TITLE: **Risk and Protective Factors for SGA-Induced Metabolic Syndrome in Bipolar Youth**

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A. SPECIFIC AIMS

The overall objective of this prospective surveillance and controlled intervention study is to test the hypotheses that (1) low long-chain omega-3 (LC*n*-3) fatty acid status increases risk for adverse cardiometabolic effects and weight gain in adolescent bipolar patients treated with second generation antipsychotic (SGA) medications by up-regulating stearoyl-CoA desaturase-1 (SCD1), a lipogenic enzyme implicated in triglyceride biosynthesis, metabolic syndrome, and obesity, and (2) that increasing LC*n*-3 fatty acid status through dietary supplementation will be protective against the onset and progression of adverse cardiometabolic effects and weight gain in SGA-treated patients by suppressing SCD1 expression/activity.

SPECIFIC AIM 1:

To prospectively evaluate low LC*n*-3 fatty acid status as a risk factor for adverse cardiometabolic effects and weight gain in SGA-treated adolescent manic patients. Prediction 1: Low baseline RBC EPA+DHA levels will be associated with greater baseline-endpoint increases in BMI and triglyceride levels following acute (6-week) SGA treatment compared with high RBC EPA+DHA levels. Prediction 2: Baseline *SCD1* expression/activity will increase significantly following 6-week SGA treatment. Prediction 3: The relationship between RBC EPA+DHA levels and SGA-induced elevations in BMI and triglyceride levels is mediated by elevations in *SCD1* expression/activity.

SPECIFIC AIM 2:

To prospectively evaluate the protective effects of increasing LC*n*-3 fatty acid status on the progression of adverse cardiometabolic effects and weight gain in SGA-treated adolescent manic patients. Prediction 4: LC*n*-3 fatty acid supplementation will be more effective than placebo for decreasing BMI and triglyceride levels in SGA-treated patients. Prediction 5: Reductions in BMI and triglyceride levels following LC*n*-3 fatty acid supplementation will be associated with reductions in *SCD1* expression/activity and elevations in RBC EPA+DHA levels. Prediction 6: LC*n*-3 fatty acid supplementation will be more effective than placebo for improving SGA safety, tolerability, and efficacy.

B. BACKGROUND AND SIGNIFICANCE

Bipolar disorder is a common psychiatric illness that is characterized by recurrent episodes of mania and depression, as well as inter-episode periods of euthymia.[71] In the United States (US), the life-time prevalence rates are 1.0% for bipolar I disorder (BD-I), 1.1% for BD-II, and 2.4% for subthreshold BD (4.4% total).[72] The initial onset of mania, and by definition BD-I, most frequently occurs during childhood and adolescence.[12-15] Outcome data indicate that bipolar youth have a relapsing and remitting illness course that is often associated with significant psychosocial morbidity[73] and a 10-fold increased risk for suicide compared with general population estimates.[74,75] Prior US studies have reported that 55%–75% of adult BD-I patients are overweight or obese, a rate that is approximately double the US national average (~30%).[4,8,76-79] This is a major concern because obesity has been associated with a worsening of psychiatric outcomes in bipolar disorder, including shorter time to recurrence of mood episodes,[28] poorer global functioning,[80] and more frequent suicide attempts.[29] Furthermore, excessive weight gain and obesity during childhood and adolescence strongly predict obesity, metabolic syndrome, hypertension, sleep apnea, osteoarthritis, type II diabetes, and cardiovascular morbidity and mortality in adulthood.[81-88] Indeed, standardized mortality rates due to cardiovascular and circulatory disease in bipolar patients are approximately twice the age-adjusted population norms.[10,11] Health care costs for cardiometabolic conditions associated with obesity are over twice as high in bipolar patients compared with the general population.[89] Accordingly, there is an urgent and currently unmet need to identify modifiable risk and protective factors for metabolic disorder, obesity, and associated cardiovascular risk in patients with bipolar disorder.

Second-generation antipsychotic (SGA) medications, including quetiapine, olanzapine, and risperidone, have been shown to be efficacious for the rapid treatment of acute manic and psychotic symptoms in pediatric and adolescent patients in controlled trials.[16] These SGA medications are currently approved by the US FDA for the treatment of acute mania and psychosis in youth.[17] The American Academy of Child and Adolescent Psychiatry (AACAP) endorses the use of SGA medications as optimal first-line agents for the treatment of acute mania, and recommend 12-24 months of maintenance therapy for the treatment of acute mania.[18,19] Epidemiological data indicate that there has been a 6-fold increase in SGA prescription rates in pediatric and adolescent (<18 years of age) patients in US office-based medical practice during the past decade.[20-22] However, several prospective studies indicate that SGA medications are associated with significant treatment-emergent weight gain and obesity, metabolic syndrome, and elevated cardiovascular risk factors in the majority of SGA-naïve first-episode pediatric and adolescent patients.[23-27] Our group reported that the prevalence of overweight among hospitalized children and adolescents with exposure to SGA medications was triple that of national norms.[90] Another study found that more than 70% of youth exhibited significant weight gain after 12 week treatment with SGA medications.[26] In one of the largest prospective studies conducted to date, pediatric and adolescent patients treated with SGA medications including quetiapine exhibited significant elevations in body weight, fat mass, waist circumference, and BMI after 4 week exposure, and these parameters as well as triglyceride levels continued to increase at 8 and 12 weeks.[23] Our group similarly found that 8-week double blind treatment with the SGA quetiapine in adolescent (ages 12-17 years) bipolar patients resulted in a significantly greater increase in weight gain and fasting triglyceride levels compared with placebo.[91] Although controversial,[23] children and adolescents may be at greater risk than adult patients for SGA-induced weight gain.[26] Together, these and other data suggest that exposure to SGA medications precipitate weight gain and lipid dysregulation in a large subset of pediatric and adolescent patients with bipolar disorder. Despite consensus and label recommendations for metabolic screening and monitoring for all patients receiving these SGA medications,[92] the majority of youth starting SGA medications do not receive recommended

regular glucose or lipid screening/monitoring in clinical practice.[93] Together, these data highlight an urgent and unmet met need to identify modifiable risk and protective factors for SGA-induced adverse cardiometabolic effects and weight gain to guide ameliorative and preventative strategies.

The principal long-chain omega-3 (LC*n*-3) fatty acids, eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3), can only be obtained through the diet, and membrane EPA+DHA composition modulates cellular signaling pathways involved in the regulation of lipid and metabolic homeostasis.[96] Specifically, evidence suggests that low LC*n*-3 fatty acid status is associated with elevated blood pressure,[33-35] increased waist circumference, overweight and obesity,[36-40] elevated fasting triglyceride levels,[41-43] and elevated C-reactive protein (CRP) levels.[44-48] Increasing LC*n*-3 fatty acid status is efficacious for lowering elevated triglyceride levels,[42] and Lovaza® (EPA+DHA, GSK) is currently approved by the US FDA for the treatment of very high triglyceride levels (≥500 mg/dl). Increasing LC*n*-3 fatty acid intake is thought to decrease triglyceride levels by reducing biosynthesis and promoting oxidation.[161-163] Moreover, there is now a substantial body of evidence implicating low LC*n*-3 fatty acid status,[95,96] as well as elevated triglyceride levels,[97-99] as risk factors for cardiovascular morbidity and mortality. Consistent with the storage of excess triglycerides in peripheral fat stores (i.e., adipocytes), a growing number of rodent studies have also found that increasing LC*n*-3 fatty acid status is also protective against diet-induced increases in adiposity and weight gain.[118-121] While there have been no clinical studies conducted to evaluate the protective effects LC*n*-3 fatty acid against the initial onset and progression of weight gain, obesity is associated with LC*n*-3 fatty acid deficiency[36-40] and preliminary intervention studies have observed reductions in total fat mass, subcutaneous adipocyte diameter, and BMI in adult overweight or obese patients following LC*n*-3 fatty acid supplmentation.[164-166] Extant translational evidence therefore supports the proposition that low LC*n*-3 fatty acid status represents a candidate risk factor for hyperlipidemia and associated increases in adiposity and BMI.

