Cover Page

Official Title: The influence of Fermented Papaya Preparation (FPP) on cerebral energy metabolism, neuroinflammation, and cognition in older adults

NCT Number: NCT02771366

Document Date: 01/24/2017

Protocol

1. Project Title:

The influence of Fermented Papaya Preparation (FPP) on cerebral energy metabolism, neuroinflammation, and cognition in older adults (Short Title: The Efficient Brain Study)

IND 109896

2. Investigators:

Adam Woods, PhD
Stephen Anton, Ph.D.
Christian Leeuwenburgh, Ph.D.
Bhanuprasad Sandesara, M.D.

3. Abstract:

Current research suggests that nutrients in fruits and vegetables produce strong anti-oxidant and anti-proliferative effects (Heber et al., 2004). Most Americans are not consuming the minimum recommendations of fruits and vegetables per day to receive these benefits (Guenther et al., 2006). Whole food-based nutritional products, such as Fermented Papaya Product (FPP), may provide a healthy alternative for individuals. FPP, which is made by biofermentation of *Carica papaya*, has been found to enhance antioxidant protection and to decrease DNA damage in healthy older adults (Marotta et al., 2006). We aim to conduct a double-blind, randomized, placebo-controlled pilot study to determine whether FPP improves cerebral energy metabolism, neuroinflammation, systemic inflammation, cognitive function, fatigue, and health-related quality of life in older adults.

4. Background:

Cerebral energy metabolism declines with advanced aging, and is implicated in age-related cognitive decline, Alzheimer's disease (AD), and other forms of neurodegenerative disease (e.g., Parkinson's disease; Sun et al., 2012; Currais & Maher, 2013). In addition, age-associated increase in systemic and neuroinflammation is associated with higher likelihood for development of Alzheimer's disease and neurodegenerative disease in older adults. Furthermore, decreased cerebral energy metabolism and increased neuroinflammation are both associated with deficits in cognitive function, even in the absence of neurodegenerative disease (Sun et al., 2012; Currais & Maher, 2013; Woods et al., 2011 & 2013). In older adults, decreased cognition is strongly associated with the development of AD, increased rates of hospitalization, loss of functional independence, and increased mortality rate (Shumay-Cook et al., 2002 & 2003; Woods et al., 2011 & 2013). Novel methods for preventing cognitive decline and neurodegenerative diseases in older adults are needed the world's aging population.

There is currently a paucity of easily administered and affordable interventions strategies addressing this need. While there are a variety of methods for reducing systemic inflammation (e.g., Omega-3), there are few intervention methods that not only decrease systemic inflammation but also pass the blood brain barrier (BBB) with a potential for enhancing cerebral energy metabolism. Fermented Papaya Preparation (FPP), which is a natural health food made by biofermentation of *Carica papaya*, has potential for meeting these criteria. Monosaccharides, such as FPP, can pass the blood brain barrier (Dick et al., 1984). Furthermore, delivery of a monosaccharide to the brain may directly supplement available energy stores, up-regulating

energy metabolism in the brain. In addition, FPP is reported to reduce systemic inflammation (Marotta et al., 2007) and may in turn decrease neuroinflammation in the brain. Each of these factors has potential for significantly benefiting cognitive function in older adults with and without neurodegenerative diseases, including mild cognitive impairment (MCI) and Alzheimer's disease. Furthermore, if FPP increases cerebral energy metabolism and down-regulates neuroinflammation, with resulting effects on cognition, dietary supplementation with FPP may have preventative benefits for age-related cognitive conditions, including MCI, AD, Parkinson's disease and other neurodegenerative diseases.

The natural health food supplement FPP can be consumed in a convenient sachet and is currently used in different parts of the world. Additionally, FPP is produced in ISO certified factories under strict quality control procedures, which meet or exceed international standards. In contrast to the vast majority of dietary supplements, FPP has both clinical and preclinical data supporting its safety and efficacy. For example, FPP was recently found to enhance antioxidant protection and decrease DNA damage in healthy older adults (Marotta et al., 2006). Another human study found FPP supplementation had beneficial effects on inflammatory markers as well as expression of heat shock protein 70 (HSP70; Marotta et al., 2007), suggesting FPP may have beneficial effects on age-related proinflammatory disease conditions. Additionally, FPP reduced oxidative-inflammatory damage to the liver in patients with cirrhosis (Marotta et al., 2007). Of note, no side effects or adverse events were reported by participants in any of these studies. In rodents, FPP has been found to modulate oxidative stress levels in the brain (Aruoma et al., 2006) and potentially provide protection against oxidative injuries induced through ischemia-reperfusion (Santiago et al., 1993). If these beneficial effects extend to the brain and cognition, the potential positive consequences for aging populations with and/or at risk for neurodegenerative disease has the potential to reduce the adverse effects of cognitive aging and loss of function later in life. Before extending such benefits to neurodegenerative patient populations, it will be important to demonstrate the benefits of FPP in older adults showing signs of age-related cognitive decline. This discovery would provide the key data needed to test the effects of FPP in a variety of aging populations, including those with neurodegenerative diseases.

