NAPLS OMEGA 3 STUDY PROTOCOL

VERSION 2-28-17

Randomized Double-Blind Trial of Omega-3 Fatty Acid versus Placebo in Patients at Risk for Psychosis

"THE OMEGA-3 STUDY"

Tyrone D. Cannon, Ph.D., Principal Investigator
University of California, Los Angeles

Kristin Cadenhead, M.D., Co-Principal Investigator, and Ming Tsuang, M.D.
University of California, San Diego

Scott Woods, M.D., and Thomas McGlashan, M.D.
Yale University

Jean Addington, Ph.D.
University of Calgary

Barbara Cornblatt, Ph.D.
North Shore-Long Island Jewish Health System

Elaine Walker, Ph.D.
Emory University

Larry Seidman, Ph.D.
Harvard University/Massachusetts Mental Health Center

Diana Perkins, M.D., MPH
University of North Carolina, Chapel Hill

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Statement of Purpose

The overall goal of the present study is to determine whether Omega-3 Fatty Acids potentially prevent onset of psychosis and improve clinical symptoms and functional outcome in youth at elevated clinical risk for schizophrenia and related disorders. The specific aims are: (1) To determine whether the rate of progression to psychosis is lower at 12-month follow-up among patients randomly assigned to six months of treatment with Omega-3 Fatty Acids compared to to those randomly assigned to six months of treatment with placebo, (2) To determine whether Omega-3 Fatty Acids are more efficacious than placebo for prodromal symptoms, negative symptoms, and functioning, (3) To assess the safety and tolerability of Omega-3 Fatty Acids in this population, and (4) To conduct analyses of neuroimaging, neurocognitive, electrophysiological and other ancillary data to explore mechanistic explanations for the hypothesized benefits of Omega-3 Fatty Acids on clinical and functional outcomes (e.g., increases in white matter integrity and processing speed).

Background

Essential Fatty Acids: Omega-3FAs are essential for normal brain function and development[1, 2], and may also have neuroprotective properties[3, 4]. Arachidonic Acid (AA), an *n*-6 PUFA (polyunsaturated fatty acid), is synthesized from dietary sources of linoleic acid, whereas the *n*-3 PUFAs (including Docosahexaenoic acid - DHA, 22:6*n*-3) are synthesized from α-linoleic acid[5]. Interconversion of linoleic acid and α-linoleic acid is not possible in mammalian tissues; therefore, each is considered to be an essential FA. A-linoleic acid is the main PUFA in human diets; typical diets consist of 20-fold more *n*-6 fats than *n*-3 fats. The same enzymes responsible for the conversion of linoleic acid (18:2*n*-6) to AA (20:4*n*-6) mediate conversion of α-linoleic acid (18:3*n*-3) to eicosapentaenoic acid (EPA) (20:5*n*-3); thus, substrates in Omega-6 and Omega-3 pathways are competing for the same enzymes[5]. While AA and its derivatives are potent mediators of inflammation[6], Omega-3FAs such as EPA and its derivative DHA have well-documented anti-inflammatory actions[5].

Importance of Omega-3 FAs: Fish oil is the major source of the Omega-3FAs, EPA and DHA[7]. Consumption of fish oil increases EPA- and DHA-containing phospholipids at the expense of decreased AA incorporation into phospholipids[5]. DHA is the major Omega-3FA in the central nervous system (CNS) [8, 9]. Under resting conditions, essentially all DHA is esterified into membrane phospholipids. DHA is continuously required for biogenesis and maintenance of neuronal and photoreceptor membranes[10]. Dietary supplementation of EPA and DHA has beneficial effects in certain medical disorders including asthma[11], rheumatoid arthritis[12], and Crohn's disease[13]. A major benefit of Omega-3FAs is their likely role in the prevention and treatment of cardiovascular diseases[14-16]. DHA and EPA decrease plasma very-low-density lipoprotein, triglyceride levels, postprandial lipidemia and lead to antithrombotic changes[17, 18]. Moreover, Omega-3FAs have antiarrhythmic action in the heart, linked to a fall in the risk of sudden death[19, 20]. Omega-3FAs trials have also been performed in a number of psychiatric illnesses and show evidence of efficacy in depressive disorders, bipolar disorder, dementia and attention deficit disorder[21-25]. Given the well-documented metabolic abnormalities prior to onset of illness and as a consequence of antipsychotic treatment, it is evident that identifying a supplement that can potentially improve cardiovascular risk as well as clinical symptoms would be a major step forward in treatment of severe mental illness.

Fatty Acid Metabolism in Schizophrenia: Abnormal phospholipid and related fatty acid metabolism may play a role in the etiology of several psychiatric illnesses, including schizophrenia. Studies of peripheral tissues, including erythrocytes and skin fibroblasts, have shown reduced levels of phospholipids (phosphatidylcholine and phosphatidylethanolamine) in patients with schizophrenia[26-28]. Studies using ^{31}P magnetic resonance spectroscopy have shown increased levels of phosphodiesters and decreased levels of phosphomonoesters in prefrontal and temporal lobes of drug-naïve schizophrenic patients[29-32]. Since phosphomonoesters are utilized in phospholipid synthesis, and phosphodiesters are phospholipid breakdown products, these data have been taken to imply increased phospholipid turnover in patients with schizophrenia[33]. In studies of red cell membrane FAs, depletions of both the n-6 and n-3 series have been demonstrated[34-36].

