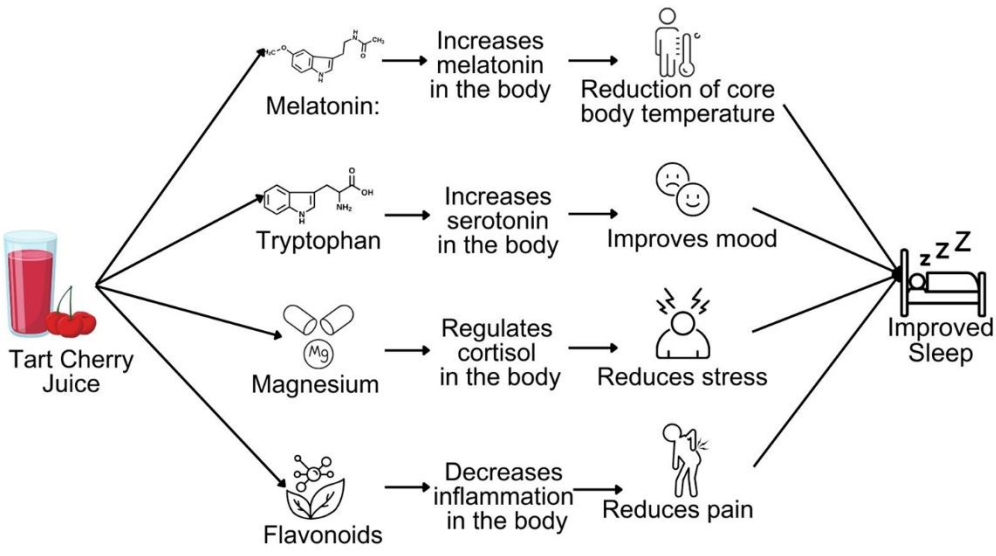

Figure 1. Theoretical model of the impact of tart cherry juice and pathways relevant to sleep. Tart cherry juice provides several compounds that hypothetically modulate specific biomarkers, which then have subsequent effects on sleep-relevant factors.

flavonoids all have specific effects on biomarkers, which consequently have downstream effects on sleep-associated factors (i.e., **reduction in core body temperature,**[10,21,22] **improved mood,**[18,23-25] **reduced stress,**[26-28] **and reduced pain,**[29-31] respectively). Clarification of the mechanisms underlying the impact of tart cherry juice on sleep will allow us to ultimately identify other potential foods and/or dietary patterns that provide similar compounds to help with sleep.

CHAPTER 3: RESEARCH DESIGN

3.1 Study Objectives and Aims

The **overall goal** of this study is to determine feasibility of a tart cherry juice intervention in older adults, and generate preliminary data of outcomes and mechanisms related to sleep. We propose a 2-arm randomized, double-blind, cross-over study in 20 older adults with self-reported insomnia. Participants will consume 240 mL of tart cherry juice or placebo (calorically matched to the tart cherry juice beverage, but theoretically devoid of melatonin, tryptophan, magnesium and flavonoids), twice/evening for approximately 4 weeks in a randomly assigned order. The aims are:

Aim 1: Determine the feasibility of recruiting individuals for and implementing a 4 week tart cherry juice intervention in older adults with self-reported insomnia. *Hypothesis:* Recruitment for and implementation of a tart cherry juice intervention in older adults with self-reported insomnia is feasible.
Aim 2: Generate preliminary data for sleep quantity, sleep quality, and relevant biomarkers in older adults with self-reported insomnia enrolled in a double-blind randomized placebo controlled tart cherry juice intervention. *Hypothesis:* Intake of tart cherry juice tends to increase sleep quantity (via sleep monitoring device), sleep quality (via validated questionnaire, e.g., the Pittsburg Sleep Quality Index) urinary melatonin, and serum serotonin, as well as decrease urinary cortisol and serum inflammatory markers.
Exploratory Aim: Explore the impact of tart cherry juice intake on sleep-related factors that may be impacted by the Aim 2 biomarkers in older adults. *Hypothesis:* Intake of tart cherry juice for 4 weeks results in changes in mood, stress, and pain.

3.2 Overview

This will be an individual-level, 2-arm, randomized, cross-over pilot in 20 older adults with self-reported insomnia to determine feasibility of a tart cherry juice intervention and to generate preliminary data on measures of sleep and related biomarkers needed to plan a future trial. Participants will complete a 1-week (approximately 7 nights of sleep) pre-intervention assessment of sleep quantity, since several nights of data are needed to reduce variation in these outcomes. They will be randomized to consume tart cherry juice or placebo, which is nutritionally matched to the cherry juice beverage, but without significant amounts of our putative active cherry compounds (e.g., melatonin, tryptophan, magnesium, or flavonoids). They will be asked to consume their assigned intervention in the evening: 240 mL (i.e., 8 ounces) at approximately 5 pm and 240 mL (i.e., 8 ounces) at approximately 1 hour before bedtime for 4 weeks. After approximately 2 weeks of washout, they will repeat the same process with the other arm of the study (**Figure 2**).