Red blood cell (RBC) membrane EPA+DHA composition represents a reliable index of habitual (1-2 months) dietary EPA+DHA intake and 'omega-3 status',[100] and we recently reported that adult medication-withdrawn patients with bipolar disorder residing in the US exhibit robust RBC EPA+DHA deficits compared with healthy adults.[101] The latter finding is consistent with prior case-control studies also finding that adult patients with bipolar disorder exhibit significantly lower RBC EPA+DHA levels relative to healthy adults.[102,103] Importantly, our recent evidence further indicates that SGA-naïve first-episode adolescent manic patients also exhibit robust RBC EPA+DHA deficits compared with healthy adolescents (Section a.6.1). Moreover, dietary supplementation with EPA+DHA (fish oil) significantly increases RBC EPA+DHA levels in children and adolescents with bipolar disorder in association with significant reductions in manic and depressive symptom severity.[104,105] We have also obtained preliminary evidence that 12-week dietary supplementation with EPA+DHA (OmegaRx®) significantly increases RBC EPA+DHA composition from in adolescent patients. Additionally, a preliminary open-label study found that 4-week EPA+DHA treatment significantly decreased elevated fasting triglyceride levels in schizophrenic patients treated with the SGA clozapine.[106] Adjunctive LC*n*-3 fatty acid supplementation was also found to accelerate treatment response, improve tolerability, and permit a 20% reduction in SGA dose in first-episode psychotic patients,[107] and to reduce relapse rates in predominantly medicated adult patients with bipolar disorder.[108] Together, these data support our hypothesis that normalizing the low LC*n*-3 fatty acid status exhibited by bipolar youth will be protective against SGA-induced adverse cardiometabolic effects, and will have additional benefits for improving mood symptoms, tolerability, and adherence.

While the mechanisms mediating SGA-induced adverse cardiometabolic effects are poorly

understood,[109] recent evidence from *in vitro*, rodent, and clinical studies indicate that different SGA medications up-regulate the expression of stearoyl-CoA desaturase-1 (*SCD1,* delta9-desaturase),[65-70] a lipogenic enzyme implicated in triglyceride biosynthesis, metabolic syndrome, and obesity.[54-64] Specifically, preclinical studies have demonstrated that mutation of the *SCD1* gene is associated with impaired triglyceride biosynthesis,[54-57] increased insulin sensitivity,[58] and reduced visceral adiposity and resistance to high-fat diet-induced obesity in mice.[59] Similarly, selective pharmacological inhibitors of the SCD1 enzyme reduce elevated triglyceride and glucose levels in rodent metabolic disease models.[60,61] We recently reported that chronic exposure to SGA medications significantly increase *SCD1* activity indices in rats, and that this effect accounted for 56 percent of the variance in plasma triglyceride levels.[67] In human subjects, elevations in the plasma product-precursor ratio of oleic acid to stearic acid (18:1/18:0), an index of SCD1 activity (the 'desaturation index'), is positively correlated with human liver biopsy SCD1 mRNA expression,[110] plasma triglycerides levels, insulin resistance, and obesity.[54,62-64] Moreover, schizophrenic patients treated with the SGA olanzapine exhibit greater *SCD1* mRNA expression in peripheral blood cells compared with drug-free patients.[70] Together, this body of translational evidence suggests that up-regulation of *SCD1* may represent a mechanism through which SGA medications increase triglyceride levels, excess adiposity, and obesity.

In contrast to SGA medications, LC*n*-3 fatty acids down-regulate *SCD1* expression at the level of transcription and mRNA stability *in vitro*,[50,51] and we recently reported that dietary-induced LC*n*-3 fatty acid deficiency robustly up-regulates rat liver *SCD1* mRNA expression, and that this effect is positively correlated with plasma triglyceride levels.[53] Additionally, we found that normalization of LC*n*-3 fatty acid status through dietary supplementation normalized elevated liver *SCD1* mRNA expression and plasma triglyceride levels. These data are consistent with prior findings that dietary LC*n*-3 fatty acid supplementation decrease *SCD1* activity in liver microsomes *ex vivo*[111] and reduce liver triglyceride synthesis and/or secretion in different rodent models.[112-116] Other studies have similarly found that EPA supplementation decreases mouse liver *SCD1* mRNA expression and hepatic triglyceride content,[117] and dose-dependently decreases plasma triglyceride levels in schizophrenic patients treated with the SGA clozapine.[159] We have recently obtained proof-of-concept evidence that dietary-induced EPA+DHA deficits robustly augment SGA-induced elevations in rat liver *SCD1* expression/activity and liver and plasma triglyceride levels in rats maintained on standard diets.[160] Together, these translational data suggest that LC*n*-3 fatty acids and SGA medications have opposing effects on *SCD1* expression/activity which may account for their opposing effects on triglyceride biosynthesis and weight gain.

C. PRELIMINARY STUDIES

C.1. First-episode SGA-naïve manic youth exhibit LC*n*-3 fatty acid deficiency. In ongoing studies, we have found that SGA-naive first-episode pediatric/adolescent (10-18 yrs) manic patients (n=30, 15.2±1.6 years) exhibit RBC EPA+DHA levels that are significantly lower than those observed in age-matched healthy adolescents (n=30)(2.9±0.8 vs. 4.2±1.3 wt% total, p=0.0002). Importantly, 91% of first-episode manic patients exhibited an 'omega-3 index' (EPA+DHA) of ≤4.0% compared with 25% of healthy controls (χ^2 p=0.001). Based on prospective observational studies finding that an omega-3 index of ≤4.0% confers a 10-fold greater risk for primary cardiac arrest compared with an omega-3 index of >8.0%,[123] these data suggest that the majority of these first-episode manic patients are at increased risk for cardiovascular morbidity and mortality. Moreover, the RBC EPA+DHA levels observed in first-episode pediatric/adolescent manic patients (2.9±0.8%) is similar to that previously observed in adult patients suffering acute coronary syndrome (3.4±1.6%),[124] as well as adolescents at ultra-high-risk for developing psychosis (3.1±1.2%).[125] It is of note that the baseline BMI of first-

episode manic patients (24.8±0.9 kg/m^2) did not differ significantly from healthy pediatric/adolescent controls (24.0±1.0 kg/m^2)(p=0.67), a finding consistent with prior prospective trials.[23] This finding suggests that LC*n*-3 fatty acid deficiency alone is not sufficient to induce weight gain, but rather, increases risk for greater weight gain in response to a metabolic challenge (i.e., SGA-induced lipogenesis).

C.2. Omega-3 fatty acid supplementation increases RBC EPA+DHA levels in adolescent patients. While the etiological factors contributing to LC*n*-3 fatty acid deficiency observed in bipolar youth are poorly understood and likely multifactorial, dietary EPA+DHA insufficiency plays a key role.[126] Specifically, studies have found that pediatric and adolescent bipolar patients consume less preformed EPA+DHA in their diet compared with healthy controls,[127] and that dietary EPA+DHA (fish oil) supplementation significantly increases RBC EPA+DHA composition in bipolar youth.[104,105] In a pilot study of adolescent patients with MDD (n=30, 15.2±3.5 years), we found that 12-week open-label EPA+DHA (OmegaRx) supplementation (2.4 g/d) significantly increased RBC EPA+DHA composition from 3.1±0.3% at baseline to 7.5±1.2% at study endpoint (+49%, p<0.0001). Based on prospective observational studies, the level of EPA+DHA observed in these patients following 12-week EPA+DHA treatment would confer a ~10-fold reduced risk for primary cardiac arrest compared with their baseline values.[123] Consistent with prior studies,[104,128] we found that baseline depressive (CDRS) symptom severity scores (primary outcome measure) declined significantly following 12-week EPA+DHA supplementation (-18%, p=0.01). Importantly, in this and other LC*n*-3 fatty acid supplementation trials conducted in children and adolescents,[104,105,128] adverse events (predominantly gastrointestinal-related) were few and rated as mild by patients. Together, these preliminary findings suggest that dietary EPA+DHA supplementation is efficacious and well-tolerated for increasing LC*n*-3 fatty acid status in pediatric and adolescent patients, and provide preliminary evidence for beneficial effects for mood symptoms.