Trials of Omega-3 Fatty Acid in Schizophrenia: Since an altered FA metabolism could be involved in the etiology of schizophrenia, controlled trials have been done in this population. Three studies have found beneficial effects of Omega-3FA supplementation in schizophrenia[37-39]. Peet et al[38] studied 45 symptomatic patients on stable antipsychotic medication treated with either 2g/day of EPA, 2g/day of DHA or placebo (corn oil) for 3 months. Improvement, measured by the Positive and Negative Syndrome Scale (PANSS)[40], was statistically superior with EPA compared to DHA and placebo. In another trial[38], Peet et al used EPA as a sole treatment in initially unmedicated patients. By the end of the study, all 12 patients on placebo, but only 8 of 14 patients on EPA, were taking antipsychotic drugs. Despite this difference in antipsychotic use between the groups, patients taking EPA had significantly lower scores on the PANSS by the end of the study. Emsley et al[37] found that 3 g/day of EPA was effective in improving PANSS total score compared to placebo (medicinal liquid paraffin) in a 12 week trial of medicated chronic patients with schizophrenia and this difference remained significant after controlling for effects of dietary EPA, medication, duration of illness, and gender.

Two other studies reported negative Omega-3FA findings[41, 42]: Fenton et al[41] assessed whether augmentation of antipsychotics with 3 g/day of EPA improved symptoms and cognition in 87 patients with schizophrenia. Patients who had residual symptoms despite antipsychotic treatment received either 3 g/day of EPA (N=43) or mineral oil as a placebo (N=44) in a 16-week, double-blind trial. No differences were found between groups in symptoms, mood, cognition, or global impression ratings. Results were similar for the intention-to-treat (N=87) and completer (N=75) groups. Peet and Horrobin[42] studied the effect of 1-4 g/day of EPA or Placebo in 115 patients with schizophrenia given for 12 weeks in addition to antipsychotic medication. Patients given 2 g/day EPA showed improvements on the PANSS, but there was also a large placebo effect in patients on antipsychotics and no difference between groups. The reason for the discrepancies in the Omega-3FA trials in schizophrenia patients could be that EPA has no beneficial effects in patients with a long history of schizophrenia who are presumably taking optimal doses of standard antipsychotic drugs[43]. The best outcomes in the current literature are found in patients with a short illness history who were not receiving standard drugs.

Trials of Omega-3 Fatty Acid in Prodromal and Early Psychosis: In a trial of 80 FEP patients, Berger et al[39] assessed whether EPA augmentation could improve antipsychotic efficacy and tolerability in a 12-week, randomized, double-blind, placebo-controlled trial of 2g/day EPA or placebo (mineral oil). The findings suggest that EPA may accelerate treatment response, improve tolerability and reduce the amount of prescribed antipsychotic medication. However, it was not possible to demonstrate a sustained symptomatic benefit of EPA in FEP, possibly due to a ceiling effect, since a high proportion of FEP achieve symptomatic remission with antipsychotic medication alone[39]. Amminger et al[44] recently published a randomized, double-blind, placebo controlled trial in 81 subjects with prodromal symptoms. The active treatment consisted of 1.2g/day of Omega-3FAs (700 mg of EPA, 480 mg of DHA, and 7.6 mg of mixed tocopherol). Coconut oil was chosen as placebo. By study's end (12 weeks), 2 of 41 individuals (4.9%) in the Omega-3FA group and 11 of 40 (27.5%) in the placebo group had transitioned to a psychotic disorder (p=0.007). The difference between the groups in risk of progression to full-threshold psychosis was 22.6%. Omega-3FAs also significantly reduced positive (p=0.01), negative (p=0.02), and general symptoms (p=0.01) and improved functioning (p=0.002) compared with placebo.

Little is known about the effects of Omega-3FAs on neurocognitive performance in schizophrenia, but it has been studied more extensively in dementia[45-49]. Accelerated cognitive decline and mild cognitive impairment correlate with lowered tissue levels of DHA/EPA, and supplementation has improved cognitive function. Cognitive improvement has been noted across other disorders affecting cognition including dyslexia (improvement in reading and spelling)[50, 51] and attention deficit disorder (reduced inattention)[51, 52]. In healthy subjects receiving a combination of 800 mg DHA and 1600 mg EPA versus olive oil placebo, the Omega-3FA group improved in measures of attention and reaction time[53]. On EEG measures, high-frequency beta-2 band activity was reduced while theta and alpha bands increased, suggesting a direct effect on the cortical function. Theoretically, these improvements could be related at least in part to increased myelination and white matter integrity; such effects have thus far been observed in animal models but not yet studied in humans.