Although studies to date suggest FPP has a number of health benefits for an aging population, additional research is needed to further evaluate the potential effects on cerebral energy metabolism, neuroinflammation, and other disease risk factors (e.g., inflammation), as well as cognitive function and health-related quality of life in older adults.

5. Specific Aims:

A growing body of literature suggests that FPP may have beneficial effects on age-related proinflammatory disease conditions (Marotta et al., 2007). However, to our knowledge, no study has tested the effects of FPP on cerebral energy metabolism, neuroinflammation and cognitive function in humans under controlled conditions. Thus, we aim to conduct a double-blind, randomized, placebo-controlled pilot study on FPP in older adults. If FPP is found to have a beneficial impact on cerebral energy metabolism, neuroinflammation and cognitive function in our study population (adults aged 65-100), then this product may be a potential treatment option to combat cognitive aging and age-related decline in brain function. The specific aims and hypotheses of the proposed pilot study are the following:

Aim #1. To determine the effect of FPP on cerebral energy metabolism.

Hypothesis: FPP will increase cerebral energy metabolism markers on alpha ATP, beta ATP, gamma ATP, and phosphocreatine (PCr).

Aim #2. To evaluate the effect of FPP on neuroinflammation, systemic inflammation, cognitive function, fatigue, and health-related quality of life.

Hypothesis: FPP will induce positive changes in neuroinflammation, systemic inflammation, cognitive function, fatigue, and health-related quality of life

6. Research Plan:

The proposed study with Osato Research Institute will evaluate the effects of FPP supplementation (dosage = 9 grams per day divided into three doses) for eight (8) weeks on markers of cerebral energy metabolism (1H-MRS markers of creatine and phosphorus [31P]-MRS markers of mitochondrial/ATP function in the human brain – alpha ATP, beta ATP, gamma ATP phosphocreatine), neuroinflammation (proton magnetic resonance spectroscopy [1H-MRS] markers of myo-inositol and choline, as well as diffusion weighted imaging based measures of extracellular freewater), systemic inflammation (i.e., C-reactive protein, TNF-α, IL-1beta, IL-6 and myeloperoxidase), cognitive performance, and health related quality of life in generally healthy, older adults (age = 65 – 100 years). All participants will have moderate cognitive limitations consistent with cognitive aging, but not neurodegenerative disease (Montreal Cognitive Assessment ≤ 28, but ≥ 23). Study participants will also not have a history of head injury or brain damage and will not have evidence of moderate to severe depression (Center for Epidemiological Studies – Depression [CESD] < 20). All inclusion/exclusion criteria are listed in Table 2.

Potential participants will participate in a brief ~10 minute phone screening that will describe the study and identify people that are not eligible for the study. If participants meet initial inclusion/exclusion criteria on the phone screen, they will be scheduled for an in-person screening visit. Participants will attend five (5) in person visits including one Screening Visit performed individually for each potential study participant, and four Assessment Visits (V1-V4). Participants will be asked to provide a fasting blood sample that will be utilized to evaluate clinical laboratory parameters at the Screening visit and all four assessment visits. The participants will complete both a treatment and a placebo condition; therefore, a cross-over design will be used. After eight (8) weeks on each of these regimens, participants will be asked to complete post-treatment outcome assessments at the University of Florida's Institute on Aging – Clinical and Translational Research Building (IOA – CTRB). Following the first post-treatment test day (V2), participants will complete a six (6) week washout period and will then return to the IOA – CTRB to complete the other study arm. The order the participants receive FPP and placebo will be determined through randomization. Participants (N = 30) will be randomized into 1 of 2 groups for 8 weeks: (1) FPP then placebo (granulated sugar), or (2) placebo then FPP.

Participants will be asked to take their study product (FPP or placebo) three times per day. Participants will consume a 3g sachet 30-40 minutes before a meal (i.e., breakfast, lunch, and dinner). Specifically, they will place the product on their tongues and let it dissolve. A small amount of water may be consumed (if needed) 3-5 minutes after sachet contents are dissolved. Participants will keep empty sachets and return them to the clinic for adherence monitoring and accountability.

Participants will be reminded up to five (5) times per month to take the supplement three times per day, once in the morning (30-40 minutes before breakfast is consumed), once mid-day (30-

40 minutes before lunch is consumed), and once in the evening (30-40 minutes before dinner is consumed).

We will use a FPP containing product and placebo made by the Osato Research Institute (Japan).

Outcome Measures.

