(BRAVO) Study: A Double Blind, Placebo-Controlled Trial of Omega-3 Fatty Acid

Supplementation among Adult Individuals at Risk of Suicide

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Abstract max 250 words (239)

Suicide remains the 10th leading cause of death among adults in the United States (U.S.). Annually, approximately 30 per 100,000 U.S. military Veterans commit suicide, compared to 14 per 100,000 U.S. civilians. Symptoms associated with suicidality can be treatment resistant and proven-effective pharmaceuticals may have adverse side-effects. Thus, a critical need remains to identify effective approaches for building psychological resiliency in at-risk individuals. Omega-3 highly unsaturated fatty acids (n-3 HUFAs) are essential nutrients, which must be consumed in the diet. N-3 HUFAs have been demonstrated to reduce symptoms of depression, anxiety, and impulsivity –which are associated with suicide risk. Here we present the design and methods for the Better Resiliency among Veterans and non-Veterans with Omega-3's (BRAVO) Study, which is a double blind, randomized, controlled trial among individuals at risk of suicide of an n-3 HUFA versus placebo supplementation in the form of all natural fruit juice beverages. The BRAVO study seeks to determine if dietary supplementation with n-3 HUFAs reduces the risk for serious suicidal behaviors, suicidal thinking, negative emotions, and symptoms associated with suicide risk. Sub-analyses will evaluate efficacy in reducing depressive symptoms, alcohol, and nicotine use. A sub-study utilizes functional magnetic resonance imaging (fMRI) to evaluate the neuropsychological and neurophysiological effects of n-3 HUFAs. We also outline selection of appropriate proxy outcome measures for detecting response to treatment and collection of ancillary data, such as diet and substance use, that are critical for interpretation of results.

Abbreviations: AMPM - Automated Multiple Pass Method; APP – As Per Protocol; BART - Balloon Analog Risk Task; BDI - Beck Depression Inventory; BHS - Beck Hopeless Scale; BRAVO - Better Resiliency among Veterans and non-Veterans with Omega-3's; BSSI - Beck Scale for Suicidal Ideation; C-SSRS - Columbia-Suicide Severity Rating Scale; DHA - docosahexaenoic acid; DHQ - Diet History Questionnaire; EAT-26 - Eating Attitudes Test-26; EPA - eicosapentaenoic acid; FFQ - food frequency questionnaire; IAT-S - Implicit Associations Test-Suicide; IPA - International Physical Activity Questionnaire; ITT - Intention to Treat; MINI - Mini International Neuropsychiatric Interview; n-3 HUFAs - Omega-3 highly unsaturated fatty acids; NESSR - New Episode of Significant Suicide Risk; PROMIS-29 - Patient Reported Outcomes Measurement Information System-29; REDCap - Research Electronic Data Capture

Keywords: suicide, omega-3 fatty acids, adults

1. Introduction max 500 word (475)

Suicide is the tenth leading cause of death in the United States (U.S.), occurring at a rate of 12.6 per 100,000 standard population in 2013 [1]. Previous suicide attempts, history of psychiatric illness, and a cluster of other traits or diagnoses contribute to suicide risk including Posttraumatic Stress Disorder (PTSD), affective disorders, borderline and aggressive/impulsive traits, hopelessness or pessimism, and alcohol and substance use disorders [2, 3]. Deficits in cognition and behavioral inhibition, often associated with psychiatric disorders, also contribute to suicide behavior [4]. Approximately 50% of those who complete suicide had at least one previous suicide attempt [5], and more than 90% of completed suicides occur in persons with diagnosable psychiatric illness [6]. Mood disorders such as major depressive and bipolar disorder are associated with roughly 60% of suicides [7]. In one large Veteran population study, those with PTSD were greater than 4 times more likely to endorse suicidal ideation than those without PTSD, and those with a PTSD comorbid condition (e.g., depression) were at 5.7 times the risk [8].

Omega-3 fatty acids are essential for neural function, as they affect emotional state, cognitive function, and mental health. Evidence exists at many levels that omega-3 highly unsaturated fatty acids (n-3 HUFAs) may have a salutary effect on mental health. N-3 HUFAs, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are concentrated in neural tissues and are essential for normative neurodevelopment, neurotransmitter (including dopaminergic, serotonergic, and endocannabinoid) function, and hypothalamic-pituitary-adrenal axis functioning [9]. A series of studies [10, 11] demonstrated that low DHA plasma levels strongly predicted future suicide risk and were associated with hyperfunction of the limbic forebrain and hypofunction of the parietal and temporal cortex. A meta-analysis of 10 randomized control trials (RCTs) in children found that n-3 HUFAs effectively reduced attention deficit

hyperactivity disorder (ADHD) symptoms with low heterogeneity [12], and several meta-analyses have reported efficacy in treating depression with n-3 HUFAs with effect sizes comparable to antidepressants [13].