Omega-3 Fatty Acids and Prepulse Inhibition (PPI) of the Startle Response: PPI is an operational measure of sensorimotor gating and has been shown to be deficient in patients with schizophrenia, their first degree

relatives, schizotypal personality disorder and now the prodrome of schizophrenia[54-56]. Omega-3FA deficiency has been associated with PPI deficits in mice supporting the role of Omega-3FA in neurodevelopment[57]. Mice who are deficient in Fabp7 (fatty acid binding protein 7), a gene with functional links to the *N*-methyl-D-aspartate (NMDA) receptor, show decreased PPI [58]. Human FABP7 has been shown to have altered expression in the brains of schizophrenia patients and to be associated with schizophrenia[58]. Pax6 (+/-) rats have impaired postnatal neurogensis and PPI deficits, both of which improve after administration of AA[59]. These results suggest the potential benefit of FAs in ameliorating PPI deficits relevant to psychiatric disorders. The effects of Omega-3FAs on PPI have not been assessed in humans.

Hypotheses

Primary Hypothesis: The rate of conversion to psychosis at 12 months as defined by SIPS criteria among prodromal patients assigned at random to Omega-3 Fatty Acids will be significantly lower than that among patients assigned to placebo.

Secondary Hypothesis: The reduction from baseline in the Scale of Prodromal Symptoms (SOPS) total score (indexing severity of positive, negative, and general symptoms) at 6 and 12 months will be significantly greater in prodromal patients assigned at random to Omega-3 Fatty Acids than in patients assigned to placebo.

Tertiary Hypothesis: The improvement from baseline in the General Functioning Scales (indexing social and role functioning) at 6 and 12 months will be significantly greater in prodromal patients assigned at random to Omega-3 Fatty Acids than in patients assigned to placebo.

Study Design

Experimental Treatment: This will be a 24-week, randomized, double-blind, placebo, fixed dose-controlled study designed to determine the acceptability and feasibility of Omega-3FA versus placebo in prodromal subjects assigned to treatments on a 1:1 basis. Omega-3FA will be administered as oral capsules given twice daily (BID). Ocean Nutrition Canada will prepare identical-looking Omega-3FA and placebo capsules. We will include 128 prodromal subjects in the 6-month trial. All subjects will receive monthly clinical assessment. The study design and Omega-3FA formulation are designed to be compatible with that of another on-going trial conducted by investigators in Australia and Europe (the Neuropro Study sponsored by the Stanley Foundation), which would enable the data from the two studies to be combined for analysis on the primary (conversion) and most of the secondary and tertiary (symptoms, functioning) endpoints.

The proposed study will potentially further the development of novel treatment approach for the prodrome of psychosis. This research will provide empirical data regarding a lower risk, broad spectrum treatment, that could have important implications for public health as a pre-emptive intervention or treatment augmentation because of the potential to effect functional outcome. It is possible that subjects in the treatment group will show an improvement in symptoms. Study participants will receive extensive clinical evaluations and consultation from investigators with knowledge regarding psychosis.

The results of this study will increase the ability to provide alternative treatments with potentially fewer side effects for the prodrome of psychosis. The potential implication for primary and tertiary prevention of psychosis from these treatments is immeasurable. Through the development and utilization of strategies such as those proposed in this research we could potentially discover the ability to delay the onset of psychosis and manage treatment more effectively with fewer side effects seen with traditional antipsychotic medication treatment. The importance of this to public health would be tremendous.

The use of Omega-3FA in an adolescent and young adult prodromal population is novel. Therefore the level of risk is considered moderate. Although this study is greater than minimal risk, it offers subjects direct benefit from participation because of the Omega-3FA and placebo treatments. It is also possible that the concurrent use of Omega-3FA will improve metabolic indices in all subjects. Because Omega-3FA is not

specifically approved by the FDA for use in early psychosis, we have received a certificate for an Investigational New Drug (IND) for the current Research Plan.

The sample of 128 prodromal subjects will be recruited from 8 sites over 16 months. The active treatment phase will be completed within 6 months, with 12-, 18-, and 24-month follow-ups. Each site will obtain institutional review board approval of the protocol. The UCLA site, directed by Dr. Cannon, and the UCSD site, directed by Dr. Cadenhead, coordinate the trial, with responsibility for acquisition of the Omega-3 and placebo pills, group assignments, and data analysis.

Sample

Adult subjects must give written informed consent, and minors must give written informed assent with consent from a parent or guardian.

Inclusion Criteria: Subjects will be included if they are treatment-seeking patients between the ages of 12 and 30 who meet diagnostic criteria for a possible prodromal syndrome and are part of the ongoing NAPLS study at each site. The enrollment targets for each site are based on enrollment into the parent NAPLS study over the past 12 months, after projecting a 50% refusal rate for participation in the clinical trial: 20 from UCLA, 20 from Emory, 10 from Harvard, 16 from Zucker Hillside Hospital, 12 from UNC, 14 from UCSD, 22 from Calgary, and 14 from Yale. In total, 128 subjects from all of the participating sites will be randomized into drug versus placebo in the Omega-3 long chain fatty acid (Omega-3FA) study. All subjects will receive clinical assessment, fluid sampling, and adverse event monitoring during the 6-month trial.

Exclusion Criteria: Subjects will be excluded for any of the following reasons:

(1) use of antipsychotic medication in the previous month.
(2) concomitant medical or neurological illness.
(3) history of significant head injury.
(4) alcohol or drug abuse (excluding nicotine) in the past month or dependence in the past three months.
(5) screening full scale estimated IQ < 80.
(6) active suicidal or homicidal ideation.
(7) pregnancy or lactation.