Cerebral energy metabolism. Cerebral energy metabolism will be assessed by evaluation of change in creatine, phosphocreatine, α-ATP, β-ATP, and γ-ATP from in vivo 1H and 31P magnetic resonance spectroscopy (MRS) in the brain. We expect that these markers will increase with FPP, but not placebo.

Neuroinflammation. Neuroinflammation will be assessed in the brain using 1H-MRS markers of myo-inositol (MI) and choline (Cho), as well as diffusion-weighted magnetic resonance imaging (MRI) based quantification of extracellular freewater (FW).

a) 1H-MRS: Elevated concentrations of myo-inositol and choline are indicative of increased neuroinflammation in the brain in both aging and medical conditions associated with elevated neuroinflammation (e.g., Alzheimer's Disease, HIV, etc.). We expect that MI and Cho will decrease with FPP, but not placebo.

b) Freewater: Elevation in the concentration of extracellular freewater (e.g., edema) has been associated with increased neuroinflammation in advanced aging, head injury, dementia, and schizophrenia. We expect that extracellular freewater will decrease with FPP, but not placebo.

Cognitive Function. Cognitive function will be assessed using 1) a battery of standardized neurocognitive/neuropsychological tests (Table 1) assessing domains previously shown to decline in older adults, and 2) performance of a working memory task during functional magnetic resonance imaging (fMRI).

a) Neurocognitive Battery: The neurocognitive battery will assess change in cognitive function in domains including: learning, memory, working memory, attention, executive function, language, and speed of processing. Table 1 shows the tests and affiliated domains tested.

b) fMRI Cognitive Assessment: The fMRI task will assess change in cognitive function by evaluating change in the functional response of the brain during performance of a difficult working memory task. This task will evaluate improvement in neural efficiency of processing with FPP versus placebo.

Table 1. Neurocognitive Assessment

MoCA	Dementia Screen
HVLT-R	Verbal Learning/Memory
Stroop Test	Attention/Executive
Trail Making A&B	Executive
Boston Naming Test	Visual Recognition
COWA	Verbal Fluency
NIH Toolbox Subtests:	
• Dimensional Card Sort	Executive Function
• Flanker Task	Attention/Executive
• Picture Sequence	Working Memory
• Auditory Verbal Learning	Episodic Memory
• Picture Vocabulary	Language
• Oral Reading	Language
• Pattern Comparison	Processing
• Oral Digit Symbol	Processing Speed
• List Sorting	Working Memory

Health-Related Quality of Life. The RAND 36-item Health Survey (SF-36) is a validated measure of health-related quality of life (Stewart et al., 1992). The SF-36 measures eight

domains: physical functioning, role limitations due to physical health, role limitations due to emotional problems, vitality (energy/fatigue), emotional well being, bodily pain, social functioning, and general health perceptions. This survey will be completed at each assessment visit.

Systemic Inflammation markers. Specific blood biomarkers will be assessed to evaluate the effect of FPP on inflammation at all four assessment visits. All the proposed biomarkers will be measured in the laboratories of the Department of Aging and Geriatric Research at the University of Florida Institute on Aging by ELISA systems in duplicate. Systemic inflammation will be assessed through evaluation of levels of TNF-α, IL-1beta, IL-6, MPO, UCHL1 and CRP.

Further use of blood samples. With the study participants' consent, any unused blood samples will be stored for future research. Future research will consist of studies on aging processes and can include researchers from institutions other than the University of Florida. No identifiable information will be available to researchers requesting use of these samples. Blood samples will be labeled only with study participant's ID number and be accompanied with demographic and anthropometric information including age, sex, and weight. The study PI will keep records of the samples and their use in future research. If a participant would like to withdraw consent to have their blood samples stored, they can do so at any time by informing the PI in writing.

Oxidative stress. Markers of oxidative stress will be assessed by evaluation of blood anti-oxidant enzymes (SOD, Glutathione Peroxidase). Total antioxidant capacity in the plasma will also be assessed. We will measure DNA and RNA oxidation levels of white blood cells (WBC). These markers will be assessed at all four assessment visits.

Self-Assessed Fatigue. Fatigue will be measured by the fatigue subscale of the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue). This 13-item scale, validated in cancer patients, is a measure of physical and functional consequences of fatigue. The FACIT-Fatigue has good test-retest reliability (r = 0.90) and internal consistency (α = 0.93-0.95; Cella et al., 2002; Yellen et al., 1997). It is scored on a reverse 4-point Likert scale, ranging from 0 to 3, with lower scores indicating more fatigue. Fatigue will also be measured with the Brief Fatigue Symptom Inventory (FSI; Hann et al., 1998) and the vitality subscale of the SF-36 (Ware & Sherbourne, 1992). These questionnaires will be completed at each assessment visit.

Anthropometrics. Participants' height, weight, and waist circumference will be taken at the Screening Visit and each assessment visit.

Safety Measures.