The primary aim of the Better Resiliency Among Veterans and non-Veterans with n-3 fatty acids (or BRAVO) study is to evaluate the efficacy of an n-3 HUFA dietary supplement among individuals at risk for suicide, who are continuing usual care. Secondary outcomes include reduction in depression and PTSD symptoms, and alcohol and substance misuse. The mechanism and timing of action of n-3 HUFAs on specific psychiatric symptoms associated with suicide risk will also be explored. A functional magnetic resonance imaging (fMRI) sub-study is embedded to determine if dietary supplementation with n-3 fatty acids increases activation of frontal brain regions associated with suicide attempt recall and risky decision making. If n-3 HUFAs are found to improve outcomes in suicidal individuals, the public health significance would be substantial. A description of the BRAVO trial is presented, including the development of a methodology that ensures safety of participants throughout the recruitment, screening, enrollment, and follow-up phases of this study. This paper describes the design and methodology of the BRAVO study and addresses implementation issues and challenges relevant to the safety of the target population being recruited.

2. Methods/Design

2.1 Study overview

The BRAVO study, a randomized, double blind, placebo controlled dietary supplementation trial, is enrolling 300 people with a recent history of suicide risk, over a period of 30 months. The study is conducted as an investigator-sponsored research study under the Division of Psychiatry Products Office of Drug Evaluation, Food and Drug Administration

Investigational New Drug investigation No. 118922. The study was approved by the Medical University of South Carolina (MUSC) Institutional Review Board and is registered as ClinicalTrials.gov protocol NCT01901887. The primary outcome is risk of suicide and suicidal symptoms as measured by New Episodes of Significant Suicide Risk (NESSR). The secondary outcomes include changes in cognitive processes associated with suicide risk, depressive symptoms, alcohol and nicotine use, PTSD symptom severity, and other neuropsychological and neurophysiological measures. Outcomes are assessed over the 6-month study: 1) screening/enrollment visit; 2) 1 month visit; 3) 3 month visit; 4) 5 month visit and 5) final assessment visit at 6 months. At 2 months, participants attend a “check-in” visit at which only adverse events and changes in medications are documented. Interested participants who are eligible are invited to participate in an fMRI study examining neurophysiological effects of n-3 HUFA supplementation as related to impulsivity and risk-taking.

2.2 Location

BRAVO is a single-site study, located at a large academic university and teaching hospital in the Southeastern United States that has an affiliated relationship with and is situated adjacent to a Veterans Administration Medical Center (VAMC).

2.3 Interventions

2.3.1 Intervention group

The intervention group receives a commercially available fruit juice smoothie containing 1,100 mg of n-3 HUFAs per 200 ml box, providing approximately 550 mg EPA plus 550 mg DHA/box taken three times a day for a total of 3,300 mg of n-3 HUFAs/day with 1,650 mg EPA plus 1,650 mg DHA per day (SMARTFISH® Nutrifriend 1100, Gaustadalléen 21, N-0349 Oslo, Norway). The dosage of n-3 HUFA was based on safety and efficacy recommendations to support

cardiovascular and mental health [14]. Blood samples are obtained at each study visit for serum fatty acid analyses to measure n-3 HUFA adherence.

2.3.2 Control group

The control group receives an identical fruit juice beverage containing 1,100 mg of macadamia nut oil per 200 ml juice box taken three times a day in place of n-3 HUFAs for a total of 3,300 mg/day. (20% 16:1 n-7, Smartfish®, Gaustadalléen 21, N-0349 Oslo, Norway). Macadamia nut oil provides a unique fatty acid blood marker to assess adherence in the placebo group. Blood samples are obtained at each study visit for macadamia nut oil.

Both groups are asked to consume one 200 ml fruit juice beverage three times per day for 6 months, preferably with each meal of the day (e.g. breakfast, lunch, and dinner). Juice containers display the BRAVO study logo, telephone contact information for the Suicide Crisis hot line, “for participant consumption only,” expiration date, batch number, and a number code on each box. Throughout the study, participants are queried about smoothie intake and presence of any adverse events. “How to use the Juice” tip sheets and a consumption tally sheet are included with the juice delivery. Fruit juice smoothies are available to participants in two flavors of their choice, fruit punch and peach, and are delivered monthly to their place of residence.