C.3. LC*n*-3 fatty acids and SGAs have opposing effects on *SCD1* mRNA expression in rat liver. As discussed above, emerging evidence suggests that stearoyl-CoA desaturase-1 (*SCD1*) is required for *de novo* triglyceride synthesis,[54,56,57] and that *SCD1* is negatively regulated by omega-3 fatty acids *in vitro*.[51,52] To evaluate this mechanism *in vivo*, we have been investigating the effects of dietary-induced LC*n*-3 fatty acid deficiency, as well as subsequent repletion, on young adult rat liver *SCD1* mRNA expression and activity indices (liver 16:1/16:0 & 18:1/18:0 ratios), and determined relationships with postprandial plasma triglyceride levels. Compared with controls, rats fed the omega-3-deficient diet exhibited significantly lower RBC and liver EPA+DHA compositions, which were highly correlated, and both were normalized in repleted rats. Liver *SCD1* mRNA expression (+82%, p=0.001) and activity indices (18:1/18:0: +60%, p<0.0001; 16:1/16:0: +75%, p<0.0001) were significantly greater in omega-3 deficient rats compared with controls. Consistent with a prior human liver biopsy study,[110] liver *SCD1* mRNA expression was positively correlated with liver 18:1/18:0 (r = +0.90, p≤0.0001) and 16:1/16:0 (r = +0.93, p≤0.0001) ratios, supporting the validity of these measures as indices of *SCD1* activity.[54] Plasma triglyceride levels were significantly greater in omega-3 fatty acid deficient rats compared with controls (+30%, p=0.02), and liver *SCD1* mRNA expression and activity indices were positively correlated with plasma triglyceride levels. Importantly, normalization of omega-3 fatty acid status corrected elevations in liver SCD1 expression and activity as well as elevated plasma triglyceride levels observed in omega-3 deficient rats. In contrast to the negative regulatory effects of LC*n*-3 fatty acid on SCD1 expression and activity, recent translational studies have found that different SGA medications robustly up-regulate *SCD1* expression and activity.[65-70] Of direct relevance to the current proposal, we recently reported that dietary-induced EPA+DHA deficits robustly augment risperidone-induced elevations in rat liver *SCD1* expression/activity and triglyceride levels in rat liver and plasma.[160] Together, these translational

data provide preliminary support for our hypothesis that SGA medications and LC*n*-3 fatty acids have opposing effects on *SCD1* expression and activity.

C.4. SGA-induced weight gain and hypertriglyceridemia in adolescent bipolar patients: Our group previously reported that 12-week open-label treatment with the SGA quetiapine resulted in a statistically significant increase in body weight in 20 adolescents at ultra-high risk for developing bipolar disorder (i.e., have a mood disorder diagnoses other than bipolar I disorder and a first-degree relative with bipolar I disorder).[129] In a subsequent double-blind placebo-controlled 8-week trial in adolescents (ages 12-17 years) with bipolar disorder, we observed a statistically significant between-group difference in baseline-endpoint change in fasting triglyceride levels (mean change in quetiapine: +30 mg/dL vs. placebo: +14 mg/dL, p=0.003), and that a greater number of subjects in the quetiapine group (24%) than in the placebo group (0%) exhibited a shift from normal to abnormal triglyceride levels. Subjects in the quetiapine group also gained more weight (mean increase: 2.3 kg) than those in the placebo group (0.9 kg), and the increase in BMI was greater for the quetiapine group (mean increase: 0.9 kg/m^2) than the placebo group (0.3 kg/m^2).[91] These data and are entirely consistent with larger prospective studies finding treatment-emergent weight gain in youth with bipolar disorder following sub-chronic exposure to quetiapine,[23] and demonstrate our experience in administering quetiapine to adolescent patients.

D. INVESTIGATOR EXPERIENCE

Dr. McNamara is Associate Professor of Psychiatry and Behavioral Neuroscience, Division of Bipolar Disorders Research, at the University of Cincinnati College of Medicine, and is Director of the Lipidomics Research Program (LRP). Dr. McNamara has been studying the pathophysiology of bipolar disorder for over 15 years, and has an active translational research program investigating the role of bioactive lipids in the pathoaetiology and treatment of psychiatric disorders. Dr. McNamara has specific expertise in lipid analytical tecnhiques as well as gene expression (i.e., qPCR) procedures outlined in this proposal. He has also been PI on prior and current omega-3 fatty acid intervention trials in pediatric and adolescent subjects. Dr. McNamara will be responsible for the overall scientific conduct of the study, providing conceptual, managerial, and administrative direction. Dr. DelBello is Associate Professor of Psychiatry, Pediatrics, and Psychology and Co-Director of the Division of Bipolar Disorders Research 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 and adolescent bipolar disorder. She has been studying the pharmacology and neurobiology of bipolar disorder for over 20 years. Dr. DelBello has several publications characterizing the effects of SGA medications on mood and metabolic parameters in adolescents with bipolar disorder. Dr. DelBello will coordinate subject recruitment and will assist with patient assessment, and will supervise training and reliability of the personnel performing diagnostic and rating instruments. Dr. DelBello will serve as the medically responsible physician for the present study. Dr. Patino is a child and adolescent psychiatrist in the Division of Bipolar Disorders Research (DBDR) at the University of Cincinnati College of Medicine. Dr. Patino has worked within the DBDR for the past year, and is currently participating in several clinical trials of children and adolescents with mood and cognitive disorders. He has extensive experience with, and has demonstrated excellent inter-rater reliabilities for, all of the proposed diagnostic and rating scales (kappa and ICCs > 0.8). Dr. Patino will assist with the clinical care and diagnostic and symptom and safety ratings for study participants. He has extensive experience with, and has demonstrated excellent inter-rater reliabilities for, all of the proposed diagnostic and rating scales (kappa and ICCs > 0.8), and will assist with the day-to-day management of the research visits and patient care. Dr.

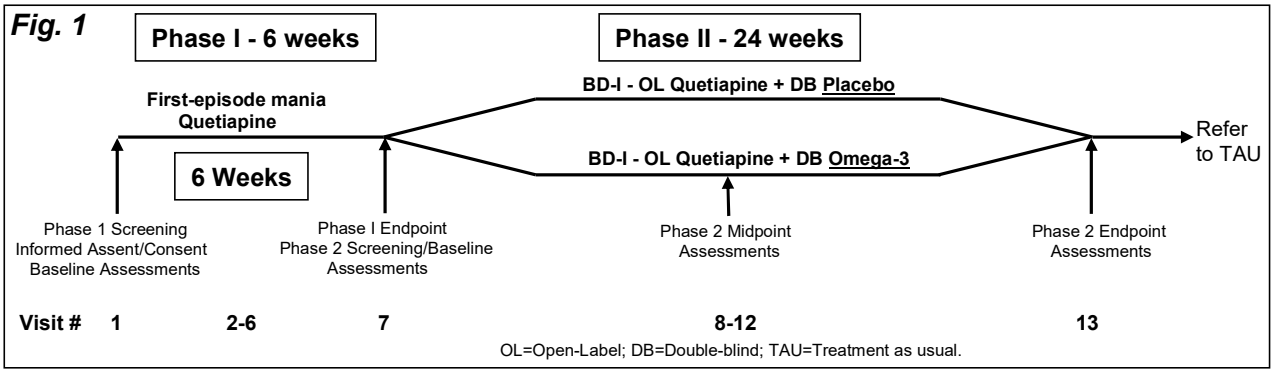
Welge, Research Assistant Professor of Psychiatry and Biostatistics and Director of the Division of Quantitative Methods in the Department of Psychiatry UCCOM, has extensive expertise in the area of psychiatry and biostatistics. Dr. Welge has specific expertise in multivariate regression mixed models and will oversee all aspects related to database design and data management, and will lead the proposed statistical analyses. Mrs. Nasrallah will be responsible for completing and maintaining regulatory documents for this study and will also serve as liaison with local and federal study monitors.

