TITLE: **Neurochemical Effects of Omega-3 Fatty Acids in Adolescents at Risk for Mania**

UC-IRB#: 09-04-15-03

SPONSOR: Investigator Initiated with NIH/NIMH

FROM: Co-Principal Investigators: Melissa DelBello, MD, MS, & Robert McNamara, PhD
Department of Psychiatry
University of Cincinnati College of Medicine
260 Stetson St.
Cincinnati, OH 45219-0516

Sub-Investigators: Richard A. Komoroski, PhD
Jeffery Welge, PhD
Jeffrey Strawn, MD
Lauren Stahl, DPsych
Wade Weber, MS
Debra Scroeck, MS, PA-C

II. RESEARCH PROTOCOL

A. Specific Aims: (1) To collect pilot data regarding the efficacy, safety, and tolerability of omega-3 fatty acid supplementation for the treatment of adolescents with active depressive symptoms and a high risk for developing mania (i.e. they have a bipolar parent and meet DSM-IV-TR criteria for major depressive disorder or depressive disorder not otherwise specified (NOS)). (2) To use proton magnetic resonance spectroscopy (^{1}H MRS) (i.e. prefrontal neurochemistry), plasma oxidation indices and cytokine concentrations, and red blood cell (RBC) omega-3 fatty acid levels to examine potential mediators of treatment response to omega-3 fatty acids in adolescents with a high risk for mania.

B. Background and Significance: The onset of bipolar disorder typically occurs during adolescence.[1-3] Family studies demonstrate that children with a bipolar parent have a 30% chance of developing a mood disorder, whereas children in whom both parents have a mood disorder (and at least one with bipolar disorder) have a 70% chance of developing a mood disorder.[4] Offspring of bipolar parents therefore have an elevated risk of developing mood disorders compared with the general population.[5-10] Moreover, when these offspring develop prominent mood symptoms or major depressive disorder, their risk of developing mania (and by definition bipolar disorder) increases even further.[11] However, antidepressants and other medications that are commonly used to treat these mood symptoms may accelerate the onset of mania or hypomania.[12-18] Therefore, studies evaluating the efficacy, safety, and tolerability of potential treatments for mood symptoms in adolescents with a bipolar parent are necessary as an initial step toward establishing early intervention, and ultimately prevention, strategies for bipolar disorder.

Disruptions in attention and affect modulation are two of the cardinal features of bipolar disorder.[19-21] These processes are regulated by the anterior limbic network, which involves the ventrolateral prefrontal cortex (VLPFC), anterior cingulate (ACC), thalamus, amygdala, and striatum.[22-23] Indeed, there are considerable data suggesting that, compared with healthy subjects,

adolescents at familial risk for developing bipolar disorder exhibit differences in the structure, function, and neurochemistry of these brain regions.[24-25] In order to extend this work, one of the primary projects of the University of Cincinnati Bipolar Disorder Imaging and Treatment Research Center (BITREC, PI-Strakowski), which is supported through a National Institute of Mental Health Center for Intervention Development and Research (CIDAR) grant, involves recruitment and longitudinal assessment of a large cohort of adolescent offspring of bipolar parents to further investigate whether these neurobiological changes are early biomarkers of incipient mood episodes. However, the BITREC does not include the resources to conduct a systematic intervention trial for bipolar offspring once they develop a mood disorder other than bipolar I disorder, nor to examine whether these neurochemical changes are useful as targets for treatments in this population.

Omega-3 fatty acids play an important role in brain maturation, function, and resilience to stress. Cross-national epidemiological surveys suggest a significant inverse correlation between per capita fish/seafood consumption (surrogates for dietary omega-3 fatty acid intake) and lifetime prevalence rates of major depressive (MDD) and bipolar disorders.[26-28] Humans cannot synthesis omega-3 fatty acids *de novo*, and therefore, are completely dependent on maternal (*in utero* & breast milk) and dietary sources to procure and maintain adequate peripheral (red blood cell, RBC) and central tissue concentrations. Studies of tissue fatty acid composition have found that patients with both unipolar depression[29-35] and bipolar disorder[36-37] exhibit peripheral (RBC) and central omega-3 fatty acid deficits relative to healthy subjects. Moreover, findings from preliminary placebo-controlled trials suggest that omega-3 fatty acid supplementation significantly reduces symptom severity in children and adolescents with MDD[38] or bipolar disorder.[39] However, whether dietary supplementation of omega-3 fatty acid slows or prevents illness onset and progression of mood disorders and associated brain changes remains unknown.

With these considerations in mind, the overall hypothesis guiding our research is that omega-3 fatty acid supplementation will delay and prevent the development of mania and associated brain changes in adolescent offspring of bipolar parents. As an initial step to test this hypothesis, we will combine magnetic resonance spectroscopy (MRS) with a controlled treatment trial in order to examine the neurophysiological effects of omega-3 fatty acid supplementation in adolescents with significant risk factors for incipient mania.

C. Preliminary Studies: The Co-Principal investigators of this study, Drs. DelBello and McNamara, are currently co-investigators of two clinical neuroimaging trials evaluating the effects the omega-3 fatty acid treatments on basal and activated PFC blood flow (fMRI) and metabolic indices (mI, NAA, Glu) in healthy (no personal or family history of psychiatric illness) male children, and in adolescent SSRI-refractory MDD patients. Dr. DelBello is a child and adolescent psychiatrist with extensive expertise in the field of adolescent bipolar disorder and magnetic resonance neuroimaging. Dr. McNamara has an active basic (translational) and clinical research program focused on the role of brain fatty acid composition in neurochemical events relevant to the pathophysiology of recurrent neuropsychiatric illness. Preliminary studies conducted by the Co-PIs have provided evidence that young patients with bipolar disorder exhibit premature age-related PFC DHA deficits, and that adolescents at-risk for developing bipolar disorder exhibit erythrocyte DHA deficits. We have also reported that treatments that are efficacious for bipolar adolescents increase PFC NAA level, and have demonstrated that hypermetabolism (increased Glx and mI and decreased NAA levels) in the ALN exists early in the course of bipolar disorder.

D. Investigator experience: Dr. DelBello is Associate Professor of Psychiatry, Pediatrics, and Psychology and Co-Director of the Division of Bipolar Disorders Research (DBDR) at the University of Cincinnati College of Medicine (UCCOM), is Director of Research Training and Education for the Division of Psychiatry at Cincinnati Children's Hospital Medical Center (CCHMC), and is Co-Director of the Mood Disorders Clinic at CCHMC. Dr. DelBello is a child and adolescent psychiatrist with extensive expertise in the field of pediatric bipolar disorder and magnetic resonance neuroimaging. She has been studying the neurobiology and pharmacology of adolescent bipolar disorder for over 10 years. Dr. DelBello will provide the clinical expertise in adolescents at-risk for developing mania, which includes supervising and training all personnel in efficacy and tolerability ratings, ensuring high ethical standards, directing subject recruitment, and maintaining high levels of inter-rater reliabilities on diagnostic and symptom assessments. Dr. DelBello will be responsible for the overall scientific conduct of the study, providing both conceptual and managerial direction. Dr. DelBello will also be directly responsible for overseeing recruitment, assessment, and management of the research subjects, supervising the training and reliability of the personnel performing diagnostic and rating instruments, and performing diagnostic and rating interviews. She will also work in collaboration with Dr. McNamara, Co-PI, and other Sub-investigators to oversee interpret results and prepare manuscripts. In addition, she will manage the research coordinator who is involved in patient recruitment and clinical follow-up.

Dr. McNamara is Associate Professor of Psychiatry in the Division of Bipolar Disorders Research (DBDR) at the University of Cincinnati College of Medicine. Dr. McNamara has been studying the molecular neuropsychopharmacology of bipolar disorder for over 10 years, and has expertise in omega-3 fatty acids and the pathoaetiology of psychiatric illness. Dr. McNamara is currently the PI on two clinical neuroimaging trials evaluating the effects the omega-3 fatty acid treatments on basal and activated PFC blood flow (fMRI) and metabolic indices (*myo*-inositol, NAA, Glx) in normal 8-10 year old male children, and in SSRI-refractory adolescent MDD patients without a family history of bipolar disorder prior to and following 10-week dietary omega-3 fatty acid treatment. Dr. McNamara also heads a basic (translational) research program focused on brain fatty acid composition and behavioral, pharmacological, neurochemical, neuroendocrine, neuroanatomical, and neuroimaging measures relevant to the pathophysiology of bipolar disorder. Dr. McNamara will directly supervise the research coordinator who is responsible for imaging and gas chromotographic analyses of RBC samples, and interpretation of the MRS data. He will be responsible for all data compilation and analyses in coordination with Dr. Welge and will work in collaboration with Drs. DelBello and Komoroski to interpret data and prepare and submit manscripts.

Dr. Komoroski is a Research Professor of Psychiatry at the University of Cincinnati College of Medicine and Associate Director of the Center for Imaging Research and is an internationally recognized authority in applications of NMR spectroscopy to biomedicine and chemistry, with over 100 publications in peer-reviewed journals. He has over 15 years of experience in the application of in vivo and in vitro NMR techniques to psychiatric disorders. Dr. Komoroski will provide expertise in neurochemistry, LCModeling, and ^{1}H-MRS data analyses and interpretation and will oversee quality control for the MRS data. He will also assist in manuscript preparation.

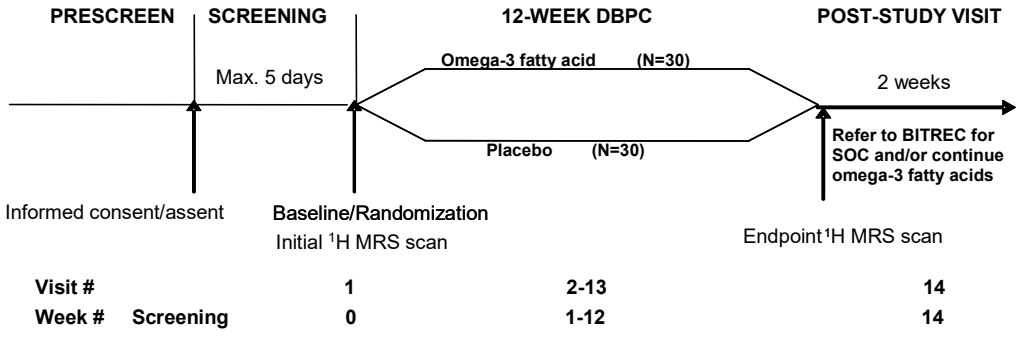
Dr. Welge, Research Assistant Professor of Psychiatry and Biostatistics and Director of the Division of Quantitative Methods in the Department of Psychiatry UCCOM, has extensive expertise in the area of psychiatry and biostatistics. Dr. Welge will oversee all aspects related to database design and data management. He will lead the statistical analyses and complete exploratory post-hoc analyses. He will also assist in preparing manuscripts of the research findings.