2.4 Participants and recruitment method

2.4.1 Participant eligibility

Table 1 displays inclusion and exclusion criteria for the target population which includes U.S. military Veterans and non-Veterans, ages 18-90 years at risk for suicidal behavior. The

upper age limit of 90 was selected to include World War II Veterans who still utilize Veteran Affairs health services.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
1) United States (U.S.) military Veterans and non-Veterans ages 18-90 years	1) any unstable medical conditions requiring immediate attention,
2) a suicide attempt in past 6 months, or a suicide attempt during the adult lifetime AND current diagnosis of an episode of depression as diagnosed on the Mini International Neuropsychiatric Interview (MINI), <u>or</u> an inpatient admission with suicide risk in the last 6 months, <u>or</u> an inpatient admission with suicide risk during the adult lifetime AND current diagnosis of an episode of depression as diagnosed on the Mini International Neuropsychiatric Interview (MINI), or positive suicidal behavior or ideation based on a psychiatrist-administered Columbia-Suicide Severity Rating Scale (C-SSRS) and psychiatrist review of participant medical history and physical, <u>or</u> a score of 0 or greater on the Implicit Associations Test-Suicide (IAT-S), <u>or</u> score of > or = 9 on the Beck Hopelessness Scale (BHS) and psychiatrist review of participant medical history and physical	2) medical conditions that preclude potential study participation for the duration of the study or any life threatening medical condition(s) or life expectancy of less than 6 months 3) history of non-febrile seizures 4) unstable or rapidly progressive neurological disease 5) diabetes mellitus and/or taking hypoglycemic agents 6) regularly taking anticoagulants (including high-dose aspirin, warfarin, Coumadin), taking the medication isotretinoin (Accutane); 7) allergy, hypersensitivity, or intolerance to fish oils or omega-3 fatty acids, any nuts, fruits, fish, rosemary, or milk protein; 8) pregnancy, lactation, or intention to become pregnant within the next 12 months;
3) currently under the care of a mental healthcare provider. A release of information from his/her mental healthcare provider is required.	9) acute intoxication or withdrawal from alcohol or other substances
4) capacity to provide written, informed consent	10) Body Mass Index (BMI) <18 kg/m ² or > 45 kg/m ² without medical co-morbidities associated with obesity and extreme obesity;
5) having a stable residence with adequate space to store juice, and ability and willingness to consume 3 fruit juice beverages per day for 6 months	11) evidence of disordered eating or risk of malnutrition based on the Eating Attitude Test-26 (EAT-26); 12) history of significant psychiatric instability 13) any unstable medical conditions requiring immediate

For the *f*MRI sub-study, 40 individuals (20 randomized to the omega-3 intervention, 20 randomized to placebo) receive an *f*MRI while completing the Balloon Analog Risk Task (BART)[15]. Interested, eligible participants sign a separate *f*MRI study consent. Each participant of the *f*MRI study completes two identical scanning sessions (baseline prior to the intervention initiation and 6-months post treatment). Exclusion criteria include inability or unwillingness to participate in an *f*MRI scan, the presence of metallic objects in the body that would interfere with the scan, pronounced claustrophobia, and body weight >300 pounds.

2.4.2 Recruitment

Recruitment efforts are geared toward attracting individuals from the surrounding community who are experiencing serious suicidal ideation. The research team developed recruitment material that include newspaper and internet ads, flyers, brochures, etc. The recruitment advertisements, study flyers, and brochures are posted in locations frequented by likely participants such as the local newspaper, Craig's List, MUSC Institute of Psychiatry inpatient and outpatient waiting rooms, units and clinics, the Veteran Health Administration mental health clinic waiting rooms, Veterans of Foreign War (VFW) facilities, military commissaries, local colleges, vocational rehabilitation centers, Veteran housing, and public transportation. Research coordinators meet with all of the regional support community based organizations and attend events in the community to talk about the study with potential participants, families, and providers. In addition to advertising materials in and around the

greater Charleston area, many participants are referred from the medical university's inpatient and outpatient psychiatric clinics. Mental health clinicians in the community and research investigators conducting other psychiatric studies are approached and informed about the study and eligibility criteria. Individuals who have been excluded from other studies because of suicidal ideation are welcome to participate.

2.4.3 BRAVO Call Center

A BRAVO Call Center was established with study research staff specifically hired and trained to receive all referrals, including self-referrals as well as family, community, and clinician referrals. The Call Center answers study questions, provides information, schedules appointments, and makes other referrals outside of the BRAVO study. When dealing with such a vulnerable population, timely communication is an essential safety component. This Center is responsible for coordinating communication between research staff, study psychiatrists, study clinicians, and community providers. The Call Center staff received special training from the American Red Cross Suicide Hotline Center to handle any crisis calls. In addition, an experienced study psychiatrist, PhD level licensed neuropsychologist, or licensed PhD-level psychiatric nurse are on site in the study clinical suite at all times that the Call Center is open (40 hours per week) and when any patients are being seen.