Procedures

During the week prior to randomization and beginning study capsules, patients will undergo eligibility and baseline examinations. After beginning study capsules, patients will be scheduled for 6 monthly follow-up visits as well as 12-, 18-, and 24-month follow-ups (Table 1).

Omega-3FA and Placebo*:* The Omega-3FA compound will be manufactured by Ocean Nutrition Canada and contain an 2:1 proportion of EPA to DHA in which each capsule includes 370 mg EPA and 200 mg DHA as well as 2 mg/g Tocopherol. The dose will be two capsules per day for a total of 740 mg of EPA and 400 mg of DHA. The ratio and dose of Omega-3FA were selected based on previous data from controlled trials that demonstrates the efficacy of EPA in trials with schizophrenia patients[37, 38] and the potential benefit of a low dose of DHA in combination with EPA per the Amminger study[60] in prodromal patients. The placebo is a soybean/corn blend. Both the Omega-3FA and placebo are colored with carob (so shell is brown) and flavored with natural lemon-lime, to mask them. Certificates of analysis for the Omega-3FA and placebo compounds are included in the Appendix. Ongoing testing by independent laboratories will assure the levels of Omega-3FA in capsules, stability, and absence of any contaminants, including toxic substances in this product. Stability and toxicology testing will be provided by Siliker Canada Co. UCLA will receive the compounds and assign coded numbers to packets before distribution to sites and thereby administer the double blind design.

***Antipsychotics**:* Prodromal patients currently on anti-psychotic medication will be excluded from the study. It is possible that prodromal subjects will develop worsening symptoms and require such treatments during the course of the trial, which is allowed. All concomitant treatment will be recorded.

***Antidepressants**:* Prodromal patients currently on anti-depressant medication will be included in the study; randomization to Omega-3FA vs placebo will be stratified on anti-depressant medication status.

***Background Diet**:* Baseline diet characterization will be assessed using a systematic checklist. We considered using an open-ended diary that requires recording of all food intake, but it seems unlikely that adolescents with early psychosis symptoms would comply to a satisfactory degree. The checklist is easy to complete and is more likely to be accurate than unstructured self-reports. The list includes foods that are rich in Omega-3FA. It will be given to the family at the first screening visit, and will be collected for the two consecutive weeks preceding entry into the treatment phase. Intake will be categorized as low (0-1 serving/wk of Omega-3FA rich diet), intermediate (2-3 servings/wk), or high (4 or more servings/wk). In addition fasting erythrocyte FA composition will be assessed to use as another means of controlling for background diet at baseline.

***Laboratory and Metabolic Measures**:* After confirming eligibility for the study, a urine sample will be taken for a drug screen, and a serum pregnancy test on females. Fasting erythrocyte FA composition will be quantified at baseline, month 3 and month 6 using capillary gas chromatography. The ratio of Omega-6 to 3FAs will be used to index pretreatment vs posttreatment FA composition as an objective measure of treatment adherence, to assess for dietary differences between subjects and assure that subjects on placebo are not taking Omega-3FA supplementation from an outside source. Thiobarbituric Acid Reactive Substances (TBARS) will be used for screening and monitoring lipid peroxidation before and after the treatment trial. In addition, to assess metabolic parameters, baseline measures of fasting glucose and lipids, weight, abdominal girth and blood pressure will be obtained and repeated at the end of the trial.

Table 1. Study Procedures and Timeline

Visit	1	2	3	4	5	6	7	8	9	10	11
Day	-7 to -1	1	30 (+/-2d)	60 (+/-5d)	90 (+/-5d)	120 (+/-5d)	150 (+/-5d)	180 (+/-5d)	360 (+/-15d)	540 (+/-15d)	720 (+/-15d)
Month		0	1	2	3	4	5	6	12	18	24
Screening											
Informed consent	X										
Inclusion/exclusion	X										
Characterization											
Medical history	X										
Demographics		X									
FIGS		X									
PAS		X									
Treatment											
Randomization		X									
Capsule dispensing		X	X	X	X	X	X				
Pill count			X	X	X	X	X	X			
Psychosocial		Up to 6 sessions of supportive case management									
Safety/Physical											
Physical exam	X										
Vital signs & weight	X		X	X	X	X	X	X	X	X	X
Labs (LFTs etc)	X				X			X			
Adverse events		X	X	X	X	X	X	X			
Treatment Logs		X	X	X	X	X	X	X	X	X	X
Clinical Outcomes											
SOPS		X	X	X	X	X	X	X	X	X	X
SCID		X						X	X	X	X
SCID-SPD		X						X	X	X	X
Depression (CDSS)		X	X	X	X	X	X	X	X	X	X
GAF		X	X	X	X	X	X	X	X	X	X
GF-Social		X	X	X	X	X	X	X	X	X	X
GF-Role		X	X	X	X	X	X	X	X	X	X
Other Outcomes											
Neuropsychology		X						X	X		X
Brain imaging		X						X	X		X
Electrophysiology		X						X	X		X
Salivary cortisol		X							X		X
DNA/RNA/plasma		X							X		X

Key: X=Existing Protocol; X=Added for Omega3 Trial

Other Concomitant Medications: Subjects may continue doses of antidepressant, mood stabilizer, or stimulant medication as prescribed independently of their participation in this trial. Antipsychotic mediation will not be permitted unless subjects develop worsening symptoms and require antipsychotic medication during the course of the trial. All concomitant treatment will be recorded.