E. EXPRIMENTAL DESIGN & METHODS

a. Methods and Procedures

Study Overview: We are proposing a two-phase study design (see **Fig. 1**) to first prospectively investigate the relationship between baseline LC*n*-3 fatty acid status and treatment-emergent adverse cardiometabolic events and weight gain in response to acute (6-week) quetiapine in adolescent manic patients (ages 10-17 years)(Phase I, SA1). During Phase II (SA2), patients from Phase I will be randomized (stratified by BMI of 30 **kg/m^2)** to double-blind adjunctive treatment with LC*n*-3 fatty acids or placebo for an additional 24 weeks to investigate protective effects on the progression and resolution of adverse cardiometabolic events and weight gain during quetiapine maintenance therapy. Patients will be recruited from the inpatient psychiatric units and outpatient clinics at Cincinnati Children's Hospital Medical Center (CCHMC). The attending physicians and then the legal guardians of adolescents with a manic or mixed mood episode and who have a diagnosis of bipolar disorder, type I, will be approached by research staff for potential study participation. Once permission is obtained from their inpatient psychiatrist and legal guardians, adolescents will be approached about study participation. If agreeable, research staff will obtain written informed consent and assent for study participation from the legal guardian and adolescent, respectively. Patients will be discharged from hospitalization when they are clinically stable at the discretion of their attending psychiatrist (typically after 7 days). Following initiation of double blind treatment, patients will have regularly scheduled visits at which time metabolic parameters (i.e., lipid profile, glucose, insulin), anthropomorphic measures (i.e., BMI), and mood symptom ratings (CDRS, YMRS) will be obtained. Primary outcome measures for both study Phases are BMI and fasting triglyceride levels. Following the completion of the 24-week Phase II study procedures (or early termination), patients will transition care to a community clinician so that there is no lapse in treatment. Patients will be recruited from the inpatient units and less commonly outpatient clinics affiliated with University and Cincinnati Children's Medical Center Hospitals. Our program, the Division of Bipolar Disorders Research, has successfully recruited hundreds of patients from these units during the past 5 years, and is therefore well-positioned to carry out the proposed study. Study visits will take place in the department of Psychiatry, Division of Bipolar Disorders

Research, University and Cincinnati College of Medicine.



OUTCOME MEASURES

We will obtain the following anthropomorphic, metabolic, safety, and mood symptom measures at selected visits during the 6 week Phase I component and the 24 week Phase II component (**Table 1**).

Table 1. Schedule of Assessments

ASSESSMENT	PHASE I BASELINE	PHASE I WEEKS 1,2,3,4,5	PHASE I (ENDPOINT) PHASE II BASELINE	PHASE II WEEKS 4,8,12,16,20	PHASE II ENDPOINT (WEEK 24)
WASH-U KSADS and WASI (IQ)	X				
Young Mania Rating Scale (YMRS)	X	X	X	X	X
Children's Depression Rating Scale-Revised (CDRS)	X	X	X	X	X
Clinical Global Impression-Severity (CGI-S)	X	X	X	X	X
Columbia Suicide Severity Rating Scale			X	X	X
Addiction Severity Index (ASI)	X		X		X
Prior medical and treatment history (con-meds)	X	X	X	X	X
Tanner scale	X				
3 d food record/Omega-3 Questionnaire			X	X (wk 12 & 20 only)	
Urine toxicology screen, urine pregnancy test	X	X (wks 1 & 6 only)	X		
Serum laboratory tests (renal profile, complete blood count, & liver tests)	X		X	X (wk 12 only)	X
Vital signs: body weight, BMI, waist circumference, blood pressure.	X	X	X	X	X
Physical exam	X				
Metabolic/lipid panel: fasting lipids [TAG, LDL-C, HDL-C], glucose, and insulin (HOMA-IR)	X		X	X	X
Adiponectin, TSH, hsCRP, leptin	X		X	X (wk 12 only)	X
Platelet function assay (PFA)			X	X (if indicated)	X
RBC/Plasma fatty acid analysis (GC)	X		X	X (wk 12 only)	X
WBC *SCD1* mRNA expression	X		X	X (wk 12 only)	X
ECG (QTc)	X		X		X
Adverse events (SEFCA)			X	X	X
Demographics	X				
Inclusion/exclusion criteria	X				
Med accountability sheet		X	X	X	X
Total duration of appointment:	2-3 hrs	2 hrs	2 hrs	2 hrs	2 hrs

Anthropomorphic and metabolic measures: We will obtain the following anthropomorphic measures: body mass index (BMI, calculated as weight in kilograms (to the nearest 0.1 kg) divided by height (to the nearest 0.1 cm) in meters squared, kg/m^2), incidence rates of clinically-

significant weight gain of ≥7% from baseline, waist circumference (measured at the level of both superior iliac crests and umbilicus, using the point of largest abdominal circumference), and sitting systolic and diastolic blood pressure. Fasting (>8 hour) venous blood (approximately 15 cc) will be collected for determination of lipids (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides), glucose, insulin, TSH, adiponectin, leptin, and hsCRP. The homeostasis model assessment of insulin resistance will be calculated [HOMA-IR: fasting insulin umol × glucose mmol/22.5]. White blood cells (lymphocytes, leukocytes, myeloid cells) will be isolated from whole blood to measure gene expression (i.e., SCD1 mRNA) by qPCR as previously described.[53,67,160] Red blood cell fatty acid composition will be determined as previously described.[101,143]

Dietary assessments: We will request that subjects maintain their current diet over the course of their study participation. During the course of the trial we will obtain 3-day food records at scheduled visits to obtain estimates of average total energy intake (kJ/kg/d) and other dietary macronutrients (i.e., fat (g/d), carbohydrate (g/d), and protein (g/d). Additionally, the Omega-3 Dietary Intake Questionnaire, an 11-item scale, will be administered at each Phase II visit to specifically assess omega-3 fatty acid intake. The Omega-3 Dietary Intake Questionnaire has been validated, and questionnaire scores (mg EPA and mg DHA per day) are positively correlated with EPA and DHA status.[130] The Addiction Severity Index (ASI) will also be administered to collect data regarding drug use (including nicotine and alcohol) at the Phase I baseline visit and at Phase II baseline and endpoint visits.

Mood symptom ratings: Symptom ratings will be obtained using the Young Mania Rating Scale (YMRS),[131-133] an 11-item scale to assess manic symptoms; the Children's Depression Rating Scale-Revised (CDRS-R), a 17-item observer-rated questionnaire, to assess depressive symptoms.[134,135] Subjects will also be rated using the Clinical Global Impression-Severity Scale (CGI-S), a clinician-rated 7-point scale (1=normal to 7=extremely ill) to assess overall illness severity.[136] A potential new manic episode will be identified when patients present with a YMRS score >16, and a depressive episode will be identified when patients present with a CDRS score >24 and YMRS score <10. Per DSM-IV criteria, two weeks of depressive symptoms or one week of manic symptoms are required to define a new episode.