Potential participants responding to any of the recruitment methods, call or are contacted by the BRAVO Call Center, are prescreened, and are scheduled for a screening/enrollment visit. The prescreening telephone interview determines preliminary study eligibility. Specifically, individuals are asked if they have ever been diagnosed with diabetes, have an established residence, are pregnant or intend to become pregnant, have any relevant food allergies, and are under the care of a mental health care professional. At this time, participants are asked to provide the name of their mental health care provider. If they do not have a mental health care provider,

Veterans are referred to the VA, while non-Veterans are put in contact with local resources and invited to participate after they have seen a provider. If potentially eligible for the study, an appointment is made for the informed consent process, the baseline screening/assessment, and for study enrollment. Potential participants are requested to bring all dietary supplements and medications they are taking to the screening/enrollment visit. Figure 1 provides an overall flow diagram of the BRAVO study.

The BRAVO Call Center staff manage the scheduling of the study visits, the juice deliveries, and all phone calls with the study participants. Due to the weight and bulk of the juice boxes, the study delivers the juice boxes to the participants' places of residence each month and collects any remaining juice boxes from the previous delivery. Deliveries are scheduled by phone and boxes are delivered at a time convenient for the participants. Within the first week after the enrollment visit, participants receive a one-month juice smoothie supply.

Fig. 1. BRAVO Study Flow Diagram

Participants receive a total of 6 allotments of 90 juice boxes (one month supply), with an additional one-week of extra juice boxes. All participants or designees are asked to provide a photo identification and sign a receipt that they have received their full allotment of juice boxes

for the month. When receiving a new allotment of juice, participants are asked to return any remaining juice boxes from their previous allotment.

2.5 Assessments

2.5.1 Screening

The BRAVO study uses two short videos to ensure that all potential participants receive the same information about the study itself and the informed consent. The BRAVO study video is shown to all potential participants when they first arrive at the study rooms. If the participant is still interested in potentially enrolling in the study after seeing the study video, then they are shown a separate video that explains the informed consent and Health Insurance Portability and Accountability Act (HIPAA) process and its interpretation for the BRAVO study. Interested individuals then are asked to sign the informed consent and release of information from their mental health providers, and complete the screening assessments on laptop computers. One of the study psychiatrists reviews the screening information collected by the study coordinator on the computer remotely, and in person conducts a brief physical exam and a suicide and diagnostic interview to determine if the individual meets the study criteria for high suicide risk. If participants are eligible based on inclusion and exclusion criteria, they proceed to completion of baseline enrollment. Table 2 displays the schedule of clinic visits and assessments and shows the “check-in” visit at month two which provides a touch-stone for the participants and records AEs.

Table 2: Schedule of Visits / Assessments

Instrument	Study visits					
	Baseline	1 m	2 m	3 m	5 m	6 m
PreScreening Telephone Call						
Exclusionary questions	X					
Screening/Enrollment Visit						
Eating Attitudes Test (EAT-26)	X					
Demographic characteristics	X					
Beck Scale for Suicidal Ideation (BSSI)	X	X		X	X	X

Implicit Association Test-Suicide (IAT-S)	X			X		X
Inclusion Exclusion checklist	X					
Brief physical	X					
Suicide Severity Rating Scale (C-SSRS)	X					X
Suicide, Negative Emotions, and Other Disorders						
Beck Hopelessness Scale	X	X		X	X	X
Posttraumatic Stress Disorder Checklist (PCL-C)	X	X		X	X	X
Profile of Mood States-Bipolar (POMS-Bi)	X			X		X
Beck Depression Inventory	X			X		X
Substance Use						
Fagerstrom-Nicotine Dependence Test	X	X		X	X	X
Time-Line Follow-Back (TLFB)	X	X		X	X	X
Obsessive Compulsive Drinking Scale (OCDS)	X			X		X
Cognitive						
Connor's Continuous Performance Test II	X			X		X
Suicide Stroop	X			X		X
Physiological/Diet/Exercise/Participant Characterization						
Mini International Neuropsychiatric Interview (MINI)	X					X
BRAVO Adverse Events Questionnaire	X	X	X	X	X	X
International Physical Activity Questionnaire (IPA)	X					X
Automated Multiple Pass Method (AMPM) 24-hour dietary recalls – 2 ^{*y}	X					X
	(twice)					(twice)
Diet History Questionnaire (DHQ) Food Frequency Questionnaire– 30 day	X					X
Patient Reported Outcomes Measurement Information System-29 (PROMIS-29)	X	X		X	X	X
Blood for fatty acids, endocannabinoids, biological assays	X	X		X	X	X