Concomitant Psychotherapy: During the 6-month active treatment phase, all subjects will be receive up to 6 sessions of supportive case management, as needed, as part of their evaluations by psychological staff. Staff members will provide support and address the young person's efforts to cope with symptoms and functional deficits. In addition, subjects will be permitted to participate in any outside of study supportive psychotherapy, with all concomitant psychological treatments recorded.

Family Focused Treatment (FFT) Study: Patients enrolled in the FFT study will be stratified prior to randomization in the Omega-3 trial, such that equal numbers of FFT participants will be assigned to the Omega-3FA and placebo groups. About one-quarter of the patients enrolled in NAPLS participate in the FFT study. Because the enrollment period for the FFT study will end on December 31, 2011, only about one-third of the participants in the Omega-3 trial would actually be eligible to participate in both studies. Thus, the overlap between patients participating in the FFT and Omega-3 trials is expected to be less than 10% (i.e., .33 x .25 = .083). Given this, we do not expect to have sufficient numbers of cases enrolled in both studies to be able to test whether participation in one moderates effects of participating in the other, but stratified group assignment

will ensure that equal numbers of cases in active treatment and control conditions are balanced for FFT participation.

Continuation Therapy: Although we considered forbidding patients to use Omega-3FAs after the 6-month active treatment phase, we determined that doing so would be impractical and potentially damaging to the parent study, because patients and families convinced of the benefits of Omega-3FAs would likely drop out at that point. For this reason, we will suggest but not enforce discontinuation, and of course, no "pills" will be provided by the study after the active treatment phase. We will, however, continue to record dietary and supplemental sources of Omega-3FAs for the full two-year follow-up.

Potential for Interference with Aims of Parent Grant: The aims of the parent NAPLS grant (elucidating predictors and mechanisms of conversion to psychosis) would be undercut if we were to implement a treatment program that was effective in eliminating conversions. However, neither FFT nor Omega-3 FAs are expected to have the potency to prevent psychosis in all individuals. At the same time, given that prodromal patients are distressed and treatment seeking, these individuals are getting treatments in the community regardless of their participation in our study. Such community-based treatments are not structured and lack control conditions. The large sample size target for the parent study (720 prodromal patients followed longitudinally) was proposed in part to take variability in exposure to such treatments into account. Since the parent grant was funded, we have had the opportunity to implement one clinical trial (FFT study) and possibly a second one (Omega-3). The primary advantages of implementing such controlled treatment trials within the context of the NAPLS study are that we can estimate conversion risks in the patients who received more intensive or active interventions compared with those who received less intensive or inactive treatments, we can determine if predictors of conversion differ according to the type or degree of interventions received, and we can test whether effectiveness of interventions is mediated by changes in neural, hormonal, and other processes being assessed in the core NAPLS study.

Study Management: Prior to study launch, study investigators and other key personnel will participate in several conference calls to provide training on study procedures, including recruitment strategies, inclusion and exclusion criteria, use of rating scales, and data management procedures.

After study launch, the co-PIs at the UCLA and UCSD sites will chair conference calls every two weeks attended by each site PI and study coordinator. Reports on enrollment and data completeness will be discussed regularly, along with issues brought up by sites.

Data Management: UCLA will provide the central data management site for the proposed study, and the University of Calgary site (Dr. Addington) will be responsible for overall NAPLS data management. This will be done through a centralized Oracle database with web based data input that will facilitate study coordination, data checks and early identification of faulty procedures and data errors. The database is secure, robust and easy for clinical investigators and staff to learn and use. Security access can be limited to site level with a range of access for different levels of personnel. Data entry will be of the highest standards such that each data element/field is defined with ranges, allowed values and size.

Post-treatment Biomarkers Assessment

Patients enrolled in this trial will be participants in the parent study "Predictors and Mechanisms of Conversion to Psychosis." In the context of that study, participants are assessed with MRI/DTI, electrophysiology (including PPI), neuropsychological testing at baseline and 12- and 24-month follow-ups. Given that participants in the Omega-3FA study will complete the active treatment phase at 6 months, it is desirable to add an additional biomarkers assessment point for those subjects at the 6-month (end of treatment) follow-up.

Assessments

Sources of material will come primarily from the participant, in the form of the results of questionnaires, clinical interviews, physical exam, and blood draws. All participants will undergo a series of clinical, functional, neurocognitive, neuroimaging and electrophysiological assessments, that are part of the overall NAPLS study, before and after the 6 months of Omega-3FA versus placebo. Therefore data collected as part of the parent study will be assessed for changes that may be attributable to these treatment trials. Biological specimens (blood) will be collected at baseline, 3 months and 6 months of the Omega-3FA trial to help evaluate the level of Omega-3FA in the diet and metabolic indices as well (urine) to evaluate substance use at time of testing.

For minors, a parent/legal guardian may provide additional information useful for clinical or historical data. Written and oral consent will be obtained from participants, or their legal guardian for minor participants. Assent will be obtained from minors. Only study personnel directly associated with the research will have access to individually identifiable information.