Vital Signs. As a safety check to ensure no adverse biological changes have occurred, we will assess blood pressure and radial pulse at each visit.

Blood Chemistry Panel. Under aseptic conditions, using standard venipuncture techniques, blood will be drawn and analyzed for a complete blood count (white blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution (RDW), platelet count, mean platelet volume (MPV) and differential (absolute and percent - neutrophils, lymphocytes, monocytes, eosinophils, and basophils).

Comprehensive Metabolic Panel. A comprehensive metabolic panel including albumin, albumin/globulin ratio (calculated), alkaline phosphatase, ALT, AST, BUN/creatinine ratio

(calculated), calcium, carbon dioxide, chloride, creatinine with GFR estimated, globulin (calculated), glucose, potassium, sodium, total bilirubin, total protein, and urea nitrogen will also be assessed.

These tests will be performed by Quest Diagnostics (Tampa, FL; CLIA regulated laboratory)

Table 2. Inclusion and exclusion criteria

INCLUSION	EXCLUSION
• Men and women aged 65-100 years; • Willing and able to participate in all aspects of the study; • Not confined to a wheelchair; • Evidence of cognitive aging based on Montreal Cognitive Assessment score less than or equal to 28, but greater than or equal to 23; • Able to swallow study product as directed.	• Failure to give consent; • Active treatment for cancer (< 3 years); • Stroke (< 6 mo); • Serious heart condition, peripheral vascular disease, coronary artery disease (myocardial infarction<6 mo), Class III, IV Congestive Heart Failure; • Dementia (e.g., Alzheimer's disease) • Severe anemia (Hgb < 8.0 g/dL); • Any blood or bleeding disorders; • Liver or renal disease; • Diabetes; • Severe osteoarthritis; • Anticoagulant therapy (aspirin use is permitted); • Parkinson's disease; • Severe psychiatric disease or psychological disorder (e.g., severe depression, bi-polar disorder, schizophrenia) or current use of antipsychotics; • Current use of anabolic medications (e.g., growth hormone or testosterone) or anticholinesterase inhibitor (i.e., Aricept); • High amounts of physical activity (i.e., running, bicycling, etc.) ≥ 120 min/week; • Excessive alcohol use (>2 drinks per day); • Use of tobacco products; • Resting heart rate > 120 bpm; • History of significant head injury leading to cognitive impairments; • Visual or hearing impairments that would interfere with testing; • Allergies to papaya or foods with similar compounds (i.e., banana, avocado, kiwi, chestnuts, hazel nuts) • Allergy to latex; • Participating in another clinical trial or has received an investigational product within 30 days prior to screening/enrollment; • Center for Epidemiological Studies – Depression Scale (CES-D) Score > 20.
Temporary Exclusion • Current consumption of any dietary products containing resveratrol, quercetin, or *P. cuspidatum*, grape seed extract, or ginko biloba. Participants may be eligible after a one-month washout period prior to Visit 1. • Blood pressure with Systolic > 160mmHg and Diastolic > 90mmHg. Participants will be referred to their physician to start (or adjust) hypertension therapy and may be eligible following a repeat measure of Systolic < 160mmHg and Diastolic < 90mmHg blood pressure one month later.	

Table 3. Timeline for tests, evaluations, and protocols to be conducted and performed.

Activities/Visit Type	Phone Screening	Screening Visit	V1 (W0)	V2 (W8)	V3 (W14)	V4 (W22)	Phone Call (W24)
Phone Screening Questionnaire	■						
Informed Consent		■					
Inclusion/Exclusion Criteria		■					
Medical History		■					
Center for Epidemiological Studies – Depression (CESD)		■					
Montreal Cognitive Assessment (MoCA)		■					
Height		■					
Weight & Waist Circumference		■	■	■	■	■	
Blood Pressure & Pulse		■	■	■	■	■	
Blood Chemistry Panel and Comprehensive Metabolic Panel		■	■	■	■	■	
Biomarkers of Inflammation (TNF-α, IL-6, IL1-beta, UCHL1, MPO)			■	■	■	■	
Biomarker of Inflammation (CRP)		■	■	■	■	■	
Biomarkers of Oxidative Stress			■	■	■	■	
Brain Magnetic Resonance Imaging/Magnetic Resonance Spectroscopy (cerebral energy metabolism, neuroinflammation, and cognitive brain function markers)			■	■	■	■	
Cognitive Testing			■	■	■	■	
Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT Fatigue)			■	■	■	■	
Brief Fatigue Symptom Inventory			■	■	■	■	
RAND 36-Item Health Survey (SF-36)			■	■	■	■	
Dispense Study Product (FPP or Placebo)			■		■		
Phone Call Follow-up							■

Notes: V1 (W0) = Baseline Visit; V2 (W8) = Visit 2, Week 8; V3 (W14) = Visit 3, Week 14; V4 (W22) = Visit 4, Week 22; Phone Call (W24) = Follow-up, Week 24.
Compliance checks and adverse event queries will occur by phone on a bi-weekly basis throughout the study.