2.5.2 Primary outcome assessments

The BRAVO study seeks to determine if dietary supplementation with n-3 HUFAs reduces the risk for serious suicidal behaviors, suicidal thinking, negative emotions, and symptoms associated with suicide risk, in those at risk for suicidal behaviors. A New Episode of Significant Suicide Risk (NESSR) is defined as the occurrence of any of the following: a) a suicide death, **or** b) a suicide attempt, **or** c) an inpatient admission with suicide risk, **or** d) clinical diagnostic equivalents as measured by the Columbia-Suicide Severity Rating Scale (C-SSRS).

The C-SSRS is a reliable and valid measure of severity of suicidal ideation and behaviors with the ability to track changes over time [16]. The other primary outcome measures that assess

suicidality include: the Beck Scale for Suicidal Ideation (BSSI) which measures suicide intent [17] and the Implicit Associations Test – Suicide (IAT-S) [18] which measures implicit or preconscious associations to death and suicide.

2.5.3 Secondary outcome assessments

Co-occurring psychiatric symptoms and specific aspects of cognitive performance are measured as secondary outcomes. Specifically, investigation of the impact of n-3 HUFA supplementation on symptoms of negative affect, PTSD, depression and cognitive functioning associated with suicide risk among individuals and alcohol and nicotine misuse are studied among BRAVO volunteers using a number of well-validated measures.

The Profile of Mood States-Bipolar (POMS-Bi) measures both positive and negative affect in terms of six bipolar affective states. [19] Hopelessness is a frequently identified risk factor for suicidal behaviors and is assessed by the Beck Hopelessness Scale (BHS) [20]. PTSD symptom severity is monitored using the PTSD Stress Disorder Checklist (PCL-C) [21]. Cognitive measures of sustained attention, response inhibition, and cognitive control is assessed using Conner's Continuous Performance Test II Version 5.2 [22] and the Suicide Stroop [23].

The severity of depressive symptoms is monitored using the Beck Depression Inventory [24]. Alcohol consumption is measured using the Time Line Follow Back (TLFB), a calendar-based method to collect drinking and drug use quantity and frequency [25]. Obsessive and compulsive thoughts about alcohol are assessed with the Obsessive Compulsive Drinking Scale [26]. The Fagerstrom-Nicotine Dependence Test (FTND) elucidates the severity of nicotine dependence [27]. Other assessments include the Mini International Neuropsychiatric Interview (MINI) for DSM V psychiatric diagnoses [28]. Physical activity is assessed with the International Physical Activity Questionnaire (IPA) [29]. Physical pain and sleep are measured using the

Patient Reported Outcomes Measurement Information System-29 (PROMIS-29). Percent disialo-carbohydrate-deficient transferrin (%dCDT) is measured as a serum biomarker of heavy alcohol use [30].

2.5.4 Dietary Assessments

Baseline dietary habits are characterized using the USDA Automated Multiple Pass Method (AMPM) 24-hour dietary recalls with one in-person interview and followed by a telephone interview within 10 days [31] and the Diet History Questionnaire (DHQ) Food Frequency Questionnaire-30 day [32]. The 30-day food frequency questionnaire (FFQ), when combined with the set of two AM/PM USDA 24-hour recalls, mathematically represents the current best estimation tool for determining usual intake of food and nutrients. To further document the nutrient status of participants, the BRAVO study collects the current Center for Disease Control and Prevention (CDC) recommended biochemical indicators to compare with national standards[34]. The AMPM and FFQ are administered again at the last visit after 6 months.

2.5.5 Blood Sample Assessments

Blood samples are obtained at baseline and all visits (with the exception of the 2 month check-in visit) for analysis of fatty acids and endocannabinoids. In addition, a number of biological assays are conducted to provide basic characterization of the sample population: total cholesterol, LDL, HDL, triglycerides, oxytocin, BDNF, epinephrine, norepinephrine, cytokines, c-reactive protein, and prostaglandins. The biochemical indicators of diet and nutrition include vitamins A, B₆, B₁₂, C, D, E, as well as folate, homocysteine and body iron [34]. Figure 2. displays the blood analyses and rationale.