E. Experimental Design and Methods:

Figure 1. Schedule of assessments for the 14-week trial

ASSESSMENT	SCREENING	BASELINE	WEEKS 1-11	WEEK 12 (TERMINATION)	WEEK 14 (FOLLOW-UP)
WASH-U KSADS	X	X			
Young Mania Rating Scale (YMRS)	X	X	X	X	X
Children's Depression Rating Scale-Revised (CDRS-R)	X	X	X	X	X
ADHD Rating Scale (ADHD Rating Scale-IV)	X	X	X	X	X
Clinical Global Impression-Severity (CGI-S)	X	X	X	X	X
Clinical Global Impression-Improvement (CGI-I)			X	X	X
Timeline Follow-Back	X	X	X	X	X
Child Behavior Checklist (CBCL)		X	X (Wk 4 & 8)	X	X
Teacher's Report Form (TRF)		X		X	
Children's Global Assessment Scale (CGAS)	X	X	X (Wk 4 & 8)	X	X
The Omega-3 Dietary Intake Questionnaire		X	X	X	
Columbia Suicide Severity Rating Scale (C-SSRS)	X	X	X	X	X
LIFE psychosocial functioning scales		X	X (Wk 4 & 8)	X	
Prior medical & treatment history, physical exam	X			X (physical exam)	
Vital signs	X	X	X	X	X
Urine toxicology screen & pregnancy test	X	X		X	
Serum laboratory tests (renal profile, complete blood count, liver & thyroid function tests, fasting lipid profile & glucose, and platelet function assay)	X			X	
Red blood cell omega-3 fatty acid levels	X			X	
Adverse events & adherence assessments	X	X	X	X	X
^{1}H-MRS scan		X		X	
Total duration of appointment	2 hours	3 hours	1 hour	3 hours	1-2 hours

Figure 2. Study Design



DBPC=Double-blind placebo-controlled; SOC=Standard of Care Treatment

a. Methods and Procedures:

Affected Subjects**:** Adolescents (ages 9-21 years) with a current DSM-IV-TR diagnosis of MDD or Depressive Disorder NOS (defined as exhibiting 4 of 5 criteria for an episode of major depression or meeting all MDD criteria except duration) will be recruited from the University of Cincinnati BITREC "at-risk" cohort (n=140, UC-IRB #07-04-10-03) of adolescent offspring of parents with bipolar I disorder. To be included in the BITREC at-risk cohort, adolescents must have no lifetime syndromal mood or psychotic disorder at their initial baseline assessment, a parent with bipolar I disorder, and no contraindication to undergoing an MRI scan. Based on data from prior studies we estimate that at least 35% of the cohort will develop MDD within the 5-year longitudinal follow-up period of BITREC.[6] Subjects for the proposed study will be recruited from the "at-risk" cohort as well as from adolescents who have already developed MDD or Depressive Disorder NOS and who are screen failures for the BITREC "at-risk" cohort. The BITREC at-risk adolescents in the BITREC are evaluated monthly for follow-up and are instructed to call study staff between visits if mood symptoms emerge, so that we will be able to identify potential subjects for the proposed study at or soon after the onset of a mood disorder. If a subject is recruited from outside of BITREC, their biological parent(s) with bipolar I disorder will be asked to complete a Structured Clinical Interview for DSM Disorders (SCID) to confirm diagnosis of bipolar I disorder. One of the main goals of BITREC is to identify neurobiological changes that occur prior to and at the onset of a mood disorder in adolescents who have a parent with bipolar I disorder. Therefore, all at-risk adolescents who develop a mood disorder will undergo a ^{1}H MRS as part of their participation in the BITREC, which is identical to the MR scans proposed for this study. This scanning session will occur during the baseline study visit for the proposed study for eligible BITREC subjects who choose to participate in the proposed trial. The omega-3 treatment trial and the week 12 MRS scan will occur exclusively as part of this study and are distinct from, although complementary to, participation in the BITREC. As part of the BITREC longitudinal illness course assessments are performed in the at-risk adolescents. However, the BITREC does not include a systematic treatment study for these individuals, other than for those who develop mania, who will be excluded from participation in the proposed study. We will give all participants the opportunity to participate in BITREC at study termination in order to provide follow-up.

Healthy Controls: Although we will not recruit healthy subjects for the proposed study, they are recruited (n=40) for BITREC "Project 3" and undergo yearly ^{1}H-MRS scans. Therefore, we will be able to interpret the direction of neurobiological changes in at-risk adolescents (i.e., whether patient values are changing toward or away from those of healthy adolescents).

Screening, Baseline, & Randomization: Following informed consent/assent and review of study inclusion/exclusion criteria, the initial visit (screening) will obtain demographic and clinical information as previously described. Typically, diagnostic interviews will have been performed during the study participant's most recent BITREC visit. We will make every effort to enroll potential subjects for the current study within one week of their most recent BITREC visit. However, if this is not feasible the diagnostic interviews will be updated at the time of screening. Ideally, the screening and baseline visits will be completed within 1-2 days of each other, but a maximum of 5 days will be permitted. A study physician will consult with each adolescent's treating clinician after permission is obtained and prior to making any changes to their medications. At no time during the study will we change medications of adolescents who are stable, i.e., only adolescents experiencing active depressive symptoms, as determined by a study physician in conjunction with the patient, their family and treating clinician, and information from the diagnostic and rating scales, will be

included in the study. In order to maximize subject safety, study participants will be monitored daily by telephone (and in person if needed) during the screening period.

A randomization schedule stratified by presence vs. absence of ADHD, presence vs. absence of concomitant stimulant treatment (within the ADHD group), and pre-pubescent vs. post pubescent (as determined by the Tanner Questionnaire with scores of 1-2 representing pre-pubescent and scores of 3-5 indicating post-pubescent) will be developed by the on-site biostatistician and will be used to assign omega-3 fatty acids (N=30) or placebo (N=30) treatment in a double-blind manner. We will stratify treatment assignment by some of the independently transmitted variables that contribute to the heterogeneity of at-risk adolescents (i.e. ADHD and concomitant use of stimulants) so that we will be able to assess the effects of these clinical factors on the neurophysiological and omega-3 fatty acid RBC level changes associated with supplementation of omega-3 fatty acids (vs. placebo) and examine potential neurophysiological markers of treatment response within clinically defined subgroups. We selected these variables for stratification because they may each affect outcome and treatment response.

Twelve-week Study Phase: During the 12-week DBPC phase subjects will participate in weekly research visits to assess symptom response and adverse side effects. Following the randomization visit, all visits will be scheduled weekly (or $\leq$ 10 days apart). The research visits will take approximately 1 hour; at these visits an experienced clinician will evaluate the patients for adverse effects and clinical response. Efficacy assessments will be obtained by a rater who is blind to side effects.

Two-week and Long-term Follow-up: At the end of the 12 weeks patients and their legal guardians (if < 18 years) will decide whether they would like to continue (or start for those receiving placebo) omega-3 fatty acids. Regardless of the treatment chosen, all patients will return for a follow-up visit two weeks following their last visit in the DBPC study to ensure that there has been no significant worsening of their mood. Importantly, all patients will have the option to continue to participate in the BITREC, which is a longitudinal 5-year study. Since some patients will opt to continue omega-3 fatty acid treatment, this will permit us to obtain long-term effectiveness and tolerability data. Safety and efficacy assessments will be performed at all follow-up visits. Subjects will be responsible for obtaining their own omega-3 fatty acids supplementation after study completion if they wish to continue with omega-3 treatment. Omega-3 fatty acids are widely available via food, health, and supplement retail stores at a relatively low cost.

Assessements:

The Children's Depression Rating Scale-Revised (CDRS-R), a 17-item observer-rated questionnaire,[40,41] and the

Young Mania Rating Scale (YMRS),[42-44] an 11-item observer-rated questionnaire to assess depressive and manic symptoms, respectively. A consensus adolescent/legal guardian score for the CDRS-R and YMRS will be determined by the rater.

Subjects will also be rated using the Clinical Global Impression-Severity Scale (CGI-S), a clinician rated 7-point scale (rated from 1=normal to 7=extremely ill) to assesses overall illness severity.[45] CDRS-R, YMRS, and CGI-S will be administered at screening, baseline (concurrent with the first imaging session) and then subsequently at each weekly follow-up visit and will be used to rate symptoms during the prior week.

The CGI-Improvement Scale (CGI-I) is a clinician rated scale that assesses overall illness change

from baseline and will be administered at all post-baseline visits.

The ADHD Rating Scale (ADHD Rating Scale-IV),[46,47] completed by the parent or guardian, will be administered at screening, baseline, and each weekly visit and will be used to assess ADHD symptom severity during the prior week. Rates of ADHD are high in adolescents at risk for developing mania and who have a parent with bipolar disorder.[6,48] Therefore, excluding these adolescents would limit the generalizability of our findings. Moreover, data suggest that omega-3 fatty acids may be effective for the treatment of ADHD symptoms.[49-52] Thus, we will include adolescents with ADHD in our study. However, to be included the adolescent must have been taking a stable dose of psychostimulants during the month prior to baseline.

The Columbia Suicide Severity Rating Scale (C-SSRS)[56], will be administered by trained raters at each visit as a measure of suicidality. All study investigators and staff have received training from one of the authors of the C-SSRS, Dr. Kelly Posner at Columbia University. We have incorporated this rating scale into several other studies of adolescents with mood disorders. If any subject experiences worsening of suicidality during the course of the study, they will be discontinued from study participation and receive appropriate clinical care.

The Children's Global Assessment Score (CGAS)[57] will be administered by a child and adolescent psychiatrist at screening, baseline, weeks 4, 8, and 12 (or termination), and the follow-up visit at week 14, will be used to assess overall function at the time of the rating.

Additionally, psychosocial function during the month prior to baseline will be assessed using the functional rating scales of the Longitudinal Interval Follow-up Evaluation (LIFE).[58,59] Trained raters with good inter-rater reliability (kappa >0.8) will evaluate each of four major areas of functioning; role performance, interpersonal relationships, recreational enjoyment, and sexual activity. To assess change in function, the same rater will evaluate each of these four major areas for the prior 4-week period at weeks 4, 8, and 12 (or termination).

In addition to the clinician rated instruments, for all adolescents ages 9 -18 years and for those adolescents ages 18-21 years, if feasible and permission is obtained from the subject, a primary caregiver will be asked to complete the Child Behavior Checklist (CBCL)[60] at baseline and weeks 4, 8, and 12 (or termination), and at the week 14 follow-up visit and to rate the period since the prior CBCL (baseline ratings will rate the prior month). If the parent with bipolar disorder is asked to complete the CBCL, we will assess their current mood state by reviewing the mood state module of the SCID. This information will be recorded and controlled for in statistical analyses.