7. Data Analysis:

Randomization. Osato Research Institute (Japan) will provide the blinded medication to the investigators. The two possible orderings (PA, AP, with P=Placebo, A=FPP dose) will be balanced throughout the study.

Study Design. (15 completers per ordering, 30 total). A cross-over (two-sample method) will be used, because it is more efficient and provides the value of approximately 3.6 participants as compared to one participant in a parallel study. The two-sample method is valid even with carryover and period effects, as it estimates the arithmetic mean of the period 1 and period 2 effects, a legitimate endpoint. Also, the method does not rely on any assumptions, as it leans on large sample theory, and except for anticipated outlier situations, the central limit theory assures rapid convergence of the *t*-statistic.

Power Analysis. Currently no data exists for in vivo change in brain markers of energy metabolism of neuroinflammation with FPP. Pilot data for systemic change in inflammation was used to obtain estimates of paired difference standard deviations for the power analysis.

Detectable differences with 80% power at $p = 0.0167$ (two-sided)

Variable	Paired Diff SD (σ)	Detectable Difference (0.62σ)
MPO	19	12
IL6	8	5
TNF-α	15	9

Study Outcomes. The hypotheses comparing the treatments will be tested by the two-sample method for cross-over studies per Shuster (2007). Intent-to-treat analysis will be used, and analysis of variance (ANOVA) will be performed.

For the primary hypothesis, markers of cerebral energy metabolism will be assessed using phosphorus magnetic resonance spectroscopy markers of ATP/mitochondrial function in brain tissue (phosphocreatine, α-ATP, β-ATP, and γ-ATP). The 3 ATP peaks will be averaged to provide an overall marker of ATP in brain tissue. Phosphocreatine will be analyzed separately. Each pairwise contrast will be made stratifying for matched versus unmatched. Because two cerebral energy metabolism markers were selected, Bonferroni corrections will be used ($p < 0.025$). This strict requirement for significance ensures that the probability of falsely declaring any pair of treatments as significantly different when there is no difference in the target population is less than 5%. The secondary outcome variables will be analyzed in a similar manner, with any significant findings needing independent verification in a future study. If there are no unpaired data for a contrast, the method will be equivalent to that given in Shuster (2007).

Confidentiality of Participants and Data.

a) Participant identifiers**.** The screening form, contact information form, consent form and the coding list (i.e., list of participant names and participant identification number) are the only study documents that will contain the participants' names and other personal data. All other study measures will be coded with a participant identification number that contains no identifying information that would connect to the participant. Screening form, contact information form, consent form, and coding list will be stored in a separate locked filing cabinet from all other study forms and materials.
b) Data management & storage. The data will be managed via the REDCap data management software that produces SAS-ready datasets. The Clinical and Translational Science Institute (CTSI) Data Services Lab offers REDCap software as well as tutorials for using the software

so that researchers will be able to easily set up secure case report forms and a web-based database with on-site and off-site back-up. De-identified neuroimaging data will be uploaded to a secure file system for processing using neuroimaging software on the UF HiperGator Supercomputer.

8. Possible Discomforts and Risks:

Risks Associated with consumption of Fermented Papaya Preparation. There are no known risks associated with the consumption of FPP. However, the primary ingredient in FPP is papaya, which is associated with some side effects. People with an allergy to papaya should not take FPP, because it may cause an allergic reaction. Large amounts of papaya can also lead to miscarriage in women and yellowing of palms and soles of feet.

Risks Associated with Placebo. There are no known risks associated with placebo.

Risks Associated with Blood Draw. There are risks of infection anytime the skin is punctured. Therefore, there is a minimal risk of infection due to the blood draw. To lower these risks, only certified phlebotomists using sterilized materials will perform the blood draw.

Risks Associated with Cognitive Function Tests. Participants may experience fatigue and feelings of frustration while completing the cognitive function tests. To minimize risk, participants will be able to skip any questions or tests that they do not wish to answer.

Risk associated with Brain Magnetic Resonance Imaging/Magnetic Resonance Spectroscopy. During the MRI/MRS procedure, participants will be able to talk with the MRI/MRS staff through a radio intercom speaker system, and, in the event of an emergency, they can tell them to stop the scan immediately. Participants will be asked frequently during the preparation and scanning period whether they are experiencing any discomfort, and corrections will be made as necessary. Participants may experience discomfort during the scanning process due to limited space inside the bore of the magnet. Subjects will be closely monitored and repeatedly checked by the investigators to ensure comfort. Participants may also become uncomfortable from lying still for an extended period of time, or if they do not like to be in close spaces (have "claustrophobia"). Padding with blankets can be used to prevent discomfort while lying in the magnet. The MRI/MRS scanner produces a loud “hammering” noise, which has been reported to produce temporary hearing loss in a very small number of people. Participants will be provided with earplugs to wear in the scanner, and also headphones to reduce this risk and possible discomfort.

