Oxidative Stress and BDNF as Putative Biomarkers of Cognitive Flexibility in Adolescent Bipolar Disorder

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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Abstract – 144/150 words

Executive dysfunction is common and impairing in bipolar disorder (BD), and its pathophysiology is poorly understood. Oxidative stress (OS) and brain-derived neurotrophic factor (BDNF) have been implicated in executive deficits of adult BD, but have not been studied in youth. Serum levels of OS and BDNF were measured in 30 BD and 25 healthy control (HC) adolescents. The intra-extra-dimensional (IED) set-shifting task assessed executive function, particularly cognitive flexibility. High and low BDNF subgroups were defined by median split. IED performance was impaired in BD subjects. LPH and BDNF had opposite correlations in BD and control subjects. LPH and IED performance was oppositely correlated between BD and control subjects. These correlations became stronger when parsed by BDNF. LPH and BDNF have an altered interaction in BD that is relevant to executive function. Antioxidant interventions to improve executive function may be warranted, especially when guided by BDNF levels.
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1. Introduction

1.1 Statement of the Problem

The prevalence of bipolar disorder (BD) among adolescents is approximately 5%, of which 1% have BD-I and the remainder have BD-spectrum disorders that are equally impairing [1]. BD is characterized by periods of mania/hypomania (elated mood and increased energy accompanied by other symptoms), depression (sad mood or loss of interest/enjoyment accompanied by other symptoms) and euthymia (normal mood) [2]. Individuals can change between these three mood states over the course of illness and often suffer from sub-threshold mood symptoms that are not as pronounced but nonetheless cause distress and functional impairment [2]. In addition to mood symptoms, adolescents with BD suffer from cognitive dysfunction across a number of domains [3]. Cognitive dysfunction in BD (both adult and youth) has been widely observed during mood episodes of BD, but a growing body of evidence demonstrates their presence during euthymia [3, 4]. Cognitive impairments in BD are a large source of psychosocial impairment in daily living, affecting relationships with friends and family, as well as academic performance [5]. There is also some evidence of reduced treatment adherence in BD subjects with cognitive dysfunction [6]. Deficits in executive function, processes that involve planning, decision making, and shifting one’s attention between sets of information, contribute substantially to the profile of cognitive deficits in BD [7]. Such impairments in executive function are one of the most consistently reported and well replicated findings in adolescent BD [7-9]. Available treatments for executive dysfunction in BD are limited, with stimulants being effective for comorbid ADHD symptoms, and lamotrigine (an anticonvulsant) and erythropoietin having some evidence of pro-cognitive effects [10, 11]. Modafinil has also shown some pro-cognitive effects in conjunction with standard BD treatments
in adults, but those results are preliminary [12]. However, these treatments are not effective for all patients, and their etiology remains poorly understood [13].

Peripheral biomarkers of cognitive deficits hold high potential clinical utility, as they may identify those at risk of such deficits, form the basis of diagnostic tests, identify novel treatment targets, or predict outcomes of other interventions. Not all of these functions will be provided by one biomarker, but they represent potential advances. Furthermore, rapid and economical measuring of such biomarkers through means such as a blood test would increase their utility in a clinical setting. Candidate biomarkers such as inflammation, oxidative stress (OS), and neurotrophins have been explored in multiple patient and population-based samples [14]. These biomarkers are particularly important given the biological relevance of redox alterations of fatty acids and neurotrophin alterations in the brain. Furthermore, OS and BDNF levels have been repeatedly implicated in the pathophysiology of BD [5, 15-21]. Biomarker studies in adults with BD have the implicit confounds of the negative cognitive effects of aging and years of disease and symptom burden [22]. Studies in adolescent populations may minimize the effect of these confounds, thus offering an opportunity for increased signal detection.

1.2 Purpose of the Study and Objectives

The purpose of the study was to assess cognitive function in adolescents with BD and to examine the utility of OS and neurotrophins as biomarkers of cognitive impairment in BD. The objectives of this study were to:

1. Compare cognitive functioning and biomarker levels between adolescents with BD and healthy controls (HC).
2. Determine the association between peripheral levels of OS markers and performance on a test of executive function.
3. Determine any interactions between OS markers and BDNF and how they relate to executive function.

1.3 Statement of Research Hypotheses and Rationale for Hypotheses

Hypothesis 1

Adolescents with BD will perform worse than HC participants on tests of executive function.