Assay	• Rationale for Evaluation
Endocannabinoids (2-AG, AEA, DHEA)	•The brain endocannabinoid (eCB) system modulates several neurobiological processes and its dysfunction is suggested to be involved in the pathophysiology of mood and drug use disorders.
Carbohydrate deficient transferrin	•Biological maker of heavy alcohol use, which is one of the sub-analyse in the study.
Cotinine	• Marker of tobacco use
Lipid Assay: Total, LDL, HDL Cholesterol and Triglycerides	• n-3 HUFAs are well known to lower triglyceride levels and to raise both HDL and LDL cholesterol levels. Low total and LDL cholesterol levels have be identified as potential risk factors for suicide, but without consistent replication and with uncertainty that lowering or raising these lipid levels actively alters risk of suicidal behaviors.
Oxytocin	•Hormone released by the pituitary gland that is well established as necessary for affiliate social behaviors.
BDNF	•Genetic variants in Brain Derived Neurotrophic Factor (BDNF) genes have been reported to interact with maltreatment as a child to increase risk of self- injurious behaviors. Animal studies indicate that omega-3 HUFA supplementation beneficially alter levels of these hormones.
Epinephrine/Norepinephrine	•These catecholamines are markers of excessive sympathetic nervous system activity, characteristic of anxiety states and implicated in impulsive responses. Prior studies of anxious medical student reported decreases in these catecholamines after omega-3 HUFA supplementation.
C-Reactive Protien, Cytokines, IL-6, TNF- α	•The cytokines IL-6 and TNF- α and the inflammatory marker C-Reactive protein have been implicated in depressive symptoms in human and animal studies. In human and animal studies supplementation with omega-3 HUFAs markedly reduce levels of these circulating cytokines.
Prostaglandins	•Omega-6 derived prostaglandin E2 stimulating the PE2 receptor induces anxiety and depressive like behaviors in animals in part through regulating inflammatory processes. Elevating omega-3 HUFA status may reduce production of omega-6 derived prostanoids.
Nutrients	•Baseline nutrient states profile that includes nutrients recommended for status assessment by the CDC.

•VO Blood Analysis

2.5.6 fMRI Sub-study

The purpose of the fMRI sub-study is to evaluate the impact of an n-3 HUFA intervention on prefrontal hypo activation to risky decision-making and recall of past suicidal events and

determine whether changes in prefrontal activation are associated with reduced suicidal ideation and behavior. Preceding the first *f*MRI session, study personnel interview participants regarding a recent suicide attempt. One narrative of a subjectively unbearable mental state associated with the suicide attempt, one suicidal ideation narrative (i.e., of suicide preparation behaviors), and one neutral control sequence (e.g., of getting up in the morning) are developed into 120 second scripts (i.e., 3 total). During the suicide recall task, participants listen to each of their prerecorded scripts during *f*MRI scan (3 blocks total) [35]. The Balloon Analogue Risk Task (BART)[15] provides an ecologically-valid assessment of risky decision making/risk taking behavior during the *f*MRI. Risky decision making on the BART is associated with activation of the dorsolateral prefrontal cortex, insula, and orbitofrontal cortex [36]. In the BART, participants choose between 1) continuing to inflate a balloon on a computer screen to bank temporary money with the risk of the balloon bursting and losing their accumulated money, or 2) saving the accumulated money to a permanent bank. Whenever a balloon bursts or the participant chooses to bank money, he or she starts with a new balloon. Participants respond to 30 balloons, each having a different bursting point. With each click, the participant must weigh the potential gain of collecting more money against the potential risk of losing all of the money accumulated with that balloon. The index of riskiness is based on the average number of clicks across balloons.

2.5.7 Participant Compensation

Participants are compensated for time spent attending all onsite visits and dietary follow-up phone calls. This compensation totals \$425 for the entire study period. Completion of the *f*MRI study can add \$100 to \$160 compensation depending upon performance on the BART.

2.6 Data Management and Analytic Approach

2.6.1 Data Acquisition and Security

With the exception of the informed consent and HIPAA documents, all data collected for the BRAVO study are entered by the study participants or study staff into laptop computers connected to a secure university server. Each participant is assigned a study ID at enrollment and all data is associated with the study ID, not personal identifiers. Communication and scheduling for participants and among BRAVO team members is handled by the Call Center staff using the ACT! contact manager software (Act! North America, 8800 N. Gainey Center Drive, Suite 200, Scottsdale, AZ 85258). The majority of the study data are collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at MUSC [37]. REDCap is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. A subset of data, primarily cognitive measures, are collected using DirectRT and MediaLab_{tm} software (Empirisoft Corporation, 2110 8th Avenue, Suite 7D, New York, NY 10026) with data transferred to REDCap. The AMPM dietary recalls are collected using the USDA provided software which is a computerized method for collecting interviewer-administered 24-hour dietary recalls either in person or by telephone that is a research-based, multiple-pass approach employing five steps designed to enhance complete and accurate food recall and reduce respondent burden [38]. Completion of study steps are documented at each visit by study coordinators throughout the visit and all data is uploaded onto the secure server at the completion of each study visit.