Risks Associated with Questionnaire Administration and Collecting Personal Health Information. A number of methods are employed to maintain confidentiality of participants; however, there is a small risk that personal health information could be revealed inappropriately or accidentally. First, questionnaire data are collected in secure spaces where the interview cannot be overheard. Secondly, only study investigators and key research staff (i.e. data manager and study programmers) have access to the study database. Third, participants are assigned a unique study identifier. Individual names will ultimately be removed from the study database and only the unique study identifier is used to distinguish participants in the database. Fourth, collected data are maintained in locked computer files and file cabinets, to which only study investigators have access. Collected data will be used only for research purposes. Access to research data is based on a “need to know” and "minimum necessary" standard. Published data will not contain any individual identifiers. Finally, all research staff members have to receive HIPAA training and sign a confidentiality certificate every year.

9. Possible Benefits:

The potential benefits to participants may include enhanced brain energy metabolism, reduced neuroinflammation and beneficial effects on age-related cognitive function. Thus, the potential health benefits associated with participation appear to outweigh the minimal risk associated with this study.

10. Compensation:

Participants will be compensated for their study visits as follows:

- Phone-Screening: No compensation at this visit
- Screening Visit: No compensation at this visit
- Visit 1: $50
- Visit 2: $50
- Visit 3: $50
- Visit 4: $50

11. Conflict of Interest:

There is no conflict of interest.

12. Data and Safety Monitoring Plan (DSMP):

12.1 Terminology:

Sponsor-Investigator means an individual who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. The term does not include any person other than an individual. Sponsor-investigators, as defined in 21 CFR 312.3(b), are required to comply with both the sponsor and the investigator responsibilities under 21 CFR part 312. With respect to safety reporting under 21 CFR 312.32, this includes examining data from reports in the scientific literature and reports from foreign commercial marketing experience.

Adverse event (AE) is any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality.

Any worsening of a pre-existing condition or illness is considered an AE. An elective surgery/procedure scheduled to occur during a study will not be considered an AE if the surgery/procedure is being performed for a pre-existing condition However, if the preexisting condition deteriorates unexpectedly during the trial (e.g., surgery has to be performed earlier than planned), then the deterioration of the condition for which the elective surgery/procedure is being done will be considered an AE.

Adverse events will be categorized into three different levels of severity:

- Mild - Transient and easily tolerated.

- Moderate - Results in a modification or interruption of the subject's usual activities or care and may require discontinuation of the study product.

- Severe - Results in considerable interference with the subject's usual activities or care and may require discontinuation of study product.

A study physician will assess the relationship of the AE to the study product using the following definitions:

- Probable: The AE has a strong temporal relationship to the study product or recurs on re-challenge, and another etiology is unlikely or significantly less likely.

- Possible: The AE has a strong temporal relationship to the study product and an alternative etiology is equally or less likely compared to the potential relationship to study product. An alternative etiology must be provided for the AE.

- Probably Not: The AE has little or no temporal relationship to the study product and/or a more likely alternative etiology exists. An alternative etiology must be provided for the AE.

- Not Related: The AE is due to an underlying or concurrent illness and is not related to the study product. An alternative etiology must be provided for the AE.

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the study product (i.e., fermented papaya preparation; FPP) **caused** the adverse event. The study physician and the Principal Investigator will evaluate the available information and decide whether there is a reasonable possibility that the study drug caused the adverse event and, therefore, that the event meets the definition of a suspected adverse reaction. Evidence to suggest a causal relationship between the study drug and adverse event includes: (1) Individual occurrence - a single occurrence of a serious adverse event that is uncommon and is known to be strongly associated with exposure to the study drug ; (2) One or more occurrences - a single occurrence or small number of occurrences of a serious adverse event that is uncommon in the study population, but not commonly associated with drug exposure may also be informative; (3) Aggregate Analysis of Specific Events - events which occur more frequently in the active treatment group than in a concurrent control group; or, if a control group is not available, estimate whether the rate is greater than in a control population, general population, or populations similar to the drug population.
For events that require more than one occurrence to assess causality and events evaluated in the aggregate, the time frame starts when the investigator-sponsor determines that the events qualify for reporting. The investigator-sponsor must report in an IND safety report any suspected adverse reaction to study treatment (i.e., including active comparators) that is both serious and unexpected:

> Unexpected suspected adverse reaction is an adverse event or adverse reaction that is not listed at the specificity or severity that has been previously observed; or, if an investigator brochure is not required or available and is not consistent with the risk information described in the general investigational plan.
>
> Serious suspected adverse reaction is an adverse event or adverse reaction that results in any of the following outcomes:

a) A life-threatening adverse event or death;
b) Inpatient hospitalization or prolongation of existing hospitalization;
c) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
d) A congenital anomaly/birth defect;
e) Medical events that require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A life-threatening suspected adverse reaction is an adverse event or adverse reaction that places the participant at immediate risk of death.