Power Calculations and Statistical Analyses

Over the 16-month enrollment period, a total of 128 patients will be consented and randomized to receive Omega-3FA or placebo for 6 months. This number is half of the expected enrollment in NAPLS during that period, leaving room for a refusal rate of 50%. Of the 128 subjects randomized (64 to Omega-3FA, 64 to placebo) based on the retention rate in the Amminger et al. and similar studies with Omega-3FA, about 102 would be expected to complete the trial (51/51). Amminger et al. found that patients on placebo were 5.6 times more likely to convert than those on Omega-3FAs (i.e., 27.5/4.9). In the proposed study, given an initial sample size of 128, a 20% attrition rate, and a 12-month conversion rate of 22% in the placebo group (as was detected in NAPLS 1), we have 80% power to detect as significant at alpha=0.05 a hazard rate of 4.9 (i.e., corresponding to a conversion rate of 4.5% in the Omega-3FA group). However, power will be lower than 80% if the observed relative risk is less than 4.9; for example, power falls to .61 when the hazard rate is 2.8, or 50% of that observed in Amminger et al.). Note that the 22% baserate of conversion to psychosis over 12-months in NAPLS I was based on patients who received a variety of treatments, including psychosocial and pharmacological, in the community. In addition, after accounting for attrition, we have 80% power to detect a difference in functional outcome or symptom scores of 0.49 standard deviation units or greater. Amminger et al. observed effects larger than .49 on measures of symptoms and functioning, suggesting that power to detect effects on continuous measures of symptoms and functioning in the proposed study is quite favorable.

For evaluation of the primary outcome measure (time to conversion), we will use Cox's proportional hazards model and Kaplan-Meier survival analysis as implemented in SAS PROC PHREG and LIFETEST. The secondary and tertiary outcome variables, all treated as continuous variables, will be examined as a function of treatment condition and site using mixed effects regression models. Mixed effects models permit the estimation of symptom trajectories at the level of the individual participant over time. Then the random coefficients from these within-participant trajectories are modeled as a function of treatment condition and appropriate baseline covariates (e.g., initial severity of symptoms; age or gender). At the level of the individual participant, time-varying covariates (e.g., antipsychotic treatment or not) can also be controlled, thus permitting the estimation of within-participant trajectories over and above any time-varying covariates. Similar approaches will be used to examine the trajectory of functional outcome scores. The advantages of mixed effects models over traditional repeated measures models are that they allow for (1) the inclusion of participants with missing data, (2) observations that are unequally spread out over time (both within and between participants), and (3) more flexible modeling of variance and covariance patterns of residuals. We will use two programs – SAS PROC MIXED and MIXREG – to examine these models.

Human Subjects

Only participants who meet eligibility criteria for and have previously been consented for the North American Prodrome Longitudinal Study (NAPLS) will be invited to participate. Participants will thus include individuals who are referred or respond to announcements because they may be experiencing symptoms that are associated with the prodrome of psychosis. These individuals will be referred by community health care providers or will be self-referred in response to media announcements or internet searches. Recruitment involves providing announcements to local primary care practitioners, including community pediatricians, family practice physicians, local clergy, educators, clinical social workers, psychologists, and psychiatrists. This information will include a letter introducing the study, and a brochure that describes prodromal symptoms.

Based on enrollment in NAPLS to date, about 65% of the sample are expected to be males. About 20% of subjects are expected to be African American and about 10% Latino.

Inclusion and Exclusion criteria are described earlier under "Sample."

Randomization Criteria: At the Baseline visit, subjects must continue to meet all inclusion and exclusion criteria in order to qualify for randomization.

Consent Procedures: Children under the age of 18 will provide written assent, and written parental consent will be obtained for their participation. Consent forms will be written in language that is comprehensible to individuals that have at minimum an eighth grade education. The consent form will contain information about the nature of the interviews, Omega3-FA, and fluid sampling.

Potential Risks:

1. There is a potential risk that confidential health information collected during the course of the study may be disclosed to others.
2. There is a potential risk that participants will be subjected to stigma and undue anxiety from identification as "at risk" of a serious mental disorder.
3. There is a potential risk that the participants may find the study questions and procedures tedious, or that they may be distressed by the discussion of personal issues.
4. There is a potential risk of pain, bruising and infection from the blood draw.
5. Since we will be evaluating and monitoring subjects for the presence and the development of a psychiatric disorder, we have instituted procedures for the management of anticipated clinical issues (see section 2 "Protection Against Risks" below for procedures).
6. There is a potential risk that subjects will experience side effects associated with Omega-3FAs. Omega-3FAs exert a dose-related effect on bleeding time; however, there are no documented cases of abnormal bleeding as a result of fish oil supplementation, even at high dosages and in combination with other anticoagulant medications. Other potential side effects of Omega-3FAs include a fishy aftertaste and gastrointestinal disturbances that are dose-dependent. There is also the risk of heavy metal contamination (e.g., Mercury) in fish oil. To avoid this risk, the Omega-3FA will be screened both by the manufacturer (Ocean Nutrition Canada) as well as an independent laboratory to assure the levels of Omega-3FA in capsules, their stability in capsules, and the absence of any contaminants, including toxic substances in this product. Stability and toxicology testing will be provided by Siliker Canada Co.