Additionally, for all adolescents who are still in school, a teacher will be asked to complete the Teacher Report Form (TRF)[61] at baseline and week 12 (or termination). We will obtain written permission from the adolescents (if 18-21 years) or their legal guardian (if < 18 years) to contact their teacher and will communicate with the teacher directly to complete the TRF. Using these procedures, we have 96% TRF return rates for our prior studies.

Nutritional Supplement Dosing: Subjects will be randomized to EPA+DHA supplements (omega-3 fatty acids) or placebo (olive oil) provided and encapsulated by the Inflammation Research Foundation. Placebo and omega-3 capsules are identical in size, shape, and color to protect the blind. Capsules will be provided to subjects. The omega-3 fish oil used in these capsules is screened by the International Fish Oil Standards (IFOS) program (**www.ifosprogram.com**) for PCBs, mercury, heavy metals, and oxidation levels in accordance with Council for Responsible Nutrition's voluntary monograph on omega-3 products and safety standards for human consumption. All capsules will be stored at manufacturer's recommendations (14-24°C), and EPA+DHA content determined upon initial receipt and every 60 d by gas chromatography to assure consistency across the trial. Individual

omega-3 capsules contain 400 mg EPA and 200 mg DHA. The selected EPA/DHA ratio, or EDR, (EDR: 2.0) was based on prior controlled trials finding that EDRs closer to 2.0 (1.8-2.0), regardless of dose, were more effective in reducing depression symptom severity relative to placebo[38,62-64] than were lower EDRs (0.25-1.4).[65-68] We will administer one fixed dose of omega-3 fatty acids for the proposed trial. The omega-3 fatty acid dose, 1,800 mg/day (3 capsules/day: EPA: 1,200 mg/d, DHA: 600 mg/d), was selected because a lower fixed dose was found to be efficacious in pediatric (mean age=10 years) MDD patients (600 mg/d)[38] and a similar fixed dose was found to be efficacious in a primary prevention trial of adolescents (mean age=16 years) at high risk for psychosis (1,540 mg/d).[69] Additionally prior treatment studies[70-72] have found that comparable doses (1,000 - 2,160 mg/d), but not lower doses (200-482 mg/d), are required to increase RBC EPA+DHA content in young adults to a level thought to exert the greatest protection based on relative cross-national lifetime prevalence rates of MDD (U.S., 16.2% vs. Japan, <3%)[73,74] and RBC EPA+DHA % of total fatty acids (U.S., 4.9% vs. Japan, 8.5%).[75,76] The 12-week treatment duration was selected because 12-weeks of omega-3 supplementation has been found to normalize brain DHA concentrations in omega-3 fatty acid-deficient primates[77] and significantly reduce symptom severity in youth with MDD.[38]

Nutritional Supplement Accountability and Subject Compliance: A record of all clinical supplies received, dispensed and destroyed will be maintained by the study staff. Study supplement supplies will be stored at controlled room temperature until time of dispensing to the study subject. Dispensing records will include a medication log for each subject detailing supplement dose received at each study visit. Study subjects will be asked to return all unused study supplement and the supplement bottle at each study visit. The amount of study supplement returned (if any) will be recorded by the study staff. Compliance will be determined by evaluating the dispensing records at the time a participant is discharged from the study.

Image acquisition and fMRI: fMRI evaluations will be obtained on subjects at the baseline visit and at 12 weeks. All subjects will be scanned at the University of Cincinnati College of Medicine's Center for Imaging Research (CIR) using a 4.0 Tesla Varian Unity INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA). The INOVA system is controlled by a SUN workstation running Varian's VNMR-J™ and SPIN-CAD™ image processing and pulse-sequence development software under a Unix-based operating system. Data are stored on a networked hard drive (for off-line analysis) and additionally backed up by burning onto CDs or DVDs.

During the scan sessions, subjects recline in a supine position on the scanner bed. Nonferromagnetic goggles are positioned to provide clear visualization of the stimuli and a radio-frequency (RF) coil is placed over the subject's head. Padding is inserted around the subject's head to minimize movement. Headphones are provided to block background noise and so that investigators can communicate with the subjects during scan acquisition. A microphone in the scanner permits subjects to communicate with the MRI technician in case of concern or discomfort.

Following a three-plane gradient echo scan for alignment and brain localization, a shim procedure is performed to generate a homogeneous magnetic field. To provide anatomical localization for activation maps, a high-resolution, T1-weighted, 3-D brain scan is obtained using a modified driven equilibrium Fourier transform (MDEFT) sequence (T_{MD}=1.1 s, TR=13 ms, TE=6 ms, FOV=25.6 x 19.2 x 19.2 cm, matrix 256 x 192 x 96 pixels, flip angle=20 degrees). A midsagittal localizer scan is obtained to place 40 contiguous 4 mm axial slices that extend from the inferior cerebellum to encompass the entire brain. Subjects then complete an fMRI session in which scans

are acquired while performing a cognitive task (i.e. a CPT-END, described subsequently) using a T2*-weighted gradient-echo echoplanar imaging (EPI) pulse sequence (TR/TE=2000/30 ms, FOV=25.6 x 25.6 cm, matrix 64 x 64 pixels, slice-thickness=4 mm, flip angle=75 degrees). The CPT-END task, which engages both attentional and emotional brain networks., is used to examine ALN activation in bipolar adolescents and adults. During this paradigm, 70% of the visual cues are simple colored squares, 10% are simple colored circles, 10% are emotionally neutral pictures, and 10% are emotionally unpleasant pictures. Each cue presentation requires a response. The circles require a unique response (button 2), whereas the squares, neutral and unpleasant pictures all require the same response (button 1).

Following acquisition, fMRI and structural images are reconstructed for analysis using AFNI (Analysis of Functional NeuroImages; http://afni.nimh.nih.gov/afni). In AFNI, MDEFT (structural) and EPI (functional) images are co-registered using scanner coordinates. The functional data are then transformed to Talairach space through adoption of the landmarks set by the MDEFT images. Activation maps are created using a deconvolution algorithm that compares the actual hemodynamic response to a canonical hemodynamic response function. AFNI then generates an estimate of the 'fit coefficient' (i.e., beta weight or scaling factor) describing the magnitude of the hemodynamic response relative to the average signal intensity. Activation maps therefore consist of fit coefficients divided by the average signal intensity to create a percentage change score, which can be compared across subjects and between groups.

Proton (^{1}H) MRS: Proton MRS will employ a two echo-time, 2D-PRESS/MRS sequence, optimized for myoinositol detection, to collect ^{1}H-MRS data from 2 cm thick oblique voxels placed in specific regions of interest. Acquisition parameters will be: data matrix size=16x16, TR=2s; TE=30/136ms; tip-angle = 90/180/180°; Rx bandwidth=±1 kHz; complex-points=1024; readout duration=512ms; field of view (FOV)=20x20cm; nominal volume=3.215ml. The averages per phase-encode step (NEX) in k-space will vary throughout the data collection in a circularly bound, weighted manner, ranging from NEX=1 to NEX=5, according to a sinc-modulated partial k-space filter for maximum signal-to-noise in the final, resolved spectra. A water-unsuppressed scan will then be acquired, using a 30ms echo-time PRESS/MRS sequence with identical parameters, except the averages per phase-step will be reduced to 1. In all ^{1}H-MRS will be approximately 40 minutes in duration.

Sagittal images that bisect the two hemispheres of the brain are used to delineate between the structure of the entire cingulate and the corpus callosum, thus permitting accurate positioning of the oblique MRS slabs in such a manner that the region of the anterior cingulate situated just anterior and superior the tip of the corpus callosum and the basal ganglia are encompassed. Then axial images acquired from within the MRS slabs may be chosen that exactly traverse the anterior/superior section of the corpus callosum, thus clearly defining the anatomical boundary between the gray matter of the anterior cingulate and the sharply contrasting tissue of the corpus callosum. These images also clearly display the left and right prefrontal lobes as well as the individual structures in the basal ganglia. For the anterior cingulate, the MRS voxels may be positioned such that the center of the cube (voxel) lies over the anterior cingulate, with the cube centered bilaterally, including both the left and right anterior cingulate. Voxels may be centered over the left and right basal ganglia regions so that the signal in the voxel profile is originating from these defined structures.

MRS image registration and voxel positioning: All in vivo MRS data will be processed offline on a SunBlade100 UNIX Workstation. Varian Nuclear Magnetic Resonance (VNMR) software, Version 6.1b and custom-designed software will be used for MRS data reconstruction and voxel positioning

to obtain anatomically relevant spectra, with the aid of MR images. To obtain maximum consistency in the placement of each voxel within the brain for repeated measures within subjects high-quality T_1-weighted axial 3D mpFLASH image sets will be used. Axial images, acquired for each subject during every scan session, will be co-registered with the corresponding MRS scan, thus allowing shifting of voxels to exactly the same brain location with a high degree of precision each time. This approach, combined with careful, consistent, positioning of patients between scans is expected to yield a high level of reproducibility for any given brain region studied within the same subject.

For the purpose of image reconstruction, segmentation and tissue partial-volume analysis, the 3D FLASH axial image data sets are first reconstructed by Fourier-Transforming in 3-dimensions using VNMR and in-house software, and then converted into FDF (flexible data format) and Analyze™ binary image file formats. The image-processing tool ANALYZE 4.0 is used to digitally remove extra-cranial tissue from every image. These images are separated into three distinct binary tissue maps: gray- and white-matter (T_1-weighted) and CSF (T_2-weighted) by thresholding based on pixel intensities. To obtain the tissue ratio contribution for each ^{1}H-MRSI region-of-interest (ROI), the three segmented image data sets are convolved with the calculated three-dimensional point-spread function (^{3}D-PSF) of the ^{1}H acquisition. The partial volume contribution for each tissue type is then derived and expressed as a percentage of total tissue contribution for each ROI.

Spectral Analysis: We have implemented a spectral fitting routine that uses an iterative, non-linear, Marquardt-Levenberg algorithm in combination with prior spectral knowledge to precisely fit acquired spectra. The spectral model allows for the inclusion of various constraints in the spectral model such as spectral linewidths, phase/frequency offsets and lineshapes. The model template will be iteratively fitted to the raw data until the minimum residual (within an acceptable level of tolerance) between the raw and modeled data is reached. The fitting algorithm accounts for both zeroth- and first-order phase, thus alleviating the need for prior filtering, Fourier-Transformation and phase-correction of the data. If the spectrum from an entire anatomical structure is to be studied, multiple spectra can be collected and summed from the structure of interest. For maximal signal coherence, each spectra will first be corrected for zeroth-order phase shifts resulting from point-spread effects, as well as for frequency shifts resulting from Bo inhomogeneity, prior to summing.

b. Data Analyses and Data Monitoring:

Data Analyses

Prediction 1: Omega-3 fatty acid supplementation will be more effective than placebo for the treatment of mood symptoms in adolescents with a high risk for developing mania. A mixed effects model repeated measures (MMRM) approach will be used to compare change over time in CDRS-R (and YMRS) scores between treatment (omega-3 vs. placebo) groups. Covariates for these models will be time of assessment (study week) and treatment group. A mixed model approach will account for missing information while adjusting for the correlation of observations over time. We will also use MMRM to compare change over time in total mood burden rating. We will evaluate other potential covariates and confounds as described and will specifically examine the effects of co-occurring ADHD and anxiety disorders, and concomitant use of stimulants within groups. We will also compare treatment group differences in change from baseline to endpoint in YMRS and CDRS-R scores using an analysis of variance (ANOVA). This analysis will provide information without the necessary assumptions of the MMRM model regarding missing data and fitting the regression. We will calculate the effect sizes of group differences for change in CDRS-R and YMRS using the formula d=(mean change from baseline to endpoint group1-mean change from baseline to endpoint

group2)/pooled standard deviation. We will use a χ^2 test to compare percent responders (defined by CGI-I $\leq$ 2) at endpoint between treatment groups.