2.6.2 Analytic Approach

All main analyses are assessed by both ‘Intention to Treat’ (ITT) and ‘As Per Protocol’ (APP) approaches. Specifically, if there is significant non-adherence, the ITT approach is less likely to find a statistically significant effect since non-adherent individuals are included with

their assigned adherent participants. The degree of adherence in both the intervention and placebo group is determined and reported. The subsequent APP approach only includes adherent participants in the active, as defined by blood elevations in n-3 HUFAs, and adherent participants in placebo groups, as defined by increases in blood 16:1 n-7, and seeks to assess whether oral supplementation is an efficacious strategy to improve outcomes. Suicide risk will be assessed by multivariable logistic regression analyses performed to determine whether the proportion of new episode of significant suicide risk (NESSR - a dichotomy) differs between the active or placebo treatment during the 6 month treatment period. Adjusted odds ratios with 95% CIs will be reported, with 2-tailed probability values of <0.05 considered statistically significant for all *a priori* defined hypotheses. To assess symptom reduction, a series of bivariate and regression models will be generated where defined groups are associated with improvements in specific variables characterizing suicide risk. Hypothesis tests will be 2-tailed, and a p-value < 0.05 will be considered statistically significant.

2.6.3 Sample Size

A total analytic sample of 260 subjects (130 per group) is required to determine whether the experimental group has a statistically significant reduction in the incidence of NESSRs during the study period, as compared to the usual care group. This sample size requirement is based on several assumptions including a 0.05% probability of a Type I error (alpha), 80% power (20% probability of a Type II error), and two sided testing. Further, it is anticipated that supplementation with omega-3 HUFA will achieve a minimum of a 60% reduction (effect size) in the primary outcome variable (NESSRs) within six months of initiation. Combining various publications, we conservatively estimate that 20% of subjects in the usual care group will re-attempt suicide during the study period and this will be reduced to at least 8% in the experimental group. We anticipate we may experience a dropout rate of up to 15% and have increased our

recruitment sample to accommodate this eventuality. Thus, a total of 300 subjects (150 per group) will be recruited for this study. Secondary sub-analysis will be conducted to examine treatment efficacy in subject subgroups (i.e. subjects with or without symptoms of depression or alcohol misuse or PTSD), although the statistical power will be lower for any such analyses given the reduced sample size.

2.7 Safety Considerations

Recruiting an extremely vulnerable population requires priority attention to participant safety, and the BRAVO team developed a number of procedures to assure the safety of all participants enrolled in the study. Staff having contact (including telephone contact) with potential participants have been trained by the American Red Cross National Suicide Prevention Hotline staff and are capable of making the appropriate emergency referrals for actively suicidal individuals.

Prior to enrollment, all participants must have a current mental health provider and continue care and medications as usual. Upon telephone screening, the BRAVO Call Center coordinator obtains the name of the mental health provider who is currently treating the patient. Prior to the baseline screening visit, a study psychiatrist/clinician contacts the participant's mental health care provider to verify the patient's clinical status and discuss study enrollment. During the enrollment and all subsequent visits, due to the possibility that study participants can present with a risk of suicide, study visits are scheduled when a study psychiatrist or appropriately trained and experienced clinician is present on site. During scheduled visits, members of the research team assess participants as being at increased risk for suicide (i.e., report of re-emergence of or increased intensity/frequency of suicidal ideation, development of a specific suicide plan, procurement of lethal means for suicide, expressed suicide intent, reported feeling unable to adhere to their existing safety plan, etc.). If a participant presents with increased risk for suicide,

the research coordinator stays with the participant and contacts the on-site study clinician who immediately examines the participant and makes recommendations, including hospitalization if indicated. If a study clinician determines that the participant is at immediate risk, the clinician contacts the hospital Public Safety Office and personally escorts the participant with a Public Safety Officer to the appropriate local emergency room (MUSC or VA). In all such instances, the study clinician communicates with the primary mental health provider directly to assure continuity of care.

All mental health providers are formally notified by the clinical team that an individual has enrolled in the BRAVO study after the individual completes the baseline enrollment visit. They are also notified in the event that a patient has not attended a scheduled visit despite phone contact attempts.