Cognitive deficits are repeatedly reported in BD patients; executive function is one of the most severely impaired domains, and can translate the strongest to real-world dysfunction [23]. Executive dysfunction is one of the most consistent cognitive deficits observed among adults with BD [16, 24, 25]. Adolescents show similar patterns of cognitive impairments to adults [7-9, 26, 27]. Executive function, specifically set shifting and reversal learning have been the focus of adolescent studies, using tasks such as the Wisconsin Card Sorting Task (WCST), and the intra-extra-dimensional set-shifting (IED) task [8, 9, 26]. The IED task will be used in this study, and it is expected that the findings in this study will be consistent with those done previously.

Hypothesis 2

Adolescents with BD will have higher levels of OS markers than HC participants, but similar BDNF levels.

Levels of OS markers are increased in adult BD, with multiple studies and meta-analyses showing the greatest effect sizes (ES) for markers of lipid peroxidation (damage to fatty acids and other lipid molecules) [17, 28-30]. There is a marked paucity of studies examining OS markers in young populations, and there are mixed findings in the small number of studies completed to date. One study demonstrated increased oxidative protein damage in young adults
with BD compared to HC [31]. However a conflicting study found no difference in central levels of the antioxidant glutathione (GSH), albeit in a mixed sample of adolescents and adults [32]. BD is being increasingly recognized as a progressive disorder, and perturbations observed in adult BD samples may be reflected in adolescents [33]. Despite these limited studies, it is predicted there will be greater levels of OS markers in participants with BD [34]. Circulating BDNF levels in BD adolescents have been shown to be similar to those in HC [35]. Although based on a single study, levels of BDNF are not expected to be different between BD and HC participants in this study.

Hypothesis 3

Performance on executive function tasks will be associated with markers of OS in the BD group, possibly mediated by an interaction with BDNF.

OS markers have been associated with executive function in studies involving populations with schizophrenia, depression, obstructive sleep apnea, substance abuse, mild cognitive impairment, and Alzheimer’s disease [36-42]. Healthy individuals with low endogenous antioxidant levels have been shown to have poorer executive function in some studies [43-46]. Given how pervasive this association appears to be across disorders, it may be that there is a common pathology as a result of OS in these populations. Studies in adult BD samples examining OS markers in conjunction with inflammatory markers and neurotrophins have demonstrated associations with cognitive dysfunction and illness severity [35, 47-49]. It is expected that similar associations will be seen in this youth BD population, potentially to a lesser extent given the shorter timeframe of disease progression.
1.4 Review of Literature

1.4.1 Cognition in Adolescent BD

There is substantial evidence in adult BD that the cognitive domains of working memory, processing speed, social cognition, and executive function are impaired [4, 34, 50]. Similar findings have been reported in adolescent BD, although executive function findings have varied depending on the cognitive task being used [7]. A recent review of neuropsychological research in BD youth, encompassing 111 original manuscripts that examined cognitive function across a number of domains, found that the profile of cognitive impairment encompasses those same domains [7]. Attentional set-shifting in particular, also referred to as cognitive flexibility is consistently impaired [9, 51-54]. In a study of 21 BD adolescents and 21 HC, BD participants made more total errors, more errors before the extra-dimensional (ED) shift stage and took more trials to complete the IED task than the HC group [9]. A large scale study of 170 BD youth, 118 non-BD-affected siblings, and 79 HC found significantly worse WCST performance in both the BD and BD sibling groups, compared to HC participants [52]. There is also a substantial body of evidence of executive function deficits using other testing paradigms such as the Reversal Learning Task and the Change Task [8, 55-57]. Executive function deficits have been shown to contribute to psychosocial dysfunction, which pose an additional problem for BD youth [5]. As mentioned, the etiology of cognitive dysfunction in BD is poorly understood, and there are currently few treatments, with limited evidence of efficacy [58]. Evidence has shown that adolescent BD is continuous with adult BD, which is consistent with the continuity of the profile of cognitive deficits discussed above [59]. Thus, understanding the beginning stages of BD will be instrumental in the prevention of its development later into life.
1.4.2 OS Generation and Free Radical Damage

OS is a complex, multifaceted process that involves the production of highly reactive free radical molecules at a sufficient rate to overwhelm a cell’s antioxidant defenses [60]. Protracted periods of OS can result in redox alterations to proteins, DNA, and lipid molecules [61]. This damage can have a wide variety of adverse effects that can perturb many cell functions, and produce changes in cellular functioning [62]. The process of oxidative phosphorylation can produce free radicals, including the particularly reactive superoxide molecule (O$_2^-$), which can diffuse into the cytosol [63]. Figure 1 illustrates a mechanism of OS generation and how superoxide overproduction can lead to damage to lipid molecules, a process referred to as lipid peroxidation. The example in figure 1 demonstrates peroxidation of ω-6 polyunsaturated fatty acids (PUFAs), since these peroxidation products lead to 4-hydroxy-nonenal (4-HNE) production [64].
Figure 1. Illustration of OS generation from mitochondrial-derived superoxide.