Prediction 2: Omega-3 fatty acid supplementation will be more effective than placebo for improving behavioral symptoms and psychosocial functioning in adolescents with a high risk for developing mania. A MMRM approach will be used to compare change over time in ADHD-RS scores between treatment groups. Additionally, change over time in CBCL and TRF externalizing and internalizing t-scores will be examined using a MMRM model. We will use a χ^2 test to determine whether the percent of subjects > 18 years old (vs. $\leq$ 18 year old) with completed CBCL and TRF ratings differs significantly between treatment groups. If there is no statistically significant difference, T-scores for 19-21 year olds will be determined using the gender-specific 18 year old t-scores, since we will be examining for group differences in change in score over time. We will use generalized linear mixed logistic regression models to examine change over time in percent responders (defined by achieving a level of functioning that is equal to or better than the month prior to baseline) in each of the four major areas of functioning; role performance, interpersonal relationships, recreational enjoyment, and sexual activity. A mixed effects model repeated measures (MMRM) approach will be used to compare change over time in CGAS scores between treatment groups. We will also evaluate change from baseline to endpoint in CGAS scores and calculate effect sizes as described. Covariates will be included in these models as described above. Additionally, we will examine the effect of change in mood symptom ratings during a specific time period (YMRS and CDRS-R) on change in behavioral and psychosocial functioning during the same time period by using, a repeated-measures framework in SAS PROC MIXED to account for the fact that multiple observations per individual on the same outcome are correlated across time.

Prediction 3: Omega-3 fatty acids will be safe and well tolerated compared with placebo. To evaluate the safety and tolerability of omega-3s we will use ANOVAs to compare change from baseline to week 12 (or endpoint) in vital signs and laboratory values. Additionally, we will determine the percentage of subjects who reported each adverse event and will examine treatment group differences in adverse events and serious adverse events using Fisher exact or χ^2 tests.

Prediction 4: Improvement in mood symptoms following treatment with omega-3 fatty acids is mediated by decreases Glu and mI levels and increases in NAA levels in the VLPFC (BA 10) and ACC (BA 24, 32)*:* Our hypothesized model predicts that treatment produces changes in metabolite levels (Glu, mI, and NAA). These metabolite changes lead to change in mood symptoms. The mediated link between treatment and mood symptoms via metabolite levels is the *indirect treatment effect*. The *direct treatment effect* represents any residual association between treatment and mood symptoms after adjusting for the *indirect effect*. This association will be smaller in magnitude than the unadjusted association between treatment and mood symptoms (which is the *total treatment effect* or the quantity of interest in Prediction 1). For the prediction of a mediated treatment effect to be supported, two conditions must be met, i.e., significant associations between treatment and metabolite levels and between metabolite levels and mood symptoms. Prediction 4(a) is that treatment with omega-3 fatty acids will lead to decreases in Glu and mI levels and increases in NAA levels within VLPFC and ACC. It is possible for this prediction to be supported in the absence of a general association between treatment and mood symptoms (Prediction 1), which would suggest that these metabolic changes do not impact clinical outcome. Prediction 4(b) is that decreases in Glu and mI levels in VLPFC and ACC, and increases in NAA in VLPFC and ACC, will lead to decreases in

mood symptoms. It is possible for this prediction to be supported in the absence of a general association between treatment and mood symptoms (Prediction 1), which would suggest that these metabolic changes impact clinical outcome but are not induced by omega-3 fatty acids (i.e., they are a treatment-independent marker for clinical improvement). If both components of Prediction 4 are supported, it will be of further interest to assess the extent of mediation: If the direct (i.e., residual) treatment effect is not statistically significant, the data are consistent with *complete mediation* (i.e., the treatment effect on mood is fully accounted for by the intermediate effect on metabolite levels). If other effects of treatment on mood symptoms not specified by this model exist, the direct (i.e., residual) treatment effect may remain significant and the data would be said to support *partial mediation*. Path analysis[82], which for linear models is a decomposition of the linear covariance matrix of the variables, will be used to analyze the fit of the proposed mediation model to our data. Path analysis can be thought of as an extension of linear regression to systems of simultaneous linear regression equations. This allows for the same variable to serve as an independent variable in some of the regressions and a dependent variable in others. In this study, changes in metabolite levels assume both roles: They are dependent variables in prediction 4(a) and independent variables in prediction 5(a). CDRS-R scores as well as other pilot mood measures will serve as the final outcome measures, with changes in metabolite levels as mediators and treatment group as an independent variable. Estimation of all the path coefficients, along with bootstrap computation of associated standard errors and significance levels (adjusted α=0.017 to control for multiple testing) will be carried out using the SAS macros,[78] which are available for download at http://www.comm.ohio-state.edu/ahayes/SPSS% 20programs/indirect.sas. We also will examine the effect of metabolite changes on behavioral symptoms (ADHD-RS scores), functional ratings and the pilot mood burden scale on these models in a set of expanded path models. The Bayesian Information Criterion (BIC) will be used to assess the goodness of fit of different path models to the data. This model comparison statistics combines the likelihood of the data will a penalty term that adjusted for the number of parameters used to fit the data. This statistic can be used to rank the mediation model against simpler models where one or both of the components of Prediction 4 are assumed to be absent, or where these paths are supported with respected to some metabolites but not others. Another comparison of interest (though secondary to our aims) is to compare the full path model (which shows partial mediation, since a path representing residual treatment effect is included) to a complete mediation model which does not have a direct path from treatment to mood symptoms (implying that that the correlation between treatment and mood symptoms, after accounting for metabolite levels, is zero). If both of the paths involving metabolite levels are null, but the correlation between treatment and mood symptoms is significant, the path model reduces to simple linear regression (i.e., a t-test, since the independent variable is binary). Comparison of overall model fit with BIC is important since a model in which the paths for predictions 4(a) and 4(b) fall somewhat close to significance, (e.g., p=0.05 for each path) may still give a better overall fit to the data than a model that omits both of these paths.

Prediction 5: The relationship between changes in RBC omega-3 fatty acid levels and changes in mood symptoms is mediated by metabolite changes. This prediction is similar to Prediction 4, but more precisely specifies the mechanism by which omega-3 fatty acids are hypothesize to influence mood symptoms. Specifically, changes in RBC omega-3 fatty acid levels (which are taken to be a surrogate for omega-3 levels in the brain) are predicted to be associated with changes in metabolite levels, which will in turn influence mood symptoms. These analyses will be performed in the sub-sample of subjects who are randomized to treatment with omega-3 fatty acids. Prediction 5(a) is that

increases in RBC omega-3 fatty acid levels will lead to decreases in Glu and mI levels and increases in NAA levels within VLPFC and ACC. While the mediation model of Prediction 4 specifies treatment assignment as the independent variable, in this set of models we treat RBC omega-3 levels within the omega-3 treated group as the predictor variable (although all subjects will receive the same nominal treatment the will display variation in their measured omega-3 levels due to differences in metabolism, diet and treatment compliance). Prediction 5(b) is that decreases in Glu and mI levels in VLPFC and ACC, and increases in NAA in VLPFC and ACC, will lead to decreases in mood symptoms. This component is the same as Prediction 4(b), but focused on the omega-3 treated group. While the metabolite level changes were dependent variables in Prediction 5(a), in Prediction 5(b) they are independent predictors of mood symptoms. As above, the covariance of these variables will be decomposed using path analysis, with α=0.017 used to assess significance of individual paths, and BIC used to rank competing models (e.g., simplified models with one or more paths deleted).

Exploratory analyses: In order to examine the potential impact of missing data on analyses, we will determine whether there are significant differences in demographic or clinical variables between completers and non-completers within and between treatment groups. We will examine the effect of any variables that differ and adjust analyses as necessary. Additionally, we will perform sensitivity analyses to determine whether missing data in the form of unknown changes in neurophysiologic parameters influenced our findings. Specifically, in regression analyses where change in mood symptom rating is predicted from change in a neurophysiologic and biological measure four analyses will be performed: No imputation (only available data is used), group mean imputation (for each missing change value, the mean change value of the corresponding treatment group will be substituted), and zero substitution (a change of zero is assumed for each missing change value). Additionally, associations between baseline neurophysiologic measures and subsequent "missingness" will be evaluated using regression analyses, predicting "missingness" on later scans from the baseline value, change in symptom ratings, and treatment assignment). We will also compare baseline neurometabolite levels (NAA, mI, Glu, Cho, and Cr) within each VOI in the subjects with a demographically matched subgroup of healthy control adolescents from the BITREC in order to determine the direction of any changes that occur in the subjects (i.e. whether they normalize following treatment with omega-3 fatty acids) using a separate regression for each VOI, with neurometabolite levels as the dependent variables and group (baseline at-risk adolescents with MDD vs. healthy controls) as the independent variable. Additionally, we will use multiple regression models to analyze associations between baseline neurochemical levels and omega-3 blood levels with change in mood and behavior rating in each treatment group. The treatment by change in rating scale interaction term will be the primary measure of interest. We will also explore the effects of nicotine use, illness duration, and change in behavioral, functional, and pilot mood burden measures on changes in omega-3 fatty acid levels and neurometabolite levels. Because these exploratory analyses are hypothesis-generating secondary analyses, we will use a standard 0.05 significance level as a threshold for interpreting interactions that may warrant further study. For all analyses, we will visually inspect plots of data points to identify outliers and will examine their clinical and neurophysiologic characteristics to determine their relevance to understanding treatment effects. We will examine the impact of clinical factors (e.g. ADHD and other co-occurring diagnoses) that may contribute to neurophysiologic, RBC fatty acid levels or efficacy findings as effect moderators. We will also conduct analyses of treatment group as a possible *moderator* of the association between changes in metabolite levels and mood symptoms. Moderator models allow the strength (possibly

even the direction) of an association between two variables to depend on the levels of moderating variables. We will assess moderation by the magnitude of the interaction terms in an ANCOVA model where change in mood symptoms is the outcome and the predictors are treatment group, change in metabolite levels, and interactions between metabolite changes and treatment group. A moderated relationship would be consistent with a pattern of results where an association between metabolite levels and mood symptoms exists in the omega-3 fatty acid group, but not in the placebo group. Such an outcome is plausible because placebo response may occur due to mechanisms that are independent of those we hypothesize as causes of treatment response.