Safety and tolerability assessments: A complete medical and treatment history, vital signs, a physical examination, and serum laboratory tests (including a renal profile, complete blood count, liver function tests) will be performed at select visits during Phases I and II. Additionally, electrocardiograms (ECGs) will be performed at selected visits to assess QTc interval. Adverse events will also be assessed at selected visits using a clinical interview as well as a structured side effect interview, the Side Effects Form for Children and Adolescents (SEFCA).[138] The SEFCA measures the frequency and severity of specific cardiovascular, gastrointestinal, central nervous system, ocular, mouth and nasal, genitourinary, dermatological, and musculoskeletal side effects. Because LC*n*-3 fatty acid treatment may be associated with increasing bleeding time, we added the following four questions to the SEFCA: "Bruising", "Nosebleeds", "Longer than usual bleeding", and "Heavy bleeding associated with menstruation". Additionally, the Platelet Function Assay (PFA), a sensitive measure of blood clotting, will be measured at Phase II baseline and endpoint. If a study participant answers positively to any of the bleeding items on the SEFCA, a PFA will be performed at that point. Patients will be discontinued from study participation if their PFA values are outside the reference range (PFA-EPI: 75.0-199.0 and PFA ADP: 68.0-112.0) on two consecutive visits and provided with follow-up care. An adverse event will be defined as a symptom that was not present at or worsened in severity from baseline. The Columbia Suicide Severity Rating Scale (C-SSRS)[139] will be administered at each Phase II visit as a measure of suicidality. We have extensive experience and established high inter-rater

reliability with these instruments (κ & ICCs >0.7 and typically >0.9 for most ratings).

TREATMENTS:

Quetiapine: Quetiapine fumarate (Seroquel) is approved by the FDA for the treatment of acute mania in pediatric and adolescent patients (ages 10-17 years), and is currently among the most frequently prescribed SGA in children and adolescents in outpatients clinics in the US.[21] Importantly, we have considerable experience administering quetiapine to pediatric and adolescent patients,[91,129,140-142] and prior prospective studies including our own have found that quetiapine, like other SGA medications, is associated with treatment-emergent elevations in our primary outcome measures (i.e., BMI, triglyceride levels) in pediatric and adolescent patients.[23,91,129,140-142] For the proposed Phase I component, acutely manic patients with limited prior SGA exposure (≤3 months) will be treated with quetiapine for 6-weeks. Patients will be started on 25 mg per day of quetiapine QHS, and the dose adjusted based on tolerability and response. The quetiapine target dose is 400-600 mg, with most subjects in the range of 200-500 mg. In our prior studies, the mean quetiapine dose was 400 mg/day.[91,140] Quetiapine will be dispensed at each visit by the investigational pharmacist, and we will monitor patient compliance by pill counts at weekly visits by patient/parental reports. During Phase I, every effort will be made to achieve an effective, well tolerated, and stable maintenance dose by Week 6. If patients are unable to tolerate therapeutic doses, they will be removed from the study and referred for alternate treatment. While it is anticipated that patients will be maintained on this dose during the 24-week double blind component (Phase II), we will permit dose adjustments if clinically indicated.

Risks of Quetiapine

Side Effect	**Percentage of children and adolescents who have experienced this side effect in previous clinical trials**
Somnolence	53%
Dizziness	18%
Fatigue	11%
Increased appetite	9%
Nausea	8%
Vomiting	8%
Tachycardia	7%
Dry mouth	7%
Weight gain	6%

LC*n*-3 fatty acid supplements: For the Phase II intervention trial, patients will be randomized to EPA+DHA supplements (OmegaRx) or placebo (safflower oil). Safflower oil was selected for the placebo because it does not contain omega-3 fatty acids, including α-linolenic acid (found in olive oil), and to minimally alter the fatty acid composition of the typical American diet. All capsules will be stored atroom temperature (60-75^{0}F) , and EPA+DHA content determined upon initial receipt and every 90 d by gas chromatography to assure consistency across the trial. Omega-3 and placebo capsules will be dispensed at each visit by the investigational pharmacist. Placebo and OmegaRx capsules are identical in caloric content (~9 kcal/capsule), size, shape, and color to protect the blind. To test the integrity of the study blind, we will ask study participants to separately identify their treatment as placebo or omega-3 fatty acids at the end of their study participation. We will also ask the raters for that subject to complete a questionnaire asking to which group (omega-3 fatty acids or placebo) they thought the subject

had been assigned. All responses will be placed in a sealed envelope and after the last subject completes the study, will be compared with actual assignments using Fisher exact tests.

We have previously confirmed that each OmegaRx capsule contains 400 mg EPA and 200 mg DHA. Subjects will be randomized to OmegaRx or placebo at a fixed dose of 3.0 g/day (EPA: 2.0 g, DHA: 1.0 g; 5 capsules/d). This fixed dose was selected based on American Heart Association recommendations to consume between 2.0-4.0 g/d for triglyceride reduction in individuals with hypertriglycerolaemia,[145] controlled dose-response studies finding that similar fixed doses (2.0-4.0 g/d) are efficacious in lowering elevated triglyceride levels,[42] a one month open-label intervention study finding that 3.0 g/day EPA+DHA significantly reduced fasting plasma triglyceride levels in patients treated with the SGA clozapine,[106] and findings that similar EPA+DHA doses (1.9-2.6 g/d) are efficacious for reducing manic and depression symptom severity in medicated bipolar adolescents.[104,105] The US FDA considers EPA+DHA doses up to 3.0 g/d to be 'generally regarded as safe' (www.cfsan.fda.gov/~dms/ds-ltr11.html). Treatment adherence will be encouraged at each visit and adherence monitored by subject interview, capsule counts, legal guardian interviews, and a capsule adherence diary. Subjects with > 50% non-adherence during 2 contiguous weeks will be discontinued from the study and provided with recommendations for follow-up care. Subjects in the placebo group will be required to take 5 pills per day to match the OmegaRx group. Patients will be advised to take 2 capsules during their morning meal and 3 capsules during their evening meal. In our previous studies we have found that this approach is effective and does not reduce compliance as evidenced by capsule counts and self-reports.

Risks of LC*n*-3 fatty acid

Side Effect	**Percentage of children and adolescents who have experienced this side effect in a previous clinical trial[105]**
Gastrointestinal Distress	25%
Cold/Flu/Allergies/Infection	20%
Headache	15%
Decreased Appetite	10%
Sleep Problems	10%
Skin Disturbance	10%
Agitation/Activation	5%
Respiratory Side Effects	5%
Tics	5%
Anxiety	5%

Concomitant Medications: 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 the subject, primary caregiver, and treating clinician report in conjunction with the study physician. In general, patients will be instructed to contact study staff prior to taking any concomitant medications during their study participation to minimize the adverse effects that might occur with medications as well as to accurately record the reason for concomitant medication use.

b. Data analysis and Data Monitoring:

Data management and analyses will be organized and performed under the direction of Dr. Welge, Director of the Division of Biostatistics in the Department of Psychiatry at the University of Cincinnati, who has participated in the development of this analytic plan. The research coordinator will be responsible for managing study flow, maintaining case report forms,

and entry of data into a database. All data will be double entered and compared every six months to ensure the integrity of the information. The principal investigator will maintain a secure database of all raw data which will be password protected. Demographic and clinical data will be managed in a database using Microsoft Access® and stored in an IBM compatible PC connected to other PCs within the Division of Bipolar Disorder Research with a LAN that is password protected and coded to meet HIPAA requirements. Backups are performed weekly to protect against data loss.