(1) Superoxide is formed as a result of oxidative phosphorylation, and is detoxified into hydrogen peroxide by superoxide dismutase (SOD) [60]. Hydrogen peroxide is degraded into hydroxyl and hydroperoxyl radicals with Fe$^{2+}$ as a catalyst [14]. Alternatively, superoxide itself can diffuse into the cytosol and, along with the hydroxyl and hydroperoxyl radicals, can create an environment of elevated OS. (2) Free radicals attack the double-bonds of PUFAs; linoleic acid is shown as an example [65]. Oxidative damage that occurs at carbons 9 and 13 (shown by the left and right arrows, respectively) leads to molecules at the beginning of 4-hydroxynonenal (4-HNE) production (11-HPODE is a minor product of this reaction and does not lead to 4-HNE) [65]. (3) 9-hydroperoxy-octadecadienoic acid (9-HPODE) and 13-HPODE (top and bottom,
respectively) result from this oxidation, these molecules are lipid hydroperoxides (LPH) [66]. For simplicity’s sake, 9 and 13-HPODE are not shown in distinct stereospecific configurations, but it should be noted that the reaction in step 2 is typically not stereospecific, resulting in 9 and 13 (Z,E) and (E,E) –HPODE being produced [65]. (4) Further oxidative damage at the location denoted “!” results in the fission of the lipid molecule and production of 4-HNE, the detailed mechanism for which is not fully understood [66].

There are several characteristics of the brain that make it particularly vulnerable to OS. Human neurons contain high numbers of mitochondria due to their dependence on oxidative phosphorylation, which makes the brain susceptible to the production of superoxide and other reactive oxygen species (ROS) [67]. In addition, the brain contains a large number of lipids by mass, and PUFAs comprise approximately 25-30% of all brain fatty acids, with ω-6 PUFAs specifically constituting approximately 17% of brain fatty acids [64]. These fats are found in both grey and white matter (the latter of which contains lipid-rich myelin), which makes the brain susceptible to lipid peroxidation by free radicals [68]. Taken together, this renders the brain a particularly vulnerable organ to OS [69, 70]. Endogenous antioxidant factors (e.g. GSH, SOD, catalase) work to either scavenge free radicals and bind them to form less reactive complexes, or enzymatically break them down [71]. GSH is a particularly important endogenous antioxidant due to its relative ubiquity throughout the body, and is the primary antioxidant molecule in the brain [72]. These endogenously produced compounds are assisted by exogenous compounds (such as carotenoid compounds and vitamin E) that are primarily derived from dietary sources [73]. The balance between oxidant and antioxidant factors determines overall OS, and the extent to which free radicals and ROS can cause damage [74].
LPH produced as a result of OS damage perturbs the normal function of lipid molecules. With the addition of a highly polar group to the hydrophobic tail of the fatty acid, membrane permeability and fluidity is altered [75]. The peroxide group on this modified fatty acid can be converted into a radical by other free radicals, and then damage other lipids in the membrane [75]. The lipid peroxidation end-product, 4-HNE, can have a variety of cytotoxic effects that depend on the levels of 4-HNE. At low levels, 4-HNE can actually stimulate the production of antioxidant enzymes through the general cellular response to stress [62]. As levels of 4-HNE increase it can form adducts to proteins and DNA which can change the function of these macromolecules and alter their interactions with other molecules [62]. This can lead to cell cycle arrest, senescence, and induction of apoptosis [62].