Safety Monitoring: The data and safety monitoring plan for the proposed study will include monitoring of efficacy data by an independent Data Safety Monitoring Board (DSMB) and monitoring of tolerability data, including adverse events and serious adverse events, by the study investigators as well as the independent DSMB and the University of Cincinnati Medical Center Institutional Review Board (IRB). Adverse events will be monitored during the study using clinical interviews as well as a structured side effects questionnaire, the Side Effect Form for Children and Adolescents (SEFCA) that will be administered by an experienced clinician with extensive experience conducting clinical investigations of adolescents with mood disorders. They also will be responsible for evaluating all adverse events during study visits, which will occur at weekly visits or more frequently as necessary. Additionally, a study related physician will be accessible by pager to patients and their legal guardians 24 hours/day, 7 days/week during study participation. Dr. DelBello will also be responsible for reviewing all laboratory and safety measures from all subjects following each visit. An adverse event (AE) is any unexpected medical occurrence in a patient or clinical investigation subject who is administered a product and which does not necessarily have a causal relationship with the treatment. This includes any clinical or laboratory change that occurs at any time following consent that does not typically occur in that subject and is considered clinically significant. The frequency and severity of all observed or volunteered AEs regardless of treatment group or suspected causal relationship to the study treatment will be recorded throughout the study. Dr. DelBello will also be responsible for determining causal relationships between the study treatment and all AEs. Additionally, an independent, non-study related physician also will provide independent assessments of the causal relationship between AEs and the study treatment during the course of the study. Withdrawal from the study as a result of an AE or because of therapeutic measures taken to treat an AE will be at the discretion of Dr. DelBello. If a subject withdraws or is withdrawn from the study for any reason, a BITREC study physician will monitor subjects with any ongoing AE until the AE is resolved or determined to be stable. All AEs (including those present during screening) will be reported. However, for analytics purposes, only post-baseline (randomization) AEs will be considered for calculating treatment group differences in AEs. A serious adverse event is any adverse experience occurring during study participation that results in any of the following outcomes: death; a life threatening adverse drug experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability/incapacity; or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse experiences when, based on appropriate medical judgment of the study physician, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes in this definition. The judgment of whether a particular AE meets the above criteria for an SAE for the proposed study will be determined by Dr. DelBello, in conjunction with the proposed DSMB. It will also be Dr. DelBello's responsibility, to manage all SAEs and to make referrals for

appropriate care, as necessary. All SAEs will be reported to the IRB, the study DSMB, and the NIMH project officer within 48 hours of their discovery. The study blind will be broken at any point throughout the study as needed to protect the safety of a subject. An independent physician is available 24 hours/day, 7 days/week if it is necessary to break the blind in an emergency situation. All subject information will be de-identified when reporting serious adverse events. All AEs and SAEs will be entered into a database that is de-identified and password protected to ensure confidentiality. All data will be entered into two separate databases and checked for accuracy. Dr. DelBello will ensure that all patients have appropriate follow-up care after their study participation, as previously described.

In order to maximize the safety of the study participants, in addition to adverse event monitoring by study investigators, the proposed study will have a DSMB. The DSMB will consist of non-study related faculty with research related experience and will include a statistician, a psychiatrist with expertise in omega-3 fatty acids, and a child and adolescent psychiatrist. The DSMB members will not have a potential conflict of interest in study related outcomes. The DSMB will meet a minimum of every 6 months during the course of the study and will review tolerability, safety and efficacy data. The DSMB will also review all SAEs at the time of their occurrence and provide recommendations based on this review (e.g., that the study may continue or recommend modifications, including additional tolerability and efficacy measures). Every six months the DSMB will formally review all adverse events as well as all efficacy and tolerability data. Drs. Welge and DelBello will be responsible for providing updated efficacy and tolerability data to the DSMB every six months and the independent physician will provide information on group assignment without breaking the blind (e.g. group A and group B). The DSMB will assess the risks and benefits of study participation to all subjects and based on this assessment the DSMB will provide a written report of their analyses and recommendation as to whether the study should continue, modification to the study as needed or if the study should be terminated. Dr. DelBello, in conjunction with the DSMB, will be responsible for making certain that the DSMB files their report to the IRB as well as to the sponsor (NIMH) of the study. The DSMB will also provide the investigator with a summary of their report that will include their recommendations.

In order to maximize subject safety, study participants will be monitored daily by telephone (and in person if needed) during the screening period. Patients will be discontinued from study participation if at any two consecutive visits during the study they have a $\geq$ 9 point increase in CDRS-R score (1 point increase in > 50% of the 17 items of the CDRS-R) or $\geq$ 6 point increase in YMRS score (1 point increase in > 50% of the 11 items of the YMRS) from baseline. If any of the above occurs, we will refer the patient for treatment in BITREC or the community.

Finally, during study participation, all study participants will be monitored at each study visit or telephone interview for suicidal or homicidal ideation, plan, or intent. Specifically, in addition to a clinical interview, subject will be monitored for suicidality using the C-SSRS. We have incorporated this rating scale into several other studies of adolescents with mood disorders and we and our research staff have received formal training from Dr. Posner, one of the authors of the scale. If any subject experiences worsening of suicidality during the course of the study, they will be discontinued from study participation and receive appropriate clinical care through BITREC or if necessary, the inpatient psychiatry units. Subjects who, in the opinion of the investigator, are in need of inpatient hospitalization for suicidal or homicidal ideation will be excluded from study participation. If any subject has increased suicidality from baseline, as measured by the C-SSRS, they will be discontinued from the study. Additionally, all study participants, (and if < 18 years old, their legal guardians) will be provided with study physician contact information and instructed to contact their

study physician at any time, 24 hours/day, 7 days/week, in between visits should suicidal or homicidal ideation or any other emergency situation occur. We have successfully followed this protocol in our prior outcome and clinical trials studies. In the event that it is determined that a study participant needs psychiatric hospitalization, a study physician will contact appropriate parties and arrange for admission to University Hospital (if ≥ 18 years) or Cincinnati Children's Hospital Medical Center (if < 18 years).

c. Data Storage and Confidentiality: The principal investigator will maintain a secure database of all raw imaging data through the CIR. Data will include subject information (coded to meet HIPAA requirements), scan parameters, behavioral measures, clinical measures, and imaging data locators. The CIR conducts regular and frequent backups of imaging data. Processed imaging, demographic and clinical data will be managed in a database using Microsoft Access® and stored in an IBM compatible PC connected to other research group PCs through a password protected LAN. All data will be double entered and compared to ensure the integrity of the information. Full backups are performed weekly to protect against data loss. All data will be password protected. Source documents (e.g. study binders) with clinical information will be kept in locked file cabinets within locked offices within a security-monitored building (One Stetson Square, which has limited key-card access after business hours).

d. Setting: Assessments will be conducted in our (The Division of Bipolar Disorders Research) office suites at the University of Cincinnati College of Medicine's One Stetson Square development (260 Stetson St., ML 0516, Cincinnati, OH 45219-0516). All MRI scans will take place in the University of Cincinnati College of Medicine's Center for Imaging Research (CIR).

e. Laboratory methods and facilities: Blood draw analytes will be obtained in exam rooms located in the One Stetson Square building or Medical Sciences Building. Serum laboratory tests (plasma oxidation indices and cytokine concentrations, renal profile, complete blood count, liver & thyroid function tests, fasting lipid profile & glucose, and platelet function assay) will be performed by clinical laboratories associated with the University Hospital and affiliated out patient clinics. Red blood cell fatty acid composition will be performed by gas chromatography conducted at the Genome Research Institute.

f. Estimated Period of Time to Complete the Study: Approximately 3 years, to perform recruitment and scanning of 60 subjects. Timeline for the 36-month period of the proposed study: Month 1-33: Train study personnel and recruit an average of 1.8 subjects per month; Months 34-36: Complete subject participation, data entry, checking and analyses.

F. Human Subjects

1. Description of Subjects:

Patients: This is a 12-week double-blind randomized investigation of omega-3 fatty acid (N=30) vs. placebo (N=30) for the treatment of major depressive disorder and depressive disorder NOS in adolescents (N_{Total}=60). Patient selection will be done by research staff in consultation with and with permission from the PI. Inclusion/Exclusion criteria are found below (see F., 3.).

Healthy Controls: Although we will not recruit healthy subjects for the proposed study, they are recruited (n=40) already for BITREC Project 3 and undergo an initial ^{1}H-MRS scan. Therefore, we will be able to interpret the direction of neurobiological changes in at-risk adolescents (i.e., whether patient values are changing toward or away from those of healthy adolescents).

2. Estimated sample size: We propose to randomize 30 subjects per treatment group, for a total of 60 subjects at the University of Cincinnati. As this is an investigator-initiated protocol, there are no other sites associated with this submission.

Based on our prior studies, we estimate that approximately 25% of patients will either discontinue study participation prior to completing study procedures or will not have usable MRS data. For the power calculations described, we considered medium to large effect sizes of $d > 0.6$-0.8 to be clinically relevant.[79]

3. Description of Inclusion/Exclusion Criteria

Inclusion Criterion:

- Ages 9 -21 years old.
- At least one biological parent with bipolar I disorder.
- Meets DSM-IV-TR [80] criteria for major depressive disorder (MDD) or Depressive Disorder NOS at screening as determined by the Washington University at St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia, WASH-U-KSADS;[81]
- Childhood Depression Rating Scale-Revised Version[40,41](CDRS-R) scores ≥28 at screening and baseline.
- Fluent in English.
- Provision of written informed consent/assent as previously described.
- Agrees to use one of the following method of birth control: complete abstinence from sexual intercourse, barrier (diaphragm or condom), or oral/injectable contraceptive.

Exclusion Criterion:

- Contraindication to an MRI scan (e.g., metal clips, braces or claustrophobia).
- Mood symptoms resulting from acute medical illness or acute intoxication or withdrawal from drugs or alcohol as determined by careful medical evaluation or rapid symptom resolution.
- Psychotic symptoms (i.e., hallucinations or delusions).
- Any lifetime history of a manic or hypomanic episode.
- Any lifetime diagnosis of bipolar disorder not otherwise specified (NOS) or cyclothymia .