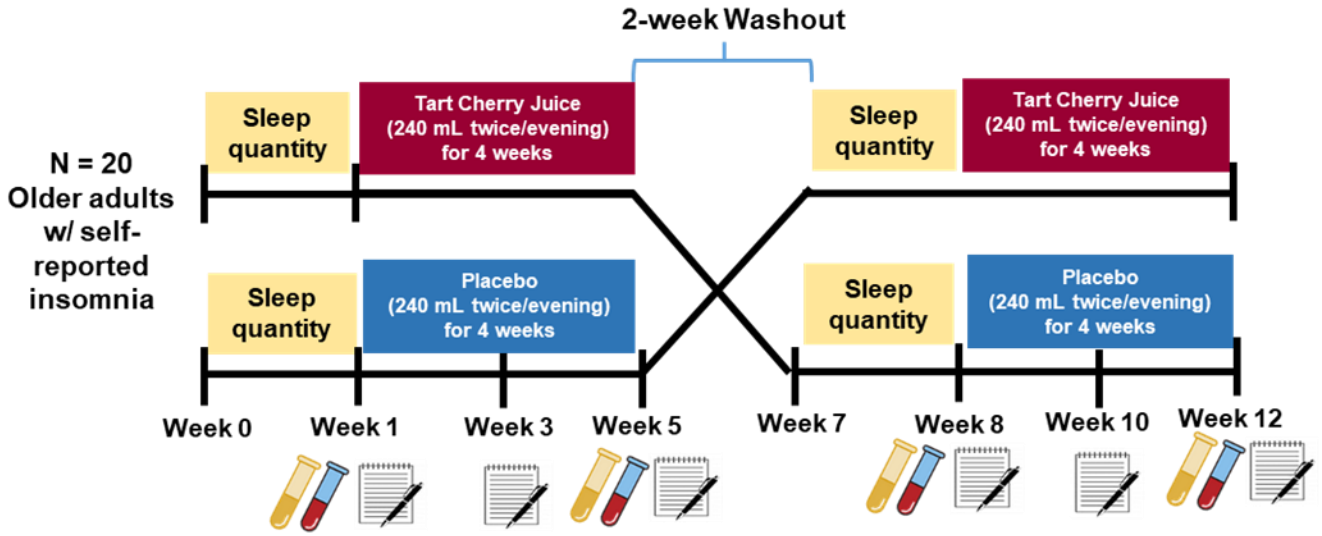

Figure 2. Proposed Cross-over Pilot Study Design. Eligible participants will consume either tart cherry juice or placebo for 4 weeks in a randomized order. Before each arm, individuals will have a one week assessment of sleep via wearable device (e.g., Garmin watch™). Blood and urine for biomarkers will be assessed before and after each arm of the intervention, while questionnaire based assessments will be administered before, during, and after each arm. After a two-week washout period, participants will be asked repeat the same process with the other arm.

3.3 Inclusion and Exclusion Criteria

Our target population is older adults, with self-reported insomnia. Individuals expressing an interest in participating will be screened to confirm that they meet the following inclusion and exclusion criteria.

Inclusion Criteria

- Men and women aged ≥65 years
- Self-reporting insomnia (e.g., trouble sleeping approximately 3 nights a week for at least 6 months and/or an Insomnia Severity Index score ≥ 10 points[32])
- Usual bed-time between 8:00 pm and 1:00 am

Exclusion Criteria

- Unwilling to follow the study protocol
- Inability to properly use the wearable device, complete smartphone-based surveys, or follow the intervention protocol
- Self-report of medically diagnosed sleep disorders except insomnia (e.g., sleep apnea)
- Current and consistent use of sleep aids or hypnotic prescriptions (e.g., trazadone)*
- Self-report of cognitive impairment, dementia, or other neurological disorder
- Are on unstable medications (i.e., change within the last 3 months) for other conditions
- Have an allergy to the intervention products
- Self-report history of diabetes
- Current alcohol (Alcohol Use Disorders Identification Test ≥4 points[33,34]) or drug use disorder (Drug Abuse Screening Test,[35,36] DAST-10>2 points)
- Are excessive caffeine drinkers (≥5 cups of caffeinated beverages a day)
- Any other reason/condition the PI and investigative team believe this intervention would be unsafe

*If individuals currently use certain sleep aids (e.g., melatonin), but are willing and able to avoid their use during the study, participants will be asked to avoid the use of the sleep aid for approximately 2-3 weeks before continuing with the study.

3.4 Number of Subjects and Study Duration

We aim to recruit a total of 20 individuals (both men and women) 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). Given that a primary goal of this study is to determine recruitment and attrition rate, we may not have exactly 20 individuals who complete the study. Participants will remain in the study for a total of approximately 12 weeks.

3.5 Study Endpoints

The primary outcome is feasibility. Secondary outcomes are sleep quantity, sleep quality, and select biomarkers. Exploratory outcomes include mood, stress, pain. See §6.2 for further information.

3.6 Study Intervention Products

Tart cherry juice will be purchased from a commercial supplier (e.g., Shoreline Fruit™). Shoreline Fruit will be responsible for preparing and bottling the tart cherry juice. Frozen or fresh tart cherries will be juiced and bottled according to the manufacturers normal processing. The intervention products will be prepared following proper food safety/handling protocols and the bottles. The bottles of tart cherry juice will be labeled with a letter, number or symbol, that distinguishes it from the placebo.