Protections Against Risk: In providing participants with information about the research, there are two chief considerations: 1) to minimize psychological discomfort and potentially damaging negative expectations, and 2) to provide information that is based on the best currently available scientific data. All of the sites in this project have considerable experience with strategies for informing potential participants about the purpose of the research and for minimizing distress and anxiety. We explain that some individuals experience changes in their

perceptions, thinking, emotions, or behavior. While some are not bothered by these changes, others may find them distressing because they interfere with their ability to function. We then explain the possible reasons for such changes, including that they may be part of normal adolescent/young adult development, a reaction to a life stressor, symptoms of drug use, symptoms of a metabolic disorder, symptoms of a mood disorder or anxiety disorder, or the early warning signs of bipolar disorder or schizophrenia. We then discuss the possible course of the "changes", including that symptoms/signs often go away, remain stable, or worsen, particularly if they are the early signs of a disorder. It is relevant to note that potential prodromal participants are usually experiencing subjective distress and are therefore motivated to take part in the clinical research assessment. Similarly, their family members typically encourage them to participate and are readily engaged in the assessment process.

We also address the following specific factors when protecting against risk:

1. Risks of stigma and undue anxiety from identification as "at risk" of a serious mental disorder will be minimized in the following ways:
    a. Information regarding possible outcomes and causes will be routinely provided. Subjects will be informed about possible outcomes, including remission, persistent symptoms, or worsening of symptoms. They will be informed that causes of symptoms include a normal adolescent or early adult maturation, a reaction to a life stressor, a symptom of drug use, symptoms of a metabolic disorder, the symptoms of a mood disorder or anxiety disorder, or the early warning signs of affective disorder or schizophrenia.
    b. Study participants who are judged to be in need of additional psychiatric or psychological evaluation/treatment, and are not currently receiving that treatment, will be referred as clinically indicated.
    c. In addition, to minimize risk of undue anxiety related to uncertainty of diagnosis at screening we will also conduct a careful systematic diagnostic interview (the Structured Clinical Interview for DSM IV, SCID) and cognitive evaluation to determine whether there is an active diagnosable condition and provide consultation to study participants.
2. The risk that the subjects may find the study questions and procedures tedious, or that they may be distressed by the discussion of personal issues will be minimized by having a study staff person monitor the subject's experiences during the study procedures, and by having a study clinician familiar with the participant available to assist the subject if she or he becomes distressed by study procedures. In addition, participants will be told that they may decline to answer any questions or to discuss any issue if they do not want to or if they find it distressing. Efforts will be made to make the study assessment procedures as pleasant as possible for the subject, and to detect and address any problems with evaluation procedures.
3. The risks of the blood draw include pain, bruising, and risk of infection that will be minimized by using sterile techniques, having blood drawn by an experienced phlebotomist, and offering the subject use of a topical anesthetic at the time of the blood draw.
4. To reduce the risk of side effects associated with Omega-3FA, all subjects will be educated about potential side-effects and queried about these side-effects at each weekly visit. If side-effects become too burdensome with the Omega-3FA, subjects will be offered the option of reducing the dose to one capsule rather than two per day or discontinuing the Omega-3FA altogether. It is possible that prodromal subjects taking Placebo will develop worsening symptoms and require alternate treatments for worsening mood, anxiety or psychotic symptoms. In these situations subjects will be offered treatment consistent with the standard of care in the community.
5. To reduce the risk of heavy metal and toxin contamination in the Omega-3FA, the company we have selected to provide the Omega-3FA performs their own screening (Ocean Nutrition Canada) and we will also use an independent laboratory to assure the levels of Omega-3FA in capsules, their stability in capsules, and the absence of any contaminants, including toxic substances in this product. Stability and toxicology testing will be provided by Siliker Canada Co.

6. In order to minimize subject discomfort and attrition, it will be important to reduce the burden of participation in the study. The full clinical assessment will take about 2 hours. Within the constraints of the research design, efforts will be made to accommodate participants' schedules and, when indicated, to avoid fatigue. Investigators from the participating sites will evaluate subject burden on an ongoing basis and adjust the assessment schedule uniformly across sites in the event any problems arise.
7. Procedures for the management of anticipated clinical issues include the following:
    a. If the screening visit the subject that is found to have a medical condition that may have caused the prodromal or psychotic symptoms, they will be excluded from further participation. If either an exclusionary medical condition or an incidental medical condition is suspected, the participant will be advised to consult with their physician or will be provided with referral information. If a psychiatric disorder is found to be present at baseline, or is found to develop during the course of the study participants would be advised to consult with their mental health care provider or provided with referral information for treatment. These participants will continue to be followed in the study, with information on treatment recorded in the database.
    b. Minor subjects and their parents or guardians will be informed that we will be doing urine drug screens as part of the routine study evaluations. We will explicitly inform parents and potential minor subjects, in the written informed consent document and orally, that we will not inform the parent of the results of the drug test, because revealing this information could affect the willingness of the minor subject to participate in the study or answer truthfully. We will tell both the minor subject and the parent or guardian our clinical policy regarding confidentiality, as follows: If a minor subject is found to have a positive drug screen, the PI will discuss the risks of drug use with the subject, and encourage the minor subject to discuss their use of drugs with their parent or guardian. If the subject is found to have a substance use disorder, we will discuss the need for treatment with the subject, and encourage the subject to allow us to speak with their parent or guardian about our concerns.
    c. Participants who are minors will be informed that their parent will be notified in the event that they are engaging in or plan to engage in behavior (i e., suicidal attempt) that is dangerous to themselves or others.
    d. To summarize, our procedures will follow routine clinical research procedures to reduce risk to participants, and follow all State regulations regarding treatment of minors and informing parents and guardians. A single clinician will attempt to engage the minor in a therapeutic relationship, and maintain confidentiality unless there is a specific, immediate safety risk (e.g., suicidal ideation).