- A current diagnosis of dysthymia will not be exclusionary, if the adolescent also has a current diagnosis of MDD or Depressive Disorder NOS.
- A history of a major medical (e.g. diabetes) or neurological illness, laboratory abnormalities, or a significant episode (> 10 minutes) of loss of consciousness that could influence the MRS results, as determined by a study physician.
- Any history of alcohol or drug dependence (nicotine dependence is permitted).
- Allergy to shellfish or seafood.
- Mental retardation (IQ<70) as determined by the Wechsler Abbreviated Scale of Intelligence (WASI), administered by a research coordinator who is a trained psychometrician.
- A positive serum pregnancy test or lactating.
- A history of intolerance, hypersensitivity or non-response to omega-3 fatty acids.
- Any history of a clotting disorder that increases risk for bleeding in themselves or a first-degree relative, since omega-3 fatty acids may be associated with anti-coagulant effects.
- Concomitant use of medications with anticoagulant effects (e.g. aspirin).
- A lithium or valproate serum level of $\geq$0.4 mEq/L and 30 mg/L, respectively at baseline.
- Use of antipsychotics, other mood stabilizers, stimulants (if opting to discontinue), or atomoxetine within 72 hours (aripiprazole within two weeks will be exclusionary because of its long half-life) or antidepressants within 5 days (fluoxetine within one month will be exclusionary because of its long half-life). Patients treated with a depot antipsychotic within one dosing interval of baseline will be excluded. Subjects diagnosed with ADHD and taking a stable dose of stimulants for the previous month will be permitted to continue if it is determined necessary by subject, primary caregiver, and treating clinician report in conjunction with the study physician.
- Concomitant use of other psychotropic medications or medications with central nervous system (CNS) effects within 5 half-lives from baseline MRI scan or prior treatment with a medication with CNS effects that requires more than 5 days of a screening period.
- Any psychiatric symptom that requires admission to an inpatient psychiatric hospital, as determined by a study physician.
- Any initiated psychotherapy within 2 months prior to the screening visit, or plans to initiate psychotherapy during study participation. Adolescents who present with their current depressive episode despite longer-term psychotherapy (i.e., >2 months) may be included. For participants who enter the study on psychotherapy, the type and frequency of therapy will remain constant during the study.

4. Inclusion of children, women, and minorities: The proposed study exclusively involves children ages 9-21 years old. As previously noted, we are not including children below age 9 years as it is not possible to control for age-related differences in brain development with the number of subjects we are proposing to study. We expect to enroll equal numbers of females and males in this study consistent with the epidemiology of offspring of parents with bipolar I disorder and of the local Cincinnati community (52.8% women and 47.2% men). Efforts will be made to have the study population reflect the ethnic distribution of the catchment area covered by the University of Cincinnati, which serves a population that is approximately 50% White, 45% African-American and 2% Asian, and 3% other racial groups, and the local Cincinnati area, which according to the 2000 Census report has a racial make-up of 53% White, 43% African American, 0.21% Native American, 1.55% Asian, 0.04% Pacific Islander, 0.63% from other races, and 1.68% from two or more races, and 1.28% of the population are Hispanic or Latino of any race.

NIH guidelines for the inclusion of females and minorities as subjects in clinical research will be followed. We will attempt to recruit equal numbers of each gender from each of the above racial groups. Most of our prior clinical trials achieved these gender, racial, and ethnic representations, and we expect the same for this study. We will analyze demographic data every four months and if we are failing to achieve these marks, we will increase our recruitment of the under-represented group by extending recruitment to other area clinics that include the under-represented group. There will be no exclusion of subjects based on gender, race, or ethnicity.

5. Study population source: Adolescents (ages 9-21 years) with a current DSM-IV-TR diagnosis of major depressive disorder (MDD) or Depressive Disorder NOS will be recruited from the University of Cincinnati BITREC "at-risk" cohort (n=140) of adolescent offspring of parents with bipolar I disorder.

6. Recruitment plans: Recruitment of subjects will be performed by study staff in consultation with Division of Bipolar Disorders Research group's physicians. Primarily, subjects will be recruited from an already UC-IRB approved study (#07-04-10-03, BITREC Project 3, PI-DelBello) and from screen fails to the same. Other potential recruitment means include referral or word-of-mouth. A study flyer is not requested at this time, but may be warranted in the future.

G. Risk/Benefit Assessment

1. Risk Level: Although previous studies involving omega-3 fatty acids have shown high tolerability and minimal clinically relevant adverse events, omega-3 fatty acids' efficacy in treating major depressive disorder has not been fully established. The US FDA has not given approval of using omega-3 fatty acids for treating mental illness in the child, adolescent or adult populations. However, using omega-3 fatty acids to treat adolescent major depressive disorder holds the prospect of a direct benefit for participants. Such potential direct benefits of study participation may include: symptom resolution, psychiatric disorder identification in a population already at-risk for developing mental illness, identification of gross brain abnormalities, as well as improvement in cardiac and brain health. It should be noted that use of a placebo in half of proposed subjects is proposed. Therefore, with the above considerations in mind, the investigators feel that the participating in this study involves more than minimal risk.

2. Anticipated Benefit Justifies the Risk: Potential benefits include a thorough psychiatric evaluation performed by investigators with expertise in the diagnosis and treatment of mood disorders, and the chance to contribute to a scientific investigation, which may be of benefit to patients with similar illnesses in the future. Additionally, study participants may also benefit from the omega-3 fatty acid treatment that they receive free of charge during study participation in that it may reduce their mood and behavioral symptoms. If requested by any study subject or their legal guardian, information obtained from this study will be available to that subject's primary clinician. Subjects and their legal guardians will be informed that the MR scan is preformed for research purposes and is not of clinical use, but that a neuroradiologist will review all MR scans for gross abnormalities and the subject and (if < 18 years old) the subject's legal guardian will be informed if an abnormality is detected. That said, the risks associated with this study are deemed more minimal to the participants with potential direct benefit from their study participation as previously described. Therefore, in the opinion of the investigators, the benefits associated with this study outweigh the risks.

3. Anticipated Benefit as Favorable as Alernative:

a. Increase Over Minimal Risk Description: This proposal represents an increase over minimal risk due to 1) study design, which involves a 50/50 chance of receiving placebo, 2) use of an US FDA supplement that is not approved for treating MDD or other mental illness, and 3) the proposed study population of adolescents.

b. Commensurate Experiences: Although omega-3 fatty acids supplementation is not a standard-of-care treatment for MDD or Depressive Disorder NOS, this proposal does involve comparable activities that one might receive while treated for MDD or Depressive Disorder NOS. Blood draws, mood interviews, and in some instances an MRI brain scan (to rule out neurological abnormality as causing mood change) are procedures one with MDD or Depressive Disorder NOS might undergo. Also, given the wide availability of omega-3 fatty acids in retail stores, receiving omega-3 fatty acids as part of this protocol might be commensurate to the public seeking holistic approaches to mental illness and general fully body health.

c. Generalizable Knowledge to Be Gained: The morbidity and mortality associated with mood disorders during adolescence is significant. The proposed study will systematically examine the therapeutic and associated neurochemical effects of omega-3 fatty acid supplementation for the treatment of mood and behavioral symptoms in adolescents with a high risk

for developing incipient mania (i.e. they are adolescents with MDD or Depressive Disorder NOS and a parent with bipolar I disorder who are thus, at an increased risk to develop bipolar disorder themselves). Since there are presently no evidence-based treatments for mood and behavioral symptoms in adolescents with a bipolar parent, studies evaluating the efficacy, tolerability and neurochemical effects of potential treatments are necessary and serve as an initial step toward establishing early intervention and ultimately preventative strategies for bipolar disorder. Moreover, understanding the neurophysiological features associated with omega-3 fatty acid supplementation and response may provide neurobiological targets for early detection, treatment development, and predicting treatment response that are currently lacking in bipolar disorder, and may be more difficult to identify later in the course of illness. Ultimately, by achieving this goal, we may be able to move toward rational early intervention strategies that will improve the lives of our patients and their families and to decrease the morbidity and mortality associated with bipolar disorder. In this proposal we outline a protocol to make these initial steps. Given that there is more than minimal risk to study participation for our subjects, it is the opinion of the investigators of this application that the importance of the knowledge to be gained as a result of the proposed research outweighs the minimal risk to the subjects.

Adolescents (and legal guardians, if < 18 years old) will receive written informed consent and assent (if <18 years old) along with verbal explanations of the entire research protocol. Adolescents (and legal guardians, if <18years old) approached for study participation will, at all times, have opportunity to ask any questions related to the study directly of the principal investigator. Subjects (and legal guardians if < 18 years old) will be told of all potential risks, benefits and alternatives to study participation, which will be fully described in the informed consent document. Consistent with the Federal guidelines, participants will be informed, verbally and in the written consent form, of the federally mandated reporting laws for child abuse and neglect. Subjects will also be told that agents of the University of Cincinnati Medical Center Institutional Review Board, the National Institutes of Health, and the Food and Drug Administration will be allowed to inspect sections of their medical and research records related to this study, if requested. Thus, except under circumstances covered under the mandated child abuse reporting laws, situations in which the adolescent and/or a family member is judged to be a danger to him- or herself or others, or the above agencies request information, no information about the adolescent or family will be shared with any individual or agency without prior written consent. If subjects are judged to be a danger to themselves or others, appropriate referrals to Cincinnati Children's Hospital Medical Center or University Hospital psychiatric emergency rooms will be made by a study related physician. The adolescents and legal guardians will be informed and it will be understood that acceptance or refusal to participate in the study will not influence their ability to receive clinical care at Children's Hospital Medical Center, University Hospital or elsewhere, and that they are free to withdraw from the study at any time. After review of the consent form with the subject (and their legal guardian, of < 18 years old), each will be given a seven-question "quiz" to make certain that they understood the main points of the consent form. Any wrong answers will be reviewed to make certain that they understand the procedures.

d. Risks/Discomforts Description: Potential risks of this study include the lack of omega-3 fatty acid efficacy in treating MDD or Depressive Disorder NOS. Study staff will have regular contact with all study participants while they are screening and over the course of the study. Clinical assessments will be performed in person as scheduled or as needed. As previously described under Safety Monitoring, patients will be discontinued from study participation if at any two consecutive visits during the study they have a $\geq$ 9 point increase in CDRS-R score (1 point

increase in > 50% of the 17 items of the CDRS-R) or ≥ 6 point increase in YMRS score (1 point increase in > 50% of the 11 items of the YMRS) from baseline. If any of the above occurs, we will refer the patient for standard-of-care treatment in BITREC (which is a collection of already UC-IRB approved treatment studies) or the community.

Another potential risk is that patients will be randomized to treatment with placebo (which is an olive oil supplement). These subjects will be monitored as stated in the previous paragraph. A subsequent decompensation in symptoms will result in removal from this protocol and refer to appropriate standard-of-care treatment.

There is also the risk of adverse events from the medication used in the proposed study. The most common reported side effects of omega-3 fatty acid supplementation include an increased in bleeding/clotting time and a "fishy" burp. Bleeding/clotting time will be measured by lab analysis to ensure that such values are within lab-determined normal limits. Burping will be recorded in the adverse event log and subjects will be asked if such event is tolerable. If the study staff learns of any new possible risk or side effects of these drugs, subjects will be notified immediately.