Unsweetened black cherry powder (e.g., Kool-aid™) will be purchased and used as the placebo. A reputable, established lab (e.g., Rutgers Food Innovation Center) will prepare the placebo, who will follow safe, good manufacturing practices. The placebo will be prepared to match the caloric/sugar content of the tart cherry juice. The Kool-aid has a similar taste and color, however does not contain significant amounts of our active ingredient (i.e., tart cherries) or active putative compounds (e.g., magnesium, melatonin, tryptophan, flavonoids). The placebo will be bottled in the same bottle type that the tart cherry juice is bottled in, however it will be marked with a letter, number, or symbol that differs from the one used to identify the tart cherry juice.

The key that decodes which of the letter/number corresponds with which product will be kept secure by an independent party (e.g., an individual at the Biostatistics and Data Sciences Core of the Marcus Institute). Thus, *the participants, study staff, and analyzers of the data will be blinded to the letter/number that is used to identify the tart cherry juice vs the placebo.* At the end of the data collection and data analysis, the study team will be unblinded. See **§**5.3 for further information on dietary intervention. Individuals will be provided with an allotment of the intervention products and additional bottles will be delivered by our study staff as needed.

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 Hebrew SeniorLife (HSL) facilities.
- We will use the Harvard Catalyst (CTSA) and 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 targeted web-based advertisements via BuildClinical), and at www.clinicaltrials.gov.

4.2 Recruitment Timeline and Randomization

Our trial proposes to enroll 20 individuals over approximately 6-months. In an attempt to account for the impact of order, we will randomly assign individuals to the order in which they receive the intervention and placebo. For example some individuals will start with the intervention and then placebo, others will start with the placebo and then the intervention. This process will be carried out while maintaining participant and investigator blinding. Block randomization will be employed as a means to reduce the impact of season/time.

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 trained 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 participant may be withdrawn from the study prior to completing all of the study related procedures due to the following conditions:

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

Withdrawn participants 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 interventions 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.

4.5 Methods to Protect Participant Privacy

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

1. Study visits will be conducted in private rooms.
2. Each participant will be given a unique study identification number and data will not include any of the participant's PHI.
3. All participant-identifying information will be stored and managed on a secured database server or in a locked cabinet. If on a database, the information will be password protected.
4. Participant confidentiality will be maintained in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations.
5. Only the PI, study personnel, and laboratory personnel approved by the IRB and authorized to view PHI will have access to the information.
6. PHI will not be used during discussion, presentation or publication of any research data.
7. 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 may be given a schedule of their study visits if requested. 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 if possible. Transportation will be provided for each visit as needed, snacks will be available, and stipends will be provided for each study milestone. If necessary/requested, reminder calls will be made to participants 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 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 and will be recognized for their contributions to the study. We will remain in close contact with individuals throughout their participation with follow-up calls each month.
- ***Incentives and Rewards:*** Participants may 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

Study visits will take place at the Clinical Research Laboratory at HRC, Roslindale, MA, an HRC-affiliated housing site, or at the participants home or via telephone (if possible). This study includes up to 7 visits. Participant eligibility will be determined during an in-person screening. If eligible and interested, the participant will be asked to continue with additional study visits. The Week numbers are approximate in order to allow for flexibility in scheduling (e.g., one participant may be on the intervention for 28 days, while another may be on the intervention for 31 days). The assessments/activities for each study visit are outlined below.

Visits	Assessments at Each Visit
Telephone Screening	We will assess: 1. Sleep Behaviors (e.g., Insomnia Severity Index) 2. Medication use (e.g., sleep aids)* 3. Medical History 4. Other exclusion criteria *If individuals currently use certain sleep aids (e.g., melatonin), but are willing and able to avoid their use during the study, participants will be asked to avoid the use of the sleep aid for approximately 2-3 weeks before continuing with the study
Week 0 (Visit 1)	We will perform/assess: 1. Informed Consent/Assessment of Understanding 2. Medical History 3. Health Behaviors 4. Substance/Alcohol Use 5. Social Network 6. Height 7. Weight If eligible, participants will be asked to: 1. Fill out a 3-day dietary record in the week prior to next visit 2. Avoid consumption of certain foods, beverages, and supplements/medications (e.g., cherries, alcohol, and sleep aids) for the entire duration of the study.

	3. Wear a digital sleep sensor (e.g, Garmin Watch) every day/night for the duration of the study and keep a sleep diary of time they went to bed and got out of bed for each night.
Week 1 and Week 8 (Visit 2 and 5)	Assessments/Activities: 1. Turn in Diet Records 2. Symptoms 3. Vitals 4. Weight 5. Sleep Quality 6. Blood Draw and Urine Sample for Biomarker 7. Depressive Symptoms 8. Stress 9. Pain 10. Additional Relevant Questionnaires Participants will: 1. Continue to wear the digital sleep sensor every day/night and maintain sleep diary 2. Start consuming assigned intervention product
Within the first 5 days of starting each intervention arm, participants will be called to confirm there is no impact of the timing of juice impact on sleep quality. If a participant discloses that they are noticing lower than usual sleep quality after consuming the juice, the timing of the dose will be modified to mitigate any negative effects.	
Week 3 and Week 10 (Visit 3 and 6)	Assessments/Activities: 1. Symptoms 2. Related Sleep Factors 3. Depressive Symptoms 4. Stress 5. Pain Participants will: 1. Continue to wear the digital sleep sensor every day/night and maintain sleep diary 2. Continue consuming assigned intervention product 3. Fill out a 3-day dietary record in the week prior to next visit
Week 5 and Week 12 (Visit 4 and 7)	Assessments/Activities: 1. Turn in Diet Records 2. Symptoms 3. Related Sleep Factors 4. Vitals 5. Weight 6. Sleep Quality 7. Blood Draw and Urine Sample for Biomarkers 8. Depressive Symptoms 9. Stress 10. Pain 11. Additional Relevant Questionnaires 12. Compliance *If Week 5, participants will continue to wear the digital sleep sensor every day/night
Week 7 (No Visit)	Participants will be asked to: 1. Continue to wear the digital sleep sensor every day/night and maintain sleep diary 2. Fill out a 3-day dietary record in the week prior to next visit

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.