For the fMRI sub-study, a psychiatrist on the fMRI research team conducts a structured debriefing at the end of each fMRI visit. The debriefing includes assessing/discussing thoughts and emotions associated with hearing the audio recordings about their suicidal experiences. Participants are not discharged until any acute distress is resolved.

The BRAVO study has a Medical Monitor and Data Safety Monitoring Committee (DSMC). The DSMC meets quarterly to discuss safety issues, adverse events, recruitment and attrition. The DSMC and study statisticians will perform an interim analysis after sufficient participants have completed data collection for viable assessment of the study variables.

3. Discussion

This paper describes the design and methodology for a dietary study that is recruiting an extremely vulnerable population often excluded from most research studies. As such, this study is designed to have a number of procedures to address the safety of participants. Suicide represents

a most tragic final action that is often made during times of severe distress, when an individual feels hopeless, is not able to consider alternatives or long-term consequences of choices, and feels compelled to act. Potential novel interventions to target suicide and mental health conditions that increase the risk for suicide are essential [39].

Fatty acid profiles in the US diets have dramatically changed over the last century reflecting less consumption of n-3 HUFA and more consumption of n-6 fatty acids [40] and [41]. N-3 HUFAs, specifically DHA, are essential for neural function [42] and thus, can impact cognitive, emotional, and mental health functioning [43]. Other studies have consistently demonstrated increased risk of depression and suicide in individuals with low n-3 HUFA intakes [44], [45], [46] and [47] and improvements in depression and suicidal ideation have been demonstrated with n-3 HUFA supplementation [48]. In one large study with US active military, lower serum n-3 HUFAs and DHA levels were associated with higher rates of suicide in Veterans [49]. This low level may be associated with an increase in dietary intake of “Western Diet” protein (i.e. red or processed meats, high fat dairy products) in order to build more muscle mass in preparation for military duty. This is in contrast to the lower rate of depression observed in individuals who follow the “Mediterranean or Healthy diet” (i.e. fish, vegetables and fruits) with higher n-3 HUFA content [46].

This study would provide information that would support the role for dietary supplementation of n-3 HUFA in reducing suicide risk and concomitant mental disorders. The safe side effect profile and minimal interactions with other food or medicine, make n-3 fatty acid supplementation ideal for implementation in the general population and among U.S. military. In addition, adherence to a dietary supplementation may be better than with prescribed psychotropic drugs. The stigma of being diagnosed with mental health disorder and prescribed a psychotropic medication carries a number of perceived adverse consequences, and fear of being labeled with a

psychiatric disorder contributing to treatment avoidance may adversely affect employment or future career goals, as well as functioning in other areas. Thus, n-3 HUFAs could, if efficacious, offer a more acceptable way of treating mental health problems for many.

The study has several unique design features and a comprehensive assessment package to not only evaluate the efficacy of n-3 HUFA on suicidal ideation/behavior but also explore mechanisms of action and potential mediators of efficacy. The study also collects information on other health conditions such as sleep, pain and quality of life. Thus, this study captures a more holistic characterization of participants over a six month period of time which is not typically done in other studies. The intervention is novel in that the experimental and control dietary supplementation is provided in all natural, flavored juices. The monthly home delivery system decreases participant inconvenience associated with picking up and returning their unused supply.

The BRAVO study is carefully designed to provide essential safeguards and precautions to assure the safety of the participants. As participants are seen by several members of the research team at baseline/follow-up visits (team of psychiatrists/clinicians, study nurse, study coordinators), the BRAVO Call Center is responsible for making study visit arrangements, reminder phone calls and re-arrangements for missed visits. One of the study challenges with this population is maintaining the continuum of care following inpatient hospitalization. Patients often use the emergency room and inpatient services when in psychiatric crisis because they do not have a mental health provider. The study team cannot enroll participants unless contact has been made with the mental health provider. Thus, facilitating this contact can ensure that these high risk patients get connected with their referred provider post inpatient discharge. Likewise, if participants are enrolled in the study but fail to show up for a follow-up appointment and cannot be contacted by phone, the study team must contact the mental health provider, thereby providing a backup alert function in potentially risky situations.

The current study has other advantages that extend beyond the study goals as it casts a wide net throughout the community to identify those individuals at risk for suicide. Those with new onset suicidal ideations are quickly identified and immediately referred to mental health services. For those individuals with suicidal ideation who already have a mental health provider, re-establishing communication with the health care provider can potentially prevent an impending suicide attempt. BRAVO is a carefully designed study that addresses a public health crisis and can potentially provide a model for future studies that involve recruiting individuals at high risk for suicide.

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