Alternatives to treatment with omega-3 fatty acids for MDD or Depressive Disorder NOS include other medications commonly used to treat depression in adolescents, such as fluoxetine, sertraline, paroxetine, citalopram, and escitalopram. All of these medications have side effects. Currently, only fluoxetine and escitalopram are US FDA approved for sure in adolescents with major depressive disorder. Omega-3 fatty acids supplementation was chosen because of the low-occurrence and lesser severity of side effects, as well as its success in some previous trials. Alternatives to treatment for MDD or Depressive Disorder NOS may also include psychological interventions, such as talk-based therapy, which have been shown efficacious in prior studies.

There is a risk that the subject's condition may worsen during the course of the study. A subject will be discontinued from the study if he/she is assessed to be at serious risk for suicide or homicide or it becomes necessary to hospitalize a subject due to exacerbation of current symptoms. The PI, along with the co-investigators and study coordinators will monitor participants' illness progression through weekly visits (which include mood rating measures, overall improvement measures, and questions about AE occurrence) and through interim-visit telephone contact. The PI and study staff will make arrangements for aftercare if the participants terminates, whether early or after study completion.

Other risks of study participation are those associated with simple venipuncture (inserting a needle into a vein to draw blood), which include discomfort and/or bruising at the site of the puncture. Less commonly, fainting, the formation of a small blood clot, swelling of the vein and surrounding tissue, bleeding from the puncture site or infection at the site where the blood was drawn may occur. To minimize the risks associated with blood draws, only staff that is trained and certified in phlebotomy will draw the blood. To guard against frustration and fatigue, interviewers will be carefully selected and trained.

Fatigue and frustration from repeated evaluations may also occur. Subjects will be asked during visits if they are agreeable to proceeding with the visit and study procedures. Breaks, when possible, will be given periodically during visits. The same may be true of receiving an MRI scan. Subjects will be asked if they are agreeable to receiving an MRI scan at the baseline and Week 16 visit.

Although a number of prior studies indicate omega-3 fatty acid supplementation is beneficial to pregnancy and a developing fetus, many studies have been inconclusive. Thus, there may be unknown teratogenic effects on a fetus exposed to high levels of omega-3. In order to decrease the risk of harm to females and their fetus if pregnant, pregnancy at any point while enrolled is

exclusionary for this protocol. Urine pregnancy screens at time points throughout study participation are performed to verify a subject's verbal declaration of not being pregnant, or verbal declaration to the contrary. All potential female participants are informed, verbally and through the informed consent document, that pregnancy is exclusionary at all times and that getting pregnant may expose that individual and her fetus to unknown risks. Participants will be reminded of pregnancy exclusion and risks during study visits.

Although there is no known risk to subjects or their fetus if receiving an MRI scan, exposing subjects or their fetus to the high magnetic fields generated by an MRI machine may involve unknown risks. Thus, subjects will not be pregnant at any point in this study.

Any subject who becomes pregnant during participation in a clinical study for which pregnancy is a standard exclusion criterion must be promptly withdrawn from the study. Long-term follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required. We will be monitoring all female subjects for pregnancy each month. Subjects receiving an MRI will receive a urine pregnancy before receiving a scan.

The effects of the drugs on the male reproduction are not entirely known. Therefore, male subjects should not cause a pregnancy. If a male subject causes a pregnancy during this study, long-term follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant may be required.

Since this study involves subjects that use an illegal substance, cannabis, and since this study involves subjects whose risk of using other illegal substances is high, there is a risk that subjects may be incarcerated while enrolled in this protocol. In the event that a currently enrolled subject is incarcerated, the University of Cincinnati Medical Center Institutional Review Board will be informed and, either the subject will be removed from the study if the PI determines that the study medicines and therapy are not tolerated or efficacious, or permission will be requested of the IRB to allow for the inclusion of prisoners and the subject will continue study treatment until follow-up care can be arranged.

As with any study, there is a potential risk of loss of confidentiality, although every attempt will be made to ensure confidentiality, unless mandated by the law or a situation where an adolescent or parent is a danger to him- or herself or others as described below. The coding of subjects immediately following provision of informed consent/assent to mask their name and identifying information minimizes this risk. All information on subjects will be stored in binders with subject codes only and these binders will be kept in a locked cabinet. In addition, all information on subjects will be stored with subject codes only on computer databases in which access is limited to research personnel. All publications resulting from the study will be presented as group data; no identifying information will be used. In the event of data sharing all subject information will be de-identified. Consistent with the Federal guidelines, participants will be informed of the federally mandated reporting laws for child abuse and neglect, verbally and in the written consent form. Therefore, if abuse or neglect is suspected the Department of Human Services will be called. Risks associated with this include embarrassment, legal consequences, and removal of the child from the legal guardians' home. Subjects will be informed during the consenting procedure that if, at any time, they or their legal guardians decide to terminate study participation, they will be given a referral for further treatment as indicated. Additionally, if the study physician determined it is unwise for the subject to continue in the study because of ineffectiveness or intolerable side effects of the study medication, their participation will be stopped. Patients who are discontinued from the study for any reason will be given a referral for further treatment as indicated.

There are no human risks known to undergoing an MRI. The risk of feeling claustrophobic while in an MRI does exist and subjects will be made aware of this prior to signing consent.

The risks to the participating subjects are reasonable in relation to proposed benefits to mankind. Indeed, the US FDA has mandated the pharmaceutical industry to further clinical studies in the pediatric population in order to provide data on currently available therapies. The information obtained from subjects participating in this protocol will potentially serve to better treat individuals with similar diagnoses and symptoms in the future. Furthermore, information from this study might provide those suffering from mental illness, especially MDD, a more holistic and cost-effective alternative to current treatment options.

H. Payment: Subjects will be reimbursed for participating in each study visit. They will receive

- $20 for completing screening procedures,
- $20 for completing baseline assessments,
- $20 for completing each follow-up visit (pro-rated at $15 for the visit + $5 additional for remembering to bring in their pill dairy and bottles for pill counts), and
- $50 from the proposed study for participation in the week 12 (or termination) MRS scan. It should be noted that, although subjects will undergo a baseline MRS scan for this proposal, subjects will do so concurrently for the BITREC Project 3 study and are thus paid $50 for that scan though BITREC Project 3.

Payment will be in the form of cash paid firsthand to the subject or check sent via mail to the subject.

I. Subject Costs: There will be no costs incurred by the subjects as a result of participating in this study. However, patients are expected to provide for clinical care that is not part of this research protocol (e.g., hospitalization costs) through standard health care payment procedures. Subjects will also be responsible for acquiring their own omega-3 fatty acids supplements, if they chose to continue such treatment, after study termination.

J. Consent/Assent Forms: See attached.

K. Literature Cited:

1. Lish JD, Dime-Meenan S, Whybrow PC, et al. The National Depressive and Manic-depressive Association (DMDA) survey of bipolar members. J Affect Disord 1994; 31:281-294.

2. Perlis RH, Miyahara S, Marangell LB, et al. STEP-BD Investigators. Long-Term implications of early onset in bipolar disorder: data from the first 1000 participants in the systematic treatment enhancement program for bipolar disorder (STEP-BD). Biol Psychiatry 2004; 55:875-881.

3. Hirschfeld RM, Vornik LA. Recognition and diagnosis of bipolar disorder. J Clin Psychiatry. 2004;15:5-9.

4. Goodwin FK, Jamison KR. Manic-Depressive Illness. New York: Oxford University Press, 1990; pp.134-136.

5. DelBello MP, Geller B. Review of studies of child and adolescent offspring of bipolar parents. Bipolar Disord. 2001;3:325-334.

6. Singh MK, DelBello MP, Stanford KE, Soutullo C, et al. Psychopathology in children of bipolar parents. J Affect Disord. 2007;102:131-136.

7. Dwyer JT, DeLong GR. A family history study of twenty probands with childhood manic-depressive illness. J Am Acad Child Adolesc Psychiatry. 1987;26:176-80.

8. Todd RD, Reich W, Petti TA, et al. Psychiatric diagnoses in the child and adolescent members of extended families identified through adult bipolar affective disorder probands. J Am Acad Child Adolesc Psychiatry. 1996;35:664-671.

9. Strober M, Morrell W, Burroughs J, et al. A family study of bipolar I disorder in adolescence. Early onset of symptoms linked to increased familial loading and lithium resistance. J Affect Disord 1988;15:255-268.

10. Chang KD, Steiner H, Ketter TA. Psychiatric phenomenology of child and adolescent bipolar offspring. J Am Acad Child Adolesc Psychiatry 2000;39:453-460.

11. Geller B, Zimerman B, Williams M, et al. Bipolar disorder at prospective follow-up of adults who had prepubertal major depressive disorder. Am J Psychiatry. 2001;158:125-127.

12. Baumer FM, Howe M, Gallelli K, Simeonova DI, et al. A pilot study of antidepressant-induced mania in pediatric bipolar disorder: Characteristics, risk factors, and the serotonin transporter gene. Biol Psychiatry 2006;60:1005-1012.

13. Cicero D, El-Mallakh RS, Holman J, Robertson J. Antidepressant exposure in bipolar children. Psychiatry. 2003;66:317-322.

14. DelBello MP, Soutullo CA, Hendricks W, Niemeier RT, et al. Prior stimulant treatment in adolescents with bipolar disorder: association with age at onset. Bipolar Disord 2001;3:53-57.

15. Faedda GL, Baldessarini RJ, Glovinsky IP, Austin NB. Treatment-emergent mania in pediatric bipolar disorder: a retrospective case review. J Affect Disord. 2004;82:149-158.

16. Martin A, Young C, Leckman JF, Mukonoweshuro C, et al. Age effects on antidepressant-induced manic conversion. Arch Pediatr Adolesc Med 2004;158:773-780.

17. Reichart CG, Nolen WA. Earlier onset of bipolar disorder in children by antidepressants or stimulants? An hypothesis. J Affect Disord. 2004;78:81-84.

18. Soutullo CA, DelBello MP, Ochsner JE, McElroy SL, et al. Severity of bipolarity in hospitalized manic adolescents with history of stimulant or antidepressant treatment. J Affect Disord. 2002;70:323-327.

19. Geller B, Williams M, Zimerman B, et al. Prepubertal and early adolescent bipolarity differentiate from ADHD by manic symptoms, grandiose delusions, ultra-rapid or ultradian cycling. J Affect Disord 1998; 51:81-91.

20. Geller B, Sun K, Zimerman B, et al. Complex and rapid-cycling in bipolar children and adolescents: a preliminary study. J Affect Disord 1995;34:259-268.

21. Martinez-Aran A, Vieta E, Colom F, et al. Cognitive dysfunctions in bipolar disorder: evidence of neuropsychological disturbances. Psychother Psychosom 2000; 69:2-18.

22. Strakowski SM, DelBello MP, Adler CM. The functional neuroanatomy of bipolar disorder: a review of neuroimaging findings. Molecular Psychiatry 2005; 10:105-116.