Planned Study Assessments at Each Visit

Visit	1	2	3	4	-	5	6	7

	Week 0	Week 1	Week 3	Week 5	Week 7	Week 8	Week 10	Week 12
Screening	X							
Update Medical History	X							
Health Behaviors and Social Network	X							
Relevant Symptoms		X	X	X		X	X	X
Vitals		X		X		X		X
Height	X							
Weight	X	X		X		X		X
Sleep Quality		X		X		X		X
Sleep Quantity (via Garmin Watch)	X	X	X	X	X	X	X	X
Related Sleep Factors			X	X			X	X
Proposed Biomarkers		X		X		X		X
Depressive Symptoms		X	X	X		X	X	X
Stress		X	X	X		X	X	X
Pain		X	X	X		X	X	X
3-Day Diet Record	X			X	X			X
Compliance				X		X		X

Eligible participants will be asked to avoid consumption of tart cherry products, as well as supplemental forms of flavonoids, magnesium, and tryptophan for the entire study in order to isolate the effects of our intervention on the proposed outcomes. They will also be advised to avoid intake of foods, beverages, and medicines that may impact sleep (e.g., alcohol, caffeine, sleep aids, medications that induce drowsiness). Participants will undergo a 1-week (approximately 7 nights) pre-intervention assessment of sleep quantity via a digital wearable device that measures aspects like sleep quantity and physical activity (e.g., Garmin Watch™), which will serve as the baseline assessment for these measures. Furthermore, participants will be asked to record the time they went to bed and the time they got out of bed (i.e., sleep diary) for the entire study. Participants will also be asked to complete a 3-day diet record. At the baseline visit, medical history, and basic anthropometric information will be collected. Participants will provide a urine sample and fasted blood samples for biomarker analyses, complete questionnaires (e.g., sleep quality, mood, etc.) and have their vitals measured. Blood samples will be collected between 8-10 am, to avoid the impact of time of day on markers. After randomization to one of the intervention arms, participants will be asked to consume their intervention product twice per day for approximately 4 weeks, while also wearing the digital sleep sensor. Half of the intervention dose is intended to be consumed at approximately 5 pm and the other approximately 1 hour before bedtime. Individuals may receive reminder messages via their digital wearable device or via email/text to remind them about their study responsibilities (e.g., charging their devices etc). All assessments that were performed at baseline will be repeated approximately 4 weeks later at follow-up (e.g., urine sample, blood draw, questionnaires). After approximately 2 weeks of a washout period (to reduce the impact of a carry-over effect from the intervention), participants will complete the same schedule with the other intervention product.

Telephone Pre-screen (~30 minutes): Volunteers will be asked about demographic information, sleep behaviors/insomnia, medication/supplement use, any past/present medical conditions and/or treatments, as well as other exclusionary criteria.

Medical History: Participants will be asked to disclose any existing or previous medical conditions vis self-report.

Insomnia Severity Index: The insomnia severity index will be administered to determine eligibility. Individuals scoring 10 points or higher will be eligible.

Screening Visit (Week 0; ~60 minutes): 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. Validated questionnaires that asses substance and alcohol use, as well as social connectedness will be administered. The participant's height and weight will be collected.

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 member is confident that the participant is completely familiar with the document and understands all the aspects of the informed consent form, it will be signed by the participant in the presence of the staff member, and 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 sleep, 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, medication use, smoking status, use of drug, use of alcohol etc.

Drug Abuse Screening Test (DAST) will evaluate drug use. DAST will be administered to participants and individuals who scores >2 points, will be excluded.[35,36]

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.[33,34]

Social Network: Subjective perception of social support and connectedness will be evaluated using a validated questionnaire.[37]

Height/Weight will be measured.

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

If eligible, all participants will be asked to avoid consumption of certain foods (e.g., tart cherries) and fill out a 3-day diet record to estimate nutrient intake in the week prior to their next visit. Participants will also be asked to wear the digital sleep sensor all day and night for the remainder of the study. They will also be given a log to record the time they go to bed and the time they get out of bed for the duration of the study.

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.

Sleep Quantity: Sleep quantity will be assessed subjectively and objectively. For the subjective measure, participants will be given a daily log or journal to document aspects of their sleep (e.g., time of sleep onset, time of rising, number of disturbances, self-rated satisfaction with sleep). Participants are asked to complete this log every day they are in the study. To objectively measure sleep quantity,

participants are asked to wear a digital device that measures sleep quantity. Participants will be asked to wear the device throughout the intervention to objectively capture quantitative aspects of sleep (e.g., time in bed, number of times awake). This device has the capacity will be connected to the participants phone via free, downloadable applications. Reminders may be sent via the device or via email during the study (e.g., reminder to charge device, reminder to open up app to sync data, etc).