Data Analysis: Demographic and clinical variables (e.g. sex, race, age, baseline BMI, quetiapine dose, dietary energy intake) will be included as covariates in the model in a stepwise manner if they are associated with primary outcome measures using a liberal p-value of <0.2. We will use independent, 2-tailed *t*-tests for continuous variables and χ^2 tests for categorical variables, and adjust for multiple comparisons as appropriate. For the Phase II DBPC component, analyses will be performed using data from the intent-to-treat (ITT) sample (all subjects receiving at least one dose of omega-3 fatty acid or placebo). All tests of hypotheses will be done at the 5% level of significance, unless otherwise specified. Prior to data analysis, the distribution of all dependent variables will be examined for normality. We will transform or apply alternative statistical models for dependent variables that are not normally distributed. Specifications for temporal correlation and random effects will be selected using AIC criterion. All analyses will be performed using the Statistical Analysis System (SAS) (SAS Institute, Cary, NC, 2002) by Dr. Welge.

Prediction 1: Low baseline RBC EPA+DHA levels will be associated with greater elevations in BMI and triglyceride levels in response to acute (6-week) SGA treatment compared with high RBC EPA+DHA levels. The relationships between baseline-endpoint change in BMI and triglyceride levels and baseline RBC EPA+DHA composition will be evaluated with a linear regression model. We will evaluate potential covariates (i.e., baseline BMI) as described above. We will also generate odd ratios and 95% confidence intervals to estimate relative risk in different quartiles of RBC EPA+DHA composition.

Prediction 2: Baseline *SCD1* expression/activity will increase significantly following 6-week SGA treatment. We will use an unpaired *t*-test (one-tail) to compare change from baseline to week 6 (or endpoint) in SCD1 mRNA expression and SCD1 activity index (plasma 18:1/18:0). We will evaluate potential covariates and confounds as described above.

Prediction 3: The relationship between RBC EPA+DHA levels and SGA-induced elevations in BMI and triglyceride levels is mediated by elevations in *SCD1* expression/activity. We predict a mediated effect in which low RBC EPA+DHA levels are associated with elevated SCD1 mRNA expression and SCD1 activity (plasma 18:1/18:0), which in turn increase baseline-endpoint change in BMI and triglyceride levels. For the prediction of a mediated effect to be supported, two conditions must be met: a significant association between RBC EPA+DHA levels and SGA-induced elevations in BMI and triglyceride levels (Prediction 1), and a significant association between RBC EPA+DHA levels and SCD1 expression/activity (Prediction 2). If there is no residual relationship between RBC EPA+DHA levels and weight and metabolic parameters, then the relationship between these measures will be inferred to exist solely through the mediating effect of SCD1 expression/activity. Path analysis,[157] an extension of linear regression to systems of simultaneous linear regression equations, will be used to analyze the fit of the proposed mediation model. This method allows for the same variable to serve as an independent variable in some of the regressions and a dependent variable in others. In this analysis, baseline-endpoint changes in BMI and triglyceride levels will serve as the final outcome measure, with changes in SCD1 expression/activity as the mediator and RBC

EPA+DHA level as the independent variable. Estimation of path coefficients and bootstrap computation of associated significance levels (α=0.05) will be carried out using SAS macros.[158] The relationship between baseline-endpoint change in baseline RBC EPA+DHA composition, and baseline-endpoint change in BMI and triglyceride levels will be evaluated with multiple regression models.

Prediction 4: LC*n*-3 fatty acid supplementation will be more effective than placebo for decreasing BMI and triglyceride levels in SGA-treated bipolar patients. A mixed effects model repeated measures (MMRM) approach will be used to compare change over time in BMI and triglyceride levels between treatment groups (placebo, omega-3). The model will contain terms for group (2 levels), time, and group-by-time interaction. Covariates will be included as described above. We will also compare treatment group differences in change from baseline to endpoint in BMI and triglyceride levels 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.

Prediction 5: Reductions in BMI and triglyceride levels following LC*n*-3 fatty acid supplementation will be correlated with reductions in *SCD1* expression/activity and increases in RBC EPA+DHA levels. The relationship between baseline-endpoint change in BMI and triglyceride levels and baseline-endpoint change in SCD1 mRNA expression and SCD1 activity index (plasma 18:1/18:0) will be evaluated with a simple linear regression model. We will evaluate potential covariates and confounds as described above.

Prediction 6: LC*n*-3 fatty acid supplementation will be more effective than placebo for improving SGA tolerability and efficacy (i.e., lower relapse and discontinuation rates). For tolerability, a MMRM approach will be used to compare change over time adverse events (SEFCA) and extrapyramidal symptoms (SAS) between treatment groups (placebo, omega-3). We will additionally measure the duration of time to exit double-blind treatment because of tolerability issues. For efficacy, we will use a MMRM approach to compare change over time in mood symptom severity (CDRS, YMRS). We will also compare treatment group differences in change from baseline to endpoint in CDRS and YMRS scores using an analysis of variance (ANOVA). A Kaplan-Meier with Mantel-Cox log-rank statistic will be used to compare duration of remission in the 2 treatment groups controlling for baseline symptom severity.

Data and Safety Monitoring plan

The data and safety monitoring plan for the proposed study will include monitoring of efficacy and safety 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 College of Medicine Institutional Review Board (IRB). 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 will meet a minimum of every 6 months during the course of the study and will formally review tolerability, safety and efficacy data. Drs. McNamara and Patino will be responsible for providing updated efficacy and tolerability data to the DSMB. The DSMB will assess the risks and benefits of study participation to all subjects. Based on this assessment the DSMB will provide a written report of their analyses and recommendation as to whether the study should continue or modification to the study as needed. Drs. McNamara, Patino, and DelBello, in conjunction with the DSMB, will be responsible for making certain that the DSMB files their report to the IRB. The DSMB will also

provide the investigator with a summary of their report that will include their recommendations.

c. Data Storage and Confidentiality

Every effort will be made to maintain the confidentiality of study records. Agents of the University of Cincinnati Medical Center Institutional Review Board and the University of Cincinnati Division of Bipolar Disorders Research may be allowed to inspect records related to this study. The data obtained from the study may be published; however, participants will not be identifiable in such publications. Participant identity will remain confidential unless disclosure is required by law. The records of this research study will be kept confidential and will not be given to anyone who is not helping conduct this study unless specifically requested and waived by the participant. To further ensure confidentiality, study records will be kept in locked file cabinets and/or in computers with passwords, all in locked rooms here at the University of Cincinnati Department of Psychiatry. Additionally, participant identification is coded with letters and numbers to de-identify individuals. As required, this study will be in full compliance of HIPAA.

d. Setting - The offices of the Division of Bipolar Disorders Research (260 Stetson St., ML 0516, Cincinnati, OH 45219-0516) is the setting for this protocol.

e. Laboratory Methods & Facilities - Red blood cell fatty acid composition will be determined in a blinded manner by gas chromatography in the laboratory of Dr. McNamara in the Department of Psychiatry, Division of Bipolar Disorders Research (260 Stetson St., ML 0516, Cincinnati, OH 45219-0516). The proposed metabolic/lipid panels and labs will be performed by LabCorp, the Platelet Function Assay (PFA) by CCHMC, and adiponectin, leptin, and hsCRP by the GCRC Biochemistry Laboratory.

f. Estimated Period of Time to Complete the Study - The following is a timeline for the 60-month period of the proposed study: Months 1-2: Train study personnel, Months 3-56: recruit an average of 2 patients per month; Months 57-60: Complete subject participation, data entry, checking and analyses.