12.2 Adverse Events Collection Period:

Adverse events will be collected from the time the informed consent form is signed until the participant exits the study.

12.3 Review and Reporting of Adverse Events:

For the duration of the study, the participants will be directed to report any AEs to the study team. All AEs, regardless of assessment of causal relationship to study drug, must be reported to the investigator or investigators' designee. The study team will report AEs (i.e., any unfavorable and unintended sign (e.g., an abnormal laboratory finding deemed to be clinically significant), symptom, or disease) that are both unexpected and judged to be related to the study product to Dr. Anton within 24 hours following discovery by the study team. All other AEs should be reported to Dr. Anton within seven business days of discovery.

- A study physician must be responsible for all trial-related medical decisions and the review of AEs throughout the study. The physician will assess AEs and will assure that they are recorded in detail. Adverse events, whether in response to a query, observed by study team, or reported spontaneously, will be reported on the appropriate CRF and/or source document.
- Study progress and safety (e.g., cumulative table of adverse events and serious adverse events) will be reviewed monthly (and more frequently if needed) by the Principal Investigator.
- Progress reports, including adverse events will be provided to an Independent Monitoring Committee for bi-annual reviews. The Data and Safety Monitoring Committee (DSMB) for this study will consist of the following individuals: (1) Ken Cusi, M.D., Professor and Chief, Adult Division of Endocrinology, Diabetes & Metabolism in the Department of Medicine, a physician who has been involved in the conduct of clinical research for many years (2) Laurence Solberg, M.D., Professor and Chief of the Division of Geriatric Medicine, a physician who has been involved in the conduct of clinical research for many years, and (3) Thomas Buford, Ph.D., an Assistant Professor who has conducted a number of clinical trials.

An independent internal Data and Safety Monitoring Committee will conduct at least one interim review of safety data from the study. The need for subsequent reviews will be determined based upon the results of the first review.

- The time frame for submitting an IND safety report to FDA is no later than 15 calendar days after the investigator-sponsor determines that the suspected adverse reaction or other information qualifies for reporting.

- Any unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA no later than 7 calendar days after the initial receipt of the information.

Dr. Anton is responsible for the submission of AE reporting forms and any other supporting documentation to the IRB Administrative Office within 5 working days of discovery. If an event, incident, experience, or outcome is life-threatening or fatal, the IRB must be notified by phone within 24 hours.

If a suspected adverse reaction occurs that is both (1) serious, and (2) unexpected, the clinical product codes may be broken to the appropriate study team member if deemed necessary (i.e., the medical care of the participant would be altered by knowing product identity). Un-blinding can be accomplished by contacting Sam Wu, Ph.D., a Biostatistician who will contact Dr Anton. The study physician will direct the process in such a way as to not un-blind the study team members.

12.4 Review of the Study Overall:

Progress reports, including participant recruitment and retention/attrition, will be provided to the DSMB for bi-annual reviews.

- An annual report will be compiled and will include a list and summary of adverse events. In addition, the annual report will address:
    1) Whether adverse events are consistent with pre-study assumptions;
    2) Reason for dropouts from the study;
    3) Whether all participants met the study enrollment criteria;
    4) Whether continuation of the study is justified on the bases that additional data are needed to accomplish the stated aims of the study; and
    5) Conditions whereby the study might be terminated prematurely.
- The annual report will be signed by the chair of the Independent Monitoring Committee and will be forwarded to the IRB. The IRB and other applicable recipients will review progress of this study on an annual basis.

This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that significantly impact the risk-benefit ratio, (2) study recruitment or retention becomes futile, (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) if the DSMB indicates stopping the trial is necessary due to adverse event frequency or severity.

References

Aruoma, O.I., Colognato, R., Fontana, I., Gartlon, J., Migliore, L., Koike, K., Coecke, S., Lamy, E., Mersch-Sundermann, V., Laurenza, I., Benzi, L., Yoshino, F., Kobayashi, K., Lee, M.C. Molecular effects of fermented papaya preparation on oxidative damage, MAP Kinase activation and modulation of the benzo[a]pyrene mediated genotoxicity. Biofactors. 2006;26(2):147-59.

Cella, D., Lai, J.S., Chang, C.H., Peterman, A., Slavin, M. Fatigue in cancer patients compared with fatigue in the general United States population. Cancer. 2002;94(2):528-38.