Week 1 and Week 8 Visits (~60 minutes): Participants will be asked to turn in their 3-day diet records. Relevant symptoms, weight, vitals, sleep quality, depressive symptoms, stress, pain and any additional relevant questionnaires will be evaluated. Additionally, up to 16 mL of blood will be taken to measure biomarkers in serum and a morning urine sample will be collected. Participants will be given their allotment of intervention products to consume each day and continue to wear their Garmin Watch.

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

Sleep Quality will be evaluated using a validated self-report questionnaire the Pittsburgh Sleep Quality Index (PSQI).[38]

Relevant Symptoms including gastrointestinal distress, appetite, pain etc., will be collected by self-report.

Blood (approximately 16 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 planned biomarkers (e.g., kynurenine/tryptophan ratio and select inflammatory markers (e.g., C-reactive protein, interleukin-6, etc.) will be measured by a reputable lab (e.g., Quest or collaborators at the University of Connecticut).

Spot Urine (approximately 30 mL) will be collected, processed, and stored at -80 degree C for future analyses. We will aim to collect urine in the morning hours between 8-10 am to avoid the influence of the circadian rhythm on cortisol. Batch analyses of planned biomarkers (e.g., cortisol and melatonin.) will be measured by a reputable lab (e.g., Quest or collaborators at the University of Connecticut).

Depressive symptoms will be evaluated using a validated self-report questionnaire (e.g., the Center for Epidemiological Studies Depression Scale (CES-D), [39] the CESD-revised (CESD-R)[40] version, and/or the Patient Health Questionnaire-9 (PHQ-9) [36]).

Stress will be evaluated by the validated 10-item questionnaire, the Perceived Stress Scale.[41]

Pain will be evaluated via a validated 24-item questionnaire, the Geriatric Pain Measure.[42]

Additional Questionnaires will be administered to evaluate other aspects that are relevant to sleep to help characterize our study population (e.g., validated self-report questionnaires that measure anxiety, affect etc.)

Compliance: Compliance with our dietary intervention will be evaluated throughout the study. Participants will be asked to log consumption of their juice and keep all of their empty bottles. Participants will return all remaining empty/full bottles to estimate number of intended doses that were consumed.

Within the first 5 days of starting each intervention arm, participants will be called to confirm there is no impact of the timing of juice impact on sleep quality. If a participant discloses that they are noticing lower than usual sleep quality after consuming the juice, the timing of the dose will be modified to mitigate any negative effects.

Week 3 and Week 10 (~30 minutes): These visits may take place over the phone. They will include an evaluation of relevant symptoms, related sleep factors, depressive symptoms, stress, pain, and compliance. Participants will be asked to fill out a 3-day diet record to estimate nutrient intake in the week prior to their next visit.

Related Sleep Factors: In addition to the assessment of sleep quality and quantity, we will also collected data about factors that are known to affect sleep (e.g., use of alcohol, room temperature via sensor given to participants), that may occur to help characterize or understand unexpected/unusual patterns in the data.

Week 5 and Week 12 Visits (~60 minutes): Participants will be asked to turn in their 3-day diet records. Relevant symptoms, weight, vitals, sleep quality, related sleep factors, depressive symptoms, stress, pain and any additional relevant questionnaires will be evaluated. Additionally, up to 16 mL of blood will be taken to measure biomarkers in serum and a morning urine sample will be collected.

At Week 7, participants will be asked to fill out a 3-day diet record to estimate nutrient intake in the week prior to their next visit. This is not an official visit.

At the end of the intervention (e.g., Week 12 Visit), participants will be asked to provide feedback via open ended questions on their experience during the intervention. They will also be asked if they would be willing to consume this intervention long-term and to guess as to which arm they were on in the beginning and ending of the intervention.

Optional Study Assessments: Participants will also have the option to partake in two optional study assessments as described below. These optional study assessments allow for further investigations of potential mechanisms relevant to sleep, including analysis of phenolic metabolites in urine and analysis of the gut microbiota in fecal samples.

24 Hour Urine Sample: If participants are willing, they will be asked to provide a 24 hour urine collection instead of the planned spot urine collection. This requires individuals to collect all of their urine for the 24 hours preceding select study visits using a provided urine container. Urine will be processed and frozen until analysis. Regardless of whether participants opt for the spot or the 24 hour urine sample, batch analyses of planned urinary biomarkers (e.g., cortisol and melatonin) will be measured by a reputable lab (e.g., Quest or collaborators at the University of Connecticut). The 24 hour urine sample will also be used to evaluate phenolic metabolites. Briefly, urine samples acidified with formic acid will be purified via solid phase extraction to isolate gut-microbially generated phenolic acid metabolites (and their subsequent phase II hepatic conjugates). Purified samples will be analyzed using LC-MS/MS with a triple-quad mass spectrometer to quantify metabolites by their specific parent to daughter ion mass transitions. Targeted metabolomics quantifies ~60 phenolics, gut microbially generated phenolic acid metabolites, and hepatic phase II metabolites of absorbed phenolics and phenolic acids.