Data and Safety Monitoring Plan: All studies conducted at this site are reviewed and approved by an Institutional Review Board (IRB). A signed informed consent, assent (as applicable), and HIPAA Authorization form is obtained from the subject or the subject's legally authorized representative prior to the initiation of any study related procedures. Handling, dispensing and administration of the investigational drug must be in accordance with all state and federal laws and regulations. The IRB requires regular updates on the status of research projects, including the number of participants enrolled, adverse events or unanticipated problems, number of withdrawals from the project, complaints about the research, and any protocol changes. Any significant adverse events (which will be reported to the IRB as soon as possible, never to exceed 10 days from when the adverse event becomes known to the PI or study staff). The IRB also monitors the consent forms and ensures that appropriate HIPAA information is included.

Adverse events in this research project are expected to be uncommon. However, should we find that a participant has an adverse event we will take the appropriate steps to deal with the issue. Staff will consult with the study physician and the PI and address patient safety. Any possible serious adverse events will be reported to the PI immediately. In addition, the PI will conduct a safety review at a weekly frequency. All subjects will be reviewed at a regularly scheduled weekly meeting and any adverse events will be discussed. Adverse events will be defined as any adverse occurrence between the signing of consent and 2 weeks after the end of study

participation. Unanticipated risks are those risks not cited in the protocol or known risks of the illness under study. Unanticipated risks include instances where anticipated risks exceed expectations in terms of their magnitude or frequency.

The Principal Investigator will attribute adverse events as follows:

- Definite: Adverse event(s) will clearly be related to investigational agent(s) or other intervention
- Probable: Adverse event(s) will likely be related to investigational agent(s)
- Possible: Adverse event(s) may be related to investigational agent(s)
- Unlikely: Adverse event(s) will doubtfully be related to investigational agent(s)
- Unrelated: Adverse event(s) will clearly not be related to the investigational agents(s)

A serious AE will be one that results in death or the immediate risk of death, hospitalization or the prolonging of an existing hospitalization, persistent or significant disability/incapacity or a congenital anomaly/birth defect. Adverse events will be graded as follows:

- No adverse event or within normal limits
- Mild adverse event
- Moderate adverse event
- Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
- Life-threatening or disabling adverse event
- Fatal adverse event

The principal investigator will evaluate the adverse event and determine whether the adverse event affects the Risk/Benefit ratio of the study, whether the blind should be broken, and whether modifications to the protocol (at Risks to Subjects, or in Procedures) or consent form (at Risks and Inconveniences) are required. Serious unanticipated adverse events will be reported within 24 hours to the local IRB, the FDA, and NIMH if unblinded on treatment course (vs. placebo), unless the attribution is that the adverse event is unrelated to study participation. Either the principal investigator or the RCP have the authority to stop or suspend the study. A summary of the adverse events will be reported to the RCP when reapproval of the protocol is sought. The summary will include number of subjects enrolled and a summary of graded adverse events to date.

We will also utilize a Data and Safety Monitoring Board (DSMB) that is already established at our Center. The Center has a system for appropriate oversight and monitoring to ensure safety and welfare of the participants and validity of the data in the form of a DSMB for studies conducted through the Center. The DSMB has one face-to-face meeting and at least three conference calls every year. This study will be reviewed three times a year by the DSMB. Their review/monitoring will include reviewing the protocol, risk:benefit considerations, informed consent documents and plans for data safety and monitoring; evaluating the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention; and other factors that can affect study outcome; considering factors external to the study such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study; protecting the safety of the study participants and reporting on the safety and scientific progress of the trial. The DSMB has the power to recommend to the PI that the study be stopped or modified.

Confidentiality: Confidentiality will be maintained by assigning each patient a study number, and coding all data collected with that number. Identifying information will not be stored on computer databases, and will not be stored with the study subject number. All computer databases are password protected, and hard copies of all data and records will be stored in locked filing cabinets. We will further protect subject confidentiality by seeking a Certificate of Confidentiality to protect the sensitive clinical information and urine drug screen data from legal discovery. All study personnel will be certified to conduct research with human subjects, and will be aware of the importance of maintaining strict confidentiality.

In Case of Injury: If a subject experiences a research injury, the site PI or designee will provide or arrange for medical treatment. This treatment will be offered for any physical injuries sustained as a consequence of participation in this research. However, UCLA will not be responsible for payment for such treatment.

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