F. HUMAN SUBJECTS

a. Description of Subjects

This is a total of 30-weeks divided into Phase I (6-weeks quetiapine) and Phase II (24-weeks double-blind adjunctive treatment with LC*n*-3 fatty acids or placebo) for the treatment of manic bipolar adolescents (ages 10-17 years)(Total N=94). Patient selection will be done by the research staff, in consultation with and with permission from Dr. DelBello and the attending physician of that patient. Inclusion and exclusion criteria are listed below.

b. Sample Size

Up to 100 patients will be enrolled in this study at the University of Cincinnati Medical Center over a total of 60 months. As this is an investigator-initiated protocol, there are no other sites associated with this submission.

c. Inclusion/Exclusion Criteria

Phase I - Inclusion Criteria:

- DSM-IV-TR criteria for bipolar disorder, type I, manic or mixed episode, diagnosed by the Washington University in St. Louis Kiddie Schedule for Affective Disorders and

Schizophrenia (WASH-U-KSADS)
- Baseline YMRS score ≥ 20
- Ages 10-17 years
- Tanner scale stages II-V
- No prior long-term (≥3 month) exposure to SGA medications
- Fluent in English
- Provision of written informed consent by a legal guardian and written assent by the subject
- Manic or depressive symptoms do not result entirely from acute medical illness or acute intoxication or withdrawal from drugs or alcohol as determined by medical evaluation and rapid symptom resolution
- If female and of child bearing potential, agrees to use one of the following method of birth control: complete abstinence from sexual intercourse, barrier (diaphragm or condom), or oral/injectable contraceptive.

Phase II, additional **Inclusion criteria**:
- Receiving a stable therapeutic dose of quetiapine for a minimum of 1 week (i.e., patients who achieved remission (YMRS total score ≤12 during Phase I)
- Not requiring concomitant use of antidepressant or mood-stabilizer medications.

Exclusion Criteria:
- IQ < 70, as determined by The Wechsler Abbreviated Scale of Intelligence (WASI)
- A positive pregnancy test (to avoid teratogenesis)
- A history of major cardiovascular or neurological illness
- Any current DSM-IV-TR substance use disorder (nicotine dependence is permitted)
- A lifetime DSM-IV-TR diagnosis of any pervasive developmental disorder

Phase II, additional **Exclusion criteria**:
- Any history of a hematological disorder in themselves or a first-degree relative will be excluded (since omega-3 fatty acids may be associated with anti-thrombotic effects). Similarly, concomitant use of medications with anticoagulant effects (e.g. aspirin) will be prohibited
- Allergy to fish/seafood
- Currently taking omega-3 fatty acid supplements.

d. Intended Demographics
Patients will be adolescents (ages 10-17 years) diagnosed with bipolar disorder. Patients from all available ethnic groups will be recruited at rates that reflect the demographics of the University Hospital Catchment Area. Consequently, we expect approximately 60% of the sample being White, 35% African-American and the remainder from other ethnic backgrounds. Because prior treatment with SGA medications would be anticipated to influence our primary outcome measures, we will recruit only patients with limited prior SGA exposure.

e. Study population source
Patients will be recruited from the inpatient units and less commonly, outpatient clinics affiliated with University and Cincinnati Children's Medical Center CCHMC Hospitals. Dr. DelBello is Director of Research Training and Education and Co-Director of the Mood Disorder Clinic at CCHMC, which will facilitate recruitment of potential subjects.

f. Recruitment Plans
Adolescents will be recruited primarily through infrastructure originally established for Dr.

Delbello's ongoing research study (R01MH080973-PI-Dr. DelBello) investigating the effects of 6-week quetiapine treatment on mood and neuroimaging measures. Since the initiation of this trial, n=73 patients who would be eligible for participation in the proposed study (i.e., they were SGA-naïve and diagnosed with DSM-IV-TR criteria for bipolar disorder, type I, manic or mixed episode) have been randomized (an average of 3 patients per month). This rate of recruitment is sufficient to meet the proposed enrollment goals of an average of 2 subjects/month. Nevertheless, we will periodically assess our recruitment and retention rates and adjust recruitment efforts accordingly.

Specifically, recruitment will be performed by study staff in consultation with CCHMC (inpatient) and UC (outpatient) physicians. Inpatients will be identified as potential study candidates by study staff with CCHMC privileges. Physicians of those identified will be asked if they deem such inpatients suitable for this research study. If so, study staff will also ask the same physician if it is permissible to approach the inpatient and their family about the research study.

G. RISK TO SUBJECTS

a. Level of Risk Description

Potential risks from study participation include possible risks from treatments, as well as other risks associated with any study participation (e.g. loss of confidentiality and fatigue and frustration from repeated evaluations). Possible risks of the proposed study design include the potential for symptom exacerbation. For the proposed study, all participants will be treated with quetiapine fumarate (Seroquel) which is the standard of care. All subjects and their legal guardians will be informed about the potential risks associated with quetiapine during the informed consent/assent process. Quetiapine is approved by the United States Food and Drug Administration for the treatment of acute mania in children and adolescents (ages 10-17 years) and is commonly prescribed for adolescents with bipolar disorder. All adolescents will be receiving quetiapine for 30 weeks total, which presently is a standard treatment for children/adolescents with acute mania.[18,19] As with any SGA medication there is a risk that quetiapine will precipitate adverse metabolic side-effects, and the principle objective of the proposed study is to elucidate risk and protective factors associated with SGA-induced adverse cardiometabolic side-effects and weight gain. Additionally, subjects who exhibit worsening of mood symptoms (they have a 9 point increase in CDRS-R score (1 point increase in > 50% of the 17 CDRS-R items) or a > 6 point increase in YMRS score (1 point increase in > 50% of the 11 YMRS items) from baseline at any two consecutive visits) will be discontinued from study participation. If in the clinical judgment of the study physician a patient exhibits increased suicidality from Phase I baseline at any two consecutive visits, he/she will be discontinued from study participation. Other adverse events may include extrapyramidal symptoms. To minimize this risk, we will permit concomitant medications (e.g. propranolol or anticholinergics) for extrapyramidal syndromes that develop. Additionally, all subjects and their legal guardians will be informed about the potential risks associated with omega-3 fatty acids during the informed consent/assent process. Patients will be informed of potential omega-3 fatty acid side-effects including nausea, diarrhea, gastrointestinal reflux, and of less common side effects, such as vomiting and increased bleeding time. We will routinely monitor for all of these side effects with clinical interviews (i.e., SEFCA), vital signs,

and laboratory measures.

In general, patients for whom further treatment with quetiapine or omega-3 fatty acids is contraindicated, either as a result of exacerbation of psychiatric symptoms or exceeding criteria for clinically-significant adverse cardiometabolic symptoms, will be terminated from the study and final study procedures performed if feasible. We will also instruct patients (and their legal guardians) to contact study staff 24 hours/day, 7 days/week, if any concerns arise during their study participation. Following study discontinuation, study physicians (Drs. DelBello and Patino) will provide clinical care to address urgent safety issues and will actively facilitate clinical referral for individualized treatment to ensure that there is no lapse in care. Alternative medication options will be discussed with the patient and their family if discontinuation is necessary.

In an effort to minimize risks associated with medication interactions, a complete history of prior medications will be obtained from the patient and their legal guardian, as well as, their treating clinician (after a release of information is obtained) by an experienced physician investigator. To minimize the potential risk associated with the screening period, patients will be monitored by study staff for worsening of mood symptoms. Patients will be evaluated in person as needed. Additionally, the screening period may be shortened at the discretion of the study physician. Patients and their families will have access to a study physician 24 hours/day, 7 days/week. The screening period is necessary to ensure the accuracy of the diagnosis. We have successfully used this approach during the screening period to maximize the safety of hundreds of adolescents with disruptive behavior and/or mood disorders in more than 20 prior studies. 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. Nevertheless, The Columbia Suicide Severity Rating Scale (C-SSRS), will be administered at each Phase II visit to measure of suicidality.