Fecal Sample: If participants are willing, they will be asked to collect at least 1 (no more than 3) separate fecal samples before select visits. Samples will be collected as is, in a sterile collection tube without any preservation medium that will be provided to participants. There will be a collection for select visits as indicated below. Participants will temporarily store the self-collected fecal samples in their own freezer (-17°C), and then transported to the lab at HSL for sample processing and long-term storage. Fecal microbiota will be analyzed using whole genome sequencing (WGS) to obtain a holistic representation of the microbes in the gut. WGS was chosen because it enables identification of microbes down to specific species or strain of bacteria whereas other methods only identify the genus level and avoid biases common with 16S rRNA sequencing. A reputable lab (e.g., the Microbial Omics Core at the Broad Institute) will perform the DNA extraction from fecal samples and WGS library construction and sequencing using NEBNext Ultra FS II (2x150bp sequencing chemistry to a minimum depth of 3 million reads per sample). Fastq sequences will be processed and analyzed by a reputable lab/core (e.g., the Harvard Chan Microbiome Analysis Core).

Optional Study Assessments

Visit	1	2	3	4	-	5	6	7
	Week 0	Week 1	Week 3	Week 5	Week 7	Week 8	Week 10	Week 12
24 Hour Urine Collection	X			X	X			X
Fecal Sample	X			X	X			X

5.3 Dietary Intervention

The duration of this study will be approximately 12 weeks. Throughout the intervention, individuals will be asked to avoid cherry containing foods/beverages.

Individuals will then be randomized to either start with the intervention or the control product. This is a double-blinded study, so the study staff nor the participant will know what juice the participants are assigned to. Those randomized to the intervention group will be asked to consume approximately 8 fluid ounces of tart cherry juice, twice per evening for approximately 4 weeks. Individuals randomized to the control group will be asked to consume approximately 8 fluid ounces of cherry-flavored Kool-aid, that is matched for color, as well as calories and sugar content. Participants will be asked to consume half of their dose (i.e., 8 fluid ounces) at around 5 pm and the remaining half (i.e., 8 fluid ounces) about an hour before bed.

The two juices will be bottled in identical bottles. The placebo will be labeled with a differing letter/number than the intervention product.

At visits 2 and 5, participants will be given an allotment of bottles of their assigned beverage, depending on their ability to store them. These bottles can remain at room temperature until they are opened. Subsequent deliveries will be made by our staff as necessary to ensure participants have a stock of their assigned beverage.

Participants will be given a premeasured cup that indicates how much 8 oz. is to allow participant to easily measure their assigned dose.

5.4 Outcome Measures

Our primary endpoints are aspects of feasibility. Secondary endpoints include sleep quantity and quality, as well as sleep-related biomarkers (e.g., melatonin, serotonin, cortisol, inflammatory markers. Exploratory outcomes include sleep-related factors (e.g., mood, stress, pain). We will also explore other alternative outcomes.

Name	**Type**	**Timeframe**	**Brief description**
Recruitment Rate	Primary	Throughout the entire study	Number of individuals that need to be screened to be enrolled that are randomized to the intervention.
Attrition	Primary	Throughout the entire study	Number of individuals randomized that withdraw from the intervention *in media res*.
Compliance of Dose	Primary	Throughout the entire study	The number of intended doses consumed.
Ability to Collect Data	Primary	Throughout the entire study	The percentage of missing data points from electronic capture data devices.
Practicality	Primary	Throughout the entire study	Self-reported willingness to continue the dietary regimen after study completion.
Sleep Quantity	Secondary	Throughout the entire study	Individuals will be asked to wear a polysomnographic device (e.g., Garmin

			watch) to continuously measure aspects related to sleep quantity (e.g., total sleep time, time spent awake).
Sleep Quality	Secondary	Week 1, 5, 8, 12	The validated self-report questionnaire, the Pittsburg Sleep Quality Index, which ranges from 0-21 points.
Melatonin	Secondary	Week 1, 5, 8, 12	Melatonin will be estimated by measuring the major urinary metabolite of melatonin, 6-sulphatoxymelatonin, utilizing a commercially available quantitative immunoassay.
Serotonin	Secondary	Week 1, 5, 8, 12	Serotonin production will be estimated by measuring the kynurenine/tryptophan ratio in blood collected from participants utilizing a commercially available quantitative immunoassay.
Cortisol	Secondary	Week 1, 5, 8, 12	Urinary cortisol will be estimated utilizing a commercially available quantitative immunoassay.
Inflammatory Markers	Secondary	Week 1, 5, 8, 12	A panel of several serum biomarkers related to inflammation (e.g., C-reactive protein, interleukin-6) will be measured in blood collected from participants utilizing a commercially available quantitative immunoassay.
Mood	Exploratory	Week 1, 3, 5, 8, 10, 12	A validated self-report questionnaire (e.g., the CES-D/CESD R, which ranges from 0-60 points or the PHQ-9, which ranges from 0-27 points)
Stress	Exploratory	Week 1, 3, 5, 8, 10, 12	The validated self-report questionnaire, the Perceived Stress Scale, which ranges from 0-56 points.
Pain	Exploratory	Week 1, 3, 5, 8, 10, 12	The validated self-report questionnaire, the Geriatric Pain Measure, which ranges from 0-42 points.

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

Below are the specific outcome variables planned for the study.