Currais, Antonio; Maher, Pamela (2013) Functional consequences of age-dependent changes in glutathione status in the brain. *Antioxidants & redox signaling* vol. 19 (8) p. 813-22

Dick, A P; Harik, S I; Klip, A; Walker, D M (1984). Identification and characterization of the glucose transporter of the blood-brain barrier by cytochalasin B binding and immunological reactivity. *Proceedings of the National Academy of Sciences of the United States of America* vol. 81 (22) p. 7233-7

Dietary Guidelines for Americans 2005. Center for Nutrition Policy and Promotion, U.S. Department of Agriculture.

Guenther, P.M., Dodd, K.W., Reedy, J., Krebs-Smith, S.M. Most Americans eat much less than recommended amounts of fruits and vegetables. J Am Diet Assoc. 2006;106:1371-1379.

Hann, D.M., Jacobsen, P.B., Azzarello, L.M., Martin, S.C., Curran, S.L., Fields, K.K., Greenberg, H., Lyman, G. Measurement of fatigue in cancer patients: development and validation of the Fatigue Symptom Inventory. Qual Life Res. 1998;7(4):301-10.

Heber, D. Phytochemicals beyond antioxidation. J Nutr. 2004;134(11):3175S-3176S.

Hofer, T., Seo, A.Y., Prudencio, M., Leeuwenburgh, C. A method to determine RNA and DNA oxidation simultaneously by HPLC-ECD: greater RNA than DNA oxidation in rat liver after doxorubicin administration. Biol Chem 2006;387:103–111.

Ji, L.L., Peterson, D.M. Aging, exercise, and phytochemicals: promises and pitfalls. Ann N Y Acad Sci. 2004;1019:453-61.

Marotta, F., Koike, K., Lorenzetti, A., Naito, Y., Fayet, F., Shimizu, H., Marandola, P. Nutraceutical strategy in aging: targeting heat shock protein and inflammatory profile through understanding interleukin-6 polymorphism. Ann N Y Acad Sci. 2007;1119:196-202.

Marotta, F., Weksler, M., Naito, Y., Yoshida, C., Yoshioka, M., Marandola, P. Nutraceutical supplementation: effect of a fermented papaya preparation on redox status and DNA damage in healthy elderly individuals and relationship with GSTM1 genotype: a randomized, placebo-controlled, cross-over study. Ann N Y Acad Sci. 2006;1067:400-7.

Marotta, F., Yoshida, C., Barreto, R., Naito Y, Packer L. Oxidative-inflammatory damage in cirrhosis: effect of vitamin E and a fermented papaya preparation. J Gastroenterol Hepatol. 2007;22(5):697-703.

Most, M. M. Estimated phytochemical content of the dietary approaches to stop hypertension (DASH) diet is higher than in the Control Study Diet. J Am Diet Assoc. 2004;104(11):1725-1727.

Santiago, L.A., Osato, J.A., Ogawa, N., Mori, A. Antioxidant protection of bio-normalizer in cerebral ischaemia-reperfusion injury in the gerbil. Neuroreport. 1993;4(8):1031-4.

Shumway-Cook A, Patla A, Stewart A, Ferrucci L, Ciol MA, Guralnik JM. Environmental components of mobility disability in community-living older persons. *JAmGeriatrSoc*. 2003;51(3):393–398.

Shumway-Cook A, Patla AE, Stewart A, Ferrucci L, Ciol MA, Guralnik JM. Environmental demands associated with community mobility in older adults with and without mobility disabilities. *PhysTher*. 2002;82(7):670–681.

Shuster, J.J. "*Design of Experiments" in Methods in Molecular Biology: Elementary Biostatistics.* WT Ambrosius (Ed.) Humana Press: Totawa, NJ. 2007; 235-260.

Stein, K.D., Martin, S.C., Hann, D.M., Jacobsen, P.B. A multidimensional measure of fatigue for use with cancer patients. Cancer Pract. 1998;6(3):143-52.

Sun, Jiya; Feng, Xuemei; Liang, Dapeng; Duan, Yong; Lei, Hongxing (2012) Down-regulation of energy metabolism in Alzheimer's disease is a protective response of neurons to the microenvironment. *Journal of Alzheimer's disease : JAD* vol. 28 (2) p. 389-402

Ware, J.E., Jr., Sherbourne, C.D. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care. 1992;30(6):473-83.

Woods AJ, Mark VW, Pitts AC, Mennemeier M. Pervasive cognitive impairment in acute rehabilitation inpatients without brain injury. *PM R*. 2011;3(5):426–32; quiz 432. doi:10.1016/j.pmrj.2011.02.018.

Woods AJ, Cohen RA, Pahor M. COMMENTARY COGNITIVE FRAILTY : FRONTIERS AND CHALLENGES. 2013;17(9):741–743.

Yellen, S.B., Cella, D.F., Webster, K., Blendowski, C., Kaplan, E. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. J Pain Symptom Manage. 1997;13(2):63-74.