To guard against the side effects of omega-3 fatty acids and quetiapine, we will first check baseline laboratory tests as described and will perform vital signs, a physical exam and an ECG to ensure that the subject is physically healthy. We will then initiate dose titration with quetiapine. To maximize safety during the 24-week double blind study phase (Phase II), body weight and fasting triglyceride and glucose levels will be obtained at selected visits, and a patient will be discontinued from study participation if they meet criteria for clinically-significant increases in body weight (≥7% from baseline) and/or clinically-significant increases in fasting triglyceride (≥450 mg/dL) and/or fasting glucose (≥125 mg/dL) on two consecutive visits during study participation. Patients and their legal guardians will also be told to contact study staff immediately if they experience any of the following; allergic reactions like skin rash, itching or hives, swelling of the face, lips, or tongue, anxiety, nervousness, changes in mood or behavior, chest pain, fast, irregular heartbeat, fever, or hot, dry skin, high blood pressure, muscle twitching, or uncontrollable head, mouth, neck, arm, or leg movements. Additionally, to minimize the gastrointestinal adverse events associated with quetiapine or omega-3 fatty acids, we will instruct subjects and their legal guardians to take their study pills with meals. To minimize difficulties associated with taking 5 capsules, patients will be advised to take two capsules during their morning meal and 3 capsules during their evening meal. We have considerable experience administering omega-3 capsules to pediatric and adolescent patients, and have found that this approach is effective and does not reduce compliance as evidenced by capsule counts and self-reports. To minimize the side effects of quetiapine, we will encourage patients to take quetiapine in the evening and we will give them the minimum effective dosage. To minimize the risk of other adverse effects from study treatment, at each study visit following baseline, study physicians (Drs. DelBello or Patino) will carefully monitor all patients for adverse effects using

a clinical interview as well as a standardized structured side effects questionnaire, the SEFCA. To maximize safety we will specifically monitor for bleeding abnormalities, and have added four questions to the SEFCA that are associated with increased bleeding time: “Bruising”, “Nosebleeds”, “Longer than usual bleeding”, and “Heavy bleeding associated with menstruation”. Additionally, the Platelet Function Assay (PFA), a sensitive measure of blood clotting. If a study participant answers positively to any of the bleeding items on the SEFCA, a PFA will be performed at any point during study participation. Patients will be discontinued from study participation if their PFA values are outside the reference range (PFA-EPI: 75.0-199.0 and PFA ADP: 68.0-112.0) on two consecutive visits and provided with follow-up care. We will advise all participants and their legal guardians about potential anti-coagulant effects of omega-3 fatty acids and will prohibit concomitant use of medications that have anticoagulant effects (e.g. aspirin or non-steroidal anti-inflammatory drugs, NSAIDs). Study related adverse events will be defined as a symptom that was not present at or worsened in severity or frequency from baseline. Patients also will be instructed to contact study staff prior to taking any concomitant medications during their study participation to minimize the adverse effects that might occur with medications as well as to accurately record the reason for concomitant medication. Moreover, rates of adverse and serious adverse events will be monitored by a Data Safety Monitoring Board (see Data and Safety Monitoring Plan) that will meet every six months to review study tolerability and efficacy measures and determine whether the study should continue.

Additionally, should the need arise to discontinue a patient from the study because of symptom worsening (as defined previously) or for any other reason, including patient/legal guardian withdrawal of consent, we will collaborate with the patient, their legal guardian, and their non-study related physician to determine treatment options for that patient following study discontinuation. At the beginning of each subject’s study participation, we will make appropriate referrals for all patients who do not have preexisting clinicians so that follow-up care will be in place at the completion of their study participation. If a study participant discontinues study participation and is not able to have an immediate appointment with the clinician to whom they are being referred, a study related physician, in consultation with their treating clinician or the clinician to whom they are being referred, will initiate appropriate treatment for the patient until they are seen by a non-study clinician. The study clinician will discuss treatment options with the patient (and their legal guardians) as well as discuss whether they would like to continue to take quetiapine. Nonetheless, following study discontinuation, the study clinician will begin treatment as indicated in collaboration with non-study related clinicians if applicable and the study participant (and their legal guardian), to maximize the safety of the study participant. Study clinicians will manage the care of study participants until appropriate follow-up treatment is in place. We have been successfully using this approach to manage patients following study participation for more than 15 years and in most situations patients receive follow-up care with a non-study related clinician within 30 days following study participation.

To minimize the risks associated with blood draws, only staff that is trained and certified in phlebotomy will draw blood. To guard against frustration and fatigue, interviewers will be carefully selected and trained. When necessary, assessments will be divided into separate interviews to decrease the time of a single session. To guard against loss of confidentiality, all subject information will be kept in locked file cabinets and in the computer system locked in the research suite. A password will be required to access computer information, and will be available only to the principal investigator and research team members who are directly involved in entering and analyzing the data. Subjects will be identified by code numbers, and the principal investigator will emphasize to the research team the responsibility of maintaining confidentiality and the seriousness of accidental or willful disclosure of confidential

information. Similarly, in the event of data sharing all information will be de-identified and no individual subject will be identifiable. Additionally, all study staff will be certified in Good Clinical Practice (GCP), which refers to a set of international ethical and scientific quality standards for designing, conducting, recording, and reporting studies that involve human subjects. No subject will be identifiable in any publication that should arise from this work. Furthermore, collateral persons who are contacted regarding any subject will be provided with a signed release of information from the legal guardian, and will typically be limited to the subject's primary clinician(s). Subjects can withdraw from this study at any time, at which point contact with collateral sources will end. Subjects will 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. By utilizing the previously described procedures we feel the risks to subjects will be minimized.

We will attempt to minimize the risks associated with reporting of suspected abuse or neglect, by attempting to inform the family prior to contacting any state/county agency. All suspected abuse or neglect will be reported to the PI or if she is unavailable, one of the other study physicians, so that the situation can be further investigated by experienced clinicians. In addition, should the need for reporting arise, the adolescents and legal guardians will be provided with supportive clinical services from the study staff until referrals to appropriate agencies are made. Following termination of study participation all patients who do not have an existing treating clinician will be given appropriate referrals for treatment. Every effort will be made so that all subjects have an appointment with a non-study treating physician prior to their last study visit so that a follow-up medication plan can be in place at the end of the study. If the appointment does not occur prior to the subject's study termination visit, and if clinically indicated and agreeable to subjects and their legal guardians, subjects will continue treatment with their medication and monitored until their follow-up appointment. We have used this approach with prior studies and typically patients are seen by a child and adolescent psychiatrist within one month of termination of study participation.

To protect against potential coercion or undue influence, all study procedures will be carefully explained to the subject and the subject's parent/legal guardian by research staff. Research staff will inform patients that it is ok if they do not want to participate in the study and that they can stop being in the study at any time. Patient's will be advised that they can take time to think about whether they want to participate in the study and that they can sign the assent at a later time. If agreeable research staff will obtain written informed consent and assent for study participation from the legal guardian and adolescent, respectively.

b. Anticipated Benefit Justifies the Risk - Potential benefits include a thorough psychiatric evaluation and monitoring 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 treatment with the quetiapine (and potentially omega-3 fatty acid treatment) that they receive free of charge during study participation in that it may reduce their mood 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. Overall, the risks associated with this study are minimal to the participants and they may

receive direct benefit from their study participation as previously described. Thus, in the opinion of the investigators, the benefits associated with this study outweigh the risks.

Anticipated Benefit as Favorable as Alternative Approaches – An alternative to study participation is not participating and receiving standard of care treatment as directed by a physician of that participant's choosing. Since this study proposes standard of care treatment, the investigators believe participation is as favorable to participants as that of the alternative approach.

H. PAYMENT

Subjects will be reimbursed $20 for each of 13 visits for their time and inconvenience.

I. SUBJECT COSTS

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 have no costs associated with research participation.

J. CONSENT/ASSENT FORMS

See attached.

K. LITERATURE CITED

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