Primary Endpoints

Study feasibility is evaluated by several identified measures listed in §5.4, however others will be explored. Feasibility outcomes will be summarized using sample quantities and corresponding 80% confidence interval estimates. The specific thresholds that will determine study feasibility will be fully described in the manual of procedures.

Secondary Endpoints

Sleep quantity will be captured via a device that has polysomnographic capacity (e.g., Garmin Watch™), which continuously measures quantitative aspects of sleep (e.g., total sleep time, number of awakenings, wake time after sleep onset, etc.). The sleep diary that a participant logs their time in bed and time out of bed will help us correct any issues with the objective measures. The primary aspect of sleep quantity will be total sleep time, however others will be explored. Participants will be asked to wear the device all day and night during the study. Change in measures of sleep quantity will be calculated between baseline and midpoint, as well as baseline and final follow-up visit. The distribution of change in sleep quantity scores will be summarized using means and standard deviations or medians and interquartile ranges, as appropriate. We will develop 80% confidence interval estimates of inter- and intra- individual variation (i.e., standard deviation) of change in these measures.

Sleep quality will be measured by a validated self-report questionnaire: the Pittsburg Sleep Quality Index (range: 0-21),[38] where higher scores indicate worse sleep. Change in sleep quality scores will be calculated between baseline and final follow-up visit. The distribution of change in sleep quality scores will be using means and standard deviations or medians and interquartile ranges, as appropriate. We will develop 80% confidence interval estimates of inter- and intra-individual variation (i.e., standard deviation) of change in these measures.

Biomarkers (i.e., melatonin, serotonin, cortisol, and inflammatory markers) will be measured by a reputable lab (e.g., University of Connecticut) utilizing commercially available quantitative immunoassays (ELISA). **Melatonin** will be estimated by measuring the major urinary metabolite of melatonin, 6-sulphatoxymelatonin. **Serotonin** production will be estimated by measuring the serum kynurenine/tryptophan ratio, which reflects the rate at which tryptophan is converted to kynurenine rather than serotonin.[18] Therefore, lower rates of the kynurenine/tryptophan ratio indicate that more tryptophan is blunted towards the serotonin pathway. **Cortisol** will be estimated by measuring the urinary cortisol. **Serum inflammatory markers** (e.g., interleukins and tumor necrosis factor) will be estimated using a panel of several biomarkers, since inflammation is a heterogeneous/complex process.[43] These markers will be consolidated into one composite inflammatory score as previously described.[44-46] Change in the biomarker measures will be calculated between baseline and final follow-up visit. The distribution of change in biomarker measures will be summarized using means and standard deviations or medians and interquartile ranges, as appropriate. We will develop 80% confidence interval estimates of inter- and intra- individual variation (i.e., standard deviation) of change in these measures.

Exploratory Outcomes

Sleep-related factors (i.e., mood, stress, and pain) will be measured. **Mood** will be evaluated by the validated questionnaire, the Center for Epidemiological Studies Depression scale[39,40], which measures severity of depressive symptoms (range: 0-60, with higher scores indicating more severe symptoms). **Stress** will be evaluated by the validated 14-item questionnaire, the Perceived Stress Scale[41] (range: 0-56, with higher scores indicating more stress). **Pain** will be reported via a validated 24-item questionnaire, the Geriatric Pain Measure[42] (range: 0-42, with higher scores indicating more pain). Change in measures of sleep-related factors will be calculated between baseline and final follow-up visit, as well as baseline and mid-point if data is available. The distribution of change in sleep-related factors will be summarized using means and standard deviations or medians and interquartile ranges, as appropriate. 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

For both arms of the study, change over time will be calculated for each outcome variable and summarized using means and standard deviations or medians and interquartile ranges, as appropriate. For the majority of outcomes, week 1 and week 8 (i.e., visits that are before intake of the intervention products) will serve as the baseline assessment and those conducted at week 5 and week 12 (i.e., visits that are 4 weeks after intake of the intervention products) will serve as the follow-up assessment. However, due to the variable nature of sleep quantity we will collect several nights of data from 1 week before the intervention products are administered for baseline and during the last week of the intervention as follow-up. The median value during those weeks will be used to avoid skewing. Then the change between the 1-week estimated medians will be calculated. Additionally, change between baseline and midpoint (week 3 and week 10) will also be calculated and explored when data is available. The specific analyses for each aim are listed below.

Aim 1: Determine the feasibility of recruiting individuals for and implementing a tart cherry juice intervention in older adults with self-reported insomnia.

The aspects of feasibility that are indicated in §5.4 will be tabulated to determine feasibility. This is a descriptive analysis, thus no formal statistical methods will be implored. However pre-specified gradient thresholds that determine if each metric is feasible, in need of modification, or not feasible are documented in the Manual of Procedures.

Aim 2: Generate preliminary data for sleep quantity, sleep quality, and relevant biomarkers in older adults with self-reported insomnia enrolled in a double blind randomized placebo controlled tart cherry juice intervention.

The changes computed for the indicated measures as described in §6.2 will be averaged within each treatment arm (i.e., tart cherry juice vs placebo) and compared with a paired Student's t-test. A p-value less than 0.05 will be used to determine statistical significance.

Exploratory Aim: Explore the impact of tart cherry juice intake on sleep-related factors that may be impacted by the Aim 2 biomarkers in older adults.