23. Strakowski SM, Adler CM, Holland SK et al. A preliminary FMRI study of sustained attention in euthymic, unmedicated bipolar disorder. Neuropsychopharmacology 2004; 29:1734-1740.

24. DelBello MP, Adler CM, Strakowski SM. The neurophysiology of childhood and adolescent bipolar disorder. CNS Spectr 2006;11:298-311.

25. DelBello MP, Strakowski SM. Neurochemical predictors of response to pharmacologic treatments for bipolar disorder. Curr Psychiatry Rep. 2004; 6:466-472.

26. Hibbeln JR. Fish consumption and major depression. Lancet 1998;351:1213.

27. Noaghiul S, Hibbeln JR. Cross-national comparisons of seafood consumption and rates of bipolar disorders. Am J Psychiatry. 2003;160:2222-2227.

28. Peet M. International variations in the outcome of schizophrenia and the prevalence of depression in relation to national dietary practices: an ecological analysis. Br J Psychiatry 2004;184:404-408.

29. Assies J, Lok A, Bockting CL, Weverling GJ, et al. Fatty acids and homocysteine levels in patients with recurrent depression: an explorative pilot study. Prostaglandins Leukot Essent Fatty Acids 2004;70:349-356.

30. Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. J Affect Disord 1998;48:149-155.

31. Frasure-Smith N, Lesperance F, Julien P. Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. Biol Psychiatry 2004;55:891-896.

32. Maes M, Christophe A, Delanghe J, Altamura C, et al. Lowered omega-3 polyunsaturated fatty acids in serum phospholipids and cholesterol esters of depressed patients. Psychiatry Res 1999;85:275-291.

33. Mamalakis G, Tornaritis M, Kafatos A. Depression and adipose essential polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids 2002;67:311-318.

34. Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. Biol Psychiatry 1998;43:315-319.

35. Tiemeier H, van Tuijl HR, Hofman A, Kiliaan AJ, et al. Plasma fatty acid composition and depression are associated in the elderly: the Rotterdam Study. Am J Clin Nutr 2003; 78:40-46.

36.Chiu CC, Huang SY, Su KP, Lu ML, et al. Polyunsaturated fatty acid deficit in patients with bipolar mania. Eur Neuropsychopharmacol 2003; 13:99-103.

37. Ranjekar PK, Hinge A, Hegde MV, Ghate M, et al. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. Psychiatry Res 2003;121:109-122.

38. Nemets H, Nemets B, Apter A, Bracha Z, et al. Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. Am J Psychiatry 2006;163:1098-1100.

39. Wozniak J, Biederman J, Mick E, Waxmonsky J. Omega-3 fatty acid monotherapy for pediatric bipolar disorder: a prospective open-label trial. Eur Neuropsychopharmacol. 2007;17:440-447.

40. Poznanski EO, Cook SC, Carroll BJ, Corzo H. Use of the Children's Depression Rating Scale in an inpatient psychiatric population. J Clin Psychiatry 1983; 44:200-203.

41. Poznanski EO, Cook SC, Carroll BJ. A depression rating scale for children. Pediatrics 1979; 64:442-450.

42. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. Br J Psychiatry 1978;133:429–435.

43. Fristad MA, Weller EB, Weller RA. The Mania Rating Scale: can it be used in children? A preliminary report. J Am Acad Child Adolesc Psychiatry 1992;31:252-257.

44. Fristad MA, Weller RA, Weller EB. The Mania Rating Scale (MRS): further reliability and validity studies with children. Ann Clin Psychiatry 1995;7:127-132.

45. Guy W. National Institute of Mental Health. In: ECDEU Assessment Manual for Psychopharmacology. Revised 1970:12-1-12-6.

46. DuPaul GJ, Power TJ, Anastopolous AD, Reid R. ADHD Rating Scale–IV: Checklists, Norms, and Clinical Interpretations. New York, NY: Guilford Press; 1998.

47. Farles DE, Yalcin I, Harder D, Heiligenstein JH. Validation of the ADHD Rating Scale as a clinician administered and scored instrument. J Atten Disord 2001;5:39-47.

48. Chang KD, Steiner H, Ketter TA. Psychiatric phenomenology of child and adolescent bipolar offspring. J Am Acad Child Adolesc Psychiatry 2000; 39:453-460.

49. Richardson AJ, Puri BK. A randomized double-blind, placebo-controlled study of the effects of supplementation with highly unsaturated fatty acids on ADHD-related symptoms in children with specific learning difficulties. Prog Neuropsychopharm and Biol Psychiatry 2002;26:233-239.

50. Stevens L, Zhang W, Peck L, Kuczek T, Grevstad N, Mahon A, Zentall SS, Arnold LE, Burgess JR. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. Lipids 2003;38:1007-1021.

51. Joshi K, Lad S, Kale M, Patwardhan B, Mahadik SP, Patni B, Chaudhary A, Bhave S, Pandit A. Supplementation with flax oil and vitamin C improves the outcome of Attention Deficit Hyperactivity Disorder (ADHD). Prostaglandins Leukot Essent Fatty Acids 2006;74:17-21.

52. Sorgi PJ, Hallowell EM, Hutchins HL, Sears B Effects of an open-label pilot study with high-dose EPA/DHA concentrates on plasma phospholipids and behavior in children with attention deficit hyperactivity disorder. Nutr J. 2007;6:16.

53. DelBello MP, Kowatch RA, Adler CM et al. A double-blind randomized pilot study comparing quetiapine and divalproex for adolescent mania. J Am Acad Child Adolesc Psychiatry 2006; 45:305-313.

54. DelBello MP, Schwiers ML, Rosenberg HL, Strakowski SM. A double-blind, randomized, placebo-controlled study of quetiapine as adjunctive treatment for adolescent mania. J Am Acad Child Adolesc Psychiatry 2002; 41:1216-1223.

55. Strakowski SM, DelBello MP, Fleck DE et al. The effects of co-occurring alcohol abuse on the course of bipolar disorder following a first hospitalization for mania. Arch Gen Psychiatry 2005;62:851-858.

56. Posner K, Brent D, Lucas C, Gould M, Stanley B, Brown G, Fisher P, Zelazny J, Burke A, Oquendo M, Mann J. Columbia-suicide severity rating scale (C-SSRS). New York State Psychiatric Institute.

57. Shaffer D, Gould MS, Brasic J, Ambrosini P, Fisher P, Bird H, Aluwahlia S: A Children's Global Assessment Scale (CGAS). Arch Gen Psychiatry 1983;40:1228-1231.

58. DelBello MP, Hanseman D, Adler CM, Fleck DE, Strakowski SM. Twelve-month outcome of adolescents with bipolar disorder following first hospitalization for a manic or mixed episode. Am J Psychiatry 2007;164:582-590.

59. Leon AC, Solomon DA, Mueller TI, Turvey CL, Endicott J, Keller MB. The Range of Impaired Functioning Tool (LIFE-RIFT): a brief measure of functional impairment. Psychol Med 1999;29:869-878.

60. Achenbach TM: Manual for the Child Behavior Checklist. 1991; Burlington University of Vermont Department of Psychiatry.

61. Achenbach TM: Manual for the Teacher's Report Form. 1991; Burlington University of Vermont Department of Psychiatry.

62. Stoll AL, Severus WE, Freeman MP, Rueter S, et al. Omega 3 fatty acids in bipolar disorder: a preliminary double-blind, placebo-controlled trial. Arch Gen Psychiatry 1999;56:407-412.

63. Su KP, Huang SY, Chiu CC, Shen WW. Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. Eur Neuropsychopharmacol 2003;13:267-271.

64. Su KP, Huang SY, Chiu TH, Huang KC, Huang CL, Chang HC, Pariante CM. Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. J Clin Psychiatry 2008;69:644-651.

65. Silvers KM, Woolley CC, Hamilton FC, Watts PM, et al. Randomised double-blind placebo-controlled trial of fish oil in the treatment of depression. Prostaglandins Leukot Essent Fatty Acids 2005;72:211-218.

66. Freeman MP, Davis M, Sinha P, Wisner KL, Hibbeln JR, Gelenberg AJ. Omega-3 fatty acids and supportive psychotherapy for perinatal depression: A randomized placebo-controlled study. J Affect Disord 2008. [Epub ahead of print].

67. Grenyer BF, Crowe T, Meyer B, Owen AJ, Grigonis-Deane EM, Caputi P, Howe PR. Fish oil supplementation in the treatment of major depression: a randomised double-blind placebo-controlled trial. Prog Neuropsychopharmacol Biol Psychiatry 2007;31:1393-1396.

68. Rogers PJ, Appleton KM, Kessler D, Peters TJ, Gunnell D, Hayward RC, Heatherley SV, Christian LM, McNaughton SA, Ness AR. No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. Br J Nutr 2008;99:421-431.

69. Amminger GP, Schaefer MR. et al. Omega-3 fatty acids reduce the risk of early transition to psychosis in ultra-high risk individuals: A double-blind randomized, placebo-controlled treatment study. International Conference of Schizophrenia Research (ICOSR), Colorado 2007.

70. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. Clin Chem 2006;52:2265-2272.

71. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. Am J Clin Nutr 2007;86:1621-1625.

72. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 2006;83:1467S-1476S.

73. Kessler RC, Merikangas KR, Wang PS. Prevalence, comorbidity, and service utilization for mood disorders in the United States at the beginning of the twenty-first century. Annu Rev Clin Psychol 2007;3:137-158.

74. Kawakami N, Shimizu H, Haratani T, Iwata N, Kitamura T. Lifetime and 6-month prevalence of DSM-III-R psychiatric disorders in an urban community in Japan. Psychiatry Res 2004;121:293-301.

75. Sands SA, Reid KJ, Windsor SL, Harris WS. The impact of age, body mass index, and fish intake on the EPA and DHA content of human erythrocytes. Lipids 2005;40:343-347.

76. Itomura M, Fujioka S, Hamazaki K, Kobayashi K, Nagasawa T, Sawazaki S, Kirihara Y, Hamazaki T. Factors influencing EPA+DHA levels in red blood cells in Japan. In Vivo 2008;22:131-135.

77. Connor WE, Neuringer M, Lin DS. Dietary effects on brain fatty acid composition: the reversibility of n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys. J Lipid Res 1990;31:237-247.

78. Preacher, K. J., & Hayes, A. F. (in press). Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. *Behavior Research Methods.*

79. Cohen. Statistical Power Analysis for the Behavioral Sciences. 1997; New York, Academic Press.

80. American Psychiatric Association: *Diagnostic and Statistical Manual of Mental Disorders. (DSM-IV-TR) (Text Revision), 4th Edition* 2000; Washington, DC: American Psychiatric Press.

81. Geller B, Zimerman B, Williams M et al. Reliability of the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) mania and rapid cycling sections. J Am Acad Child Adolesc Psychiatry 2001;40:450-455.

82. Bollen KA, Long JS. (1993) *Testing Structural Equation Models*, Sage, Newbury Park, CA.