The changes computed for the sleep related factors as described in §6.2 will be averaged within each treatment arm (i.e., tart cherry juice vs placebo) and compared with a paired Student's t-test. Additionally the changes in sleep related factors will be correlated (via Spearman Correlation) with change in biomarkers within each treatment arm (i.e., tart cherry juice vs placebo). A p-value less than 0.05 will be used to determine statistical significance.

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 Marcus 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 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 placebo products; 4) those related to sleep; and 5) those related to optional study assessments. 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 are also asked to wear a sensor to objectively measure physiological data. This device may cause slight discomfort or potential rash and be tedious to maintain charged and/or continuously wear. 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. It is also possible that since we are asking participants to consume liquids closer to bed-time, that it might cause frequent urinations during the night.

Minor Risk Related to Intervention or Control Products: We do not anticipate any major risks for the participants with consumption of either the intervention or placebo products. Participants might grow weary and uninterested in consuming the products each day over the 4-week periods.

Minor Risk Related to Sleep: Individuals are eligible for this study because they have reported difficulty sleeping. Individuals with sleep problems are more likely to have increased pain, stress, and depressive symptoms during our study.

Minor Risk Related to Optional Study Assessments: It may be tedious and inconvenient to collect a 24 hour urine sample. It may also be unpleasant to collect fecal samples for the study.

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 and/or passively obtained via wearable devices. 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 minimize risk associated with the sensor, individuals will be provided detailed instruction on how to maintain and wear the sensor.

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 the Hebrew Rehabilitation Center, 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.

To minimize any risk associated with fluid intake close to bed time, participants will be called within 5 days of starting their intervention arm to check in to see if nocturia is occurring. If the participant reports that they are urinating in the night more than usual which is disturbing their sleep, then the study staff will work with the participant to modify their consumption schedule to avoid the sleep disturbance.

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 intervention products will be well-tolerated by participants since they consist of dietary products that are regularly consumed. Regardless, 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. 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 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 Sleep: We will proactively assess pain, stress, and depressive symptoms. These scores will be regularly reviewed by the PI of the study. If any of the scores concern the PI for the participant's safety or the participant informs study staff that symptoms have become more severe than usual, they will contact the study participant and may contact their physician, providing the participant has given consent to do so. Additionally, suicidal ideation will also be monitored at visits while the participant is on the intervention products to identify 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 of Optional Study Assessments: To minimize risk associated with collecting 24-hour urine sample, individuals will be provided detailed instruction on how to collect the urine sample. They will also be reminded a few days prior of when to collect their urine. All materials for urine collection will be provided to that participants. To avoid any unpleasant experience collected a fecal sample, study staff will provide detailed instruction on how to collect the sample. Furthermore, to avoid the burden of storing it in the participants freezer, study staff will pick up the collected samples as soon as possible from the study participant.

General Risk Minimization: The proposed protocol requires up to 7 visits over a total 12 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. TV or movies can be accessed via the internet 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 during all visits.
- Provide transportation for all visits, if required.
- Provide free on-site parking.
- Compensate participants for visits.

8.3 Quality Assurance and Safety Monitoring

The PI will assume primary responsible for ensuring participants' safety on a daily basis. Since this is a single-site, phase 1/2 pilot study, without high risk, our study will not require an official Data and Safety Monitoring Board (DSMB).

8.4 Adverse Event Collection and Reporting

Any adverse event (AE) or serious adverse event (SAE) 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 AE is any untoward medical occurrence in a participant, whether or not it is causally related to the study. An AE 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 related symptom severity) that will qualify as an AE, which is outlined in our manual of procedures. All adverse events will be recorded on the appropriate case report forms, source documents and logs. The PI and/or trained staff member will evaluate all adverse events as to their severity and relation to the intervention. The severity of AEs 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 PI will also assess the relationship of any AE 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 and/or study staff will consider the symptom according to the conditions stated above and determine temporality.

Definition of a Serious Adverse Event

A serious adverse event (SAE) 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.

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 within 2 weeks of the event.

If a serious event occurs, it will be brought immediately to the attention of the Principal Investigator to decide if immediate treatment is necessary, initiate such treatment an appropriate hospital or urgent care setting, contact the primary care physician if the participant provides consent to do so, 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 appropriate. If the SAE is unexpected or unanticipated, the Principal Investigator will notify the IRB within 48 hours of being notified.

Unanticipated problems or adverse events will be reported according to the IRB's 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 the IRB per written IRB guidelines. The IRB 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 regularly updated by the IRB 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 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 participant's primary care and/or provide a letter describing the findings and need for follow-up.

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. Additionally, if a serious adverse event (SAE) occurs, it will be carefully reviewed by the PI. 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 PI 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 from the tart cherry juice. If our findings confirm that our intervention is feasible and demonstrates preliminary efficacy, subsequent trials will be appropriately designed using the variation in change in the outcomes gathered from this study. Such a study will be adequately powered to determine definitive efficacy of a dietary intervention to target biological pathways related to sleep quantity and quality.

8.7 Participant Compensation

Participants will be provided up to $550 stipend to compensate them for their time spent completing study procedures and optional assessments.

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. New staff will conduct study procedures on several adult volunteers with oversight from the PI/trained study staff to ensure consistency of raters and equipment setup.

Training will be based on standardized materials developed for the study, and coordinated by the PI. If necessary, 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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