1.4.3 OS and BD

OS has been implicated in BD pathophysiology in a number of studies, with repeated findings of increased OS markers in BD participants [17, 29, 30, 76]. Meta analyses have shown that many markers of oxidative damage are increased, namely protein carbonylation, DNA/RNA oxidation, lipid peroxidation, and nitric oxide damage [17, 30]. Lipid peroxidation has been identified as being increased to the greatest extent (i.e. has the greatest ES) out of these OS markers [17, 28]. This is especially interesting given the abundance of lipids present in the brain, suggesting that OS localized in the brain may play a specific role in BD. Markers of lipid peroxidation such as malondialdehyde (MDA, another lipid peroxidation end-product) and thiobarbituric acid-reactive substances (TBARS, a measure of OS that includes MDA levels) are increased in BD adults throughout mania, depression, and euthymia (though perturbed to the least extent in euthymia) [77-79]. Despite findings of increased peripheral levels of MDA, studies of peripheral 4-HNE levels have not found similar findings [80]. The same study found significantly greater levels of LPH in BD participants compared to HC [80].
Multiple individual studies have found decreased levels of antioxidant enzymes such as SOD and catalase in BD patients, especially during manic or depressive episodes [19, 81, 82]. However, a meta-analysis of OS markers in BD found that TBARS and nitric oxide (capable of causing nitrosative damage) were significantly increased, and no differences were found for GSH peroxidase (an enzyme that replenishes GSH), SOD, or catalase [78]. A repeat of this meta-analysis in 2014 reported similar findings for antioxidant enzymes, with no significant differences observed, however these negative results may have been influenced by heterogeneity in studies examining SOD [17]. Lipid peroxidation has been suggested as a potential biomarker to validate the staging theory of BD – those with a longer and more severe history of BD would have greater lipid peroxidation [34, 83].

OS markers have been associated with treatment response in BD as well. In animal models of OS, lithium has been shown to be able to reduce OS and prevent OS increases in the prefrontal cortex (PFC) [84]. Furthermore, a study involving early stage BD patients found that despite no significant difference in baseline TBARS levels, those who responded well to lithium treatment had decreased TBARS compared to non-responders after 6 weeks of treatment [29]. In that study both responders and non-responders had higher TBARS levels than that of HC participants at baseline [29]. Given the breadth of OS literature in BD, further depth into how OS markers associate with various symptoms and cognitive impairments is warranted.

1.4.4 OS and Cognition

Cognitive dysfunction has been associated with OS in the aging process and many neurodegenerative conditions such as MCI and dementia, which stands to reason given the vulnerability of the brain to oxidative damage [67, 85, 86]. A systematic review of populations with MCI and dementia also found repeated evidence of associations between OS and cognition
In these clinical neurological conditions there are structural changes that occur in the brain which may give rise to cognitive deficits [74]. OS has also been reported as potentially being a general peripheral biomarker of neurodegeneration or related processes [89]. However, the association between OS and cognitive function is not limited to neurodegenerative diseases, mild cognitive impairment and Alzheimer’s disease.

Cognitive dysfunction is also present in neurologically intact individuals, including healthy individuals and those with major depressive disorder, schizophrenia, obstructive sleep apnea, and BD [90, 91]. There is a growing body of literature in this field, which has rapidly expanded over the past 5 years, and a recent systematic review examining the association between OS markers and cognitive function in individuals without overt dementia or stroke found that the most common impairments in individuals were in executive function [92]. Lipid peroxidation markers, or related antioxidants, were most commonly associated with these impairments [92].

Multiple studies examining populations with schizophrenia have shown that levels of antioxidants such as SOD, thioredoxin, and measures of total antioxidant status are positively associated with attention [40-42, 93, 94]. GSH levels have also been associated with superior attention and executive functioning in schizophrenia patients [95, 96]. A study of stimulant-dependent patients found that increased levels of oxidative DNA damage were associated with poorer trail making test (TMT) and Stroop test performance – measures of executive function [39]. Population-based studies of healthy individuals found that lower levels of carotenoids, a group of lipophilic antioxidant molecules, are associated with poorer executive function as assessed by the TMT and Stroop test [44, 45].
OS has been suggested as possibly being implicated in BD neuroprogression, stemming from mitochondrial dysfunction [97]. There is evidence of decreased mitochondrial activity in pre-frontal brain regions of BD participants, which are functionally linked with executive function processes [98]. There is also abnormal expression of mitochondrial electron transport chain proteins in frontal and pre-frontal brain regions in post-mortem brain samples of BD patients [99]. Such alterations suggest that individuals with BD may have an intrinsic vulnerability to OS, especially in the brain. Furthermore, this may provide a basis for how OS relates to cognitive function in BD.

1.4.5 BDNF and its Association with BD and Cognition

BDNF is a neurotrophin that is responsible for neuronal growth and survival, maintenance of proper synapse function, and plays a role in long-term potentiation [47]. Polymorphisms in the BDNF gene have been studied in relation to a number of psychiatric disorders and neurodegenerative diseases, given the protective actions of BDNF. The val66met polymorphism (rs6265) is one of the most studied in relation to psychiatric disorders, and has been repeatedly implicated in BD pathophysiology [18]. Multiple studies examining WCST performance in BD participants have shown that carriers of at least one Met allele had significantly worse WCST performance than those with the Val/Val genotype [21, 100-102]. Met carriers have also been shown to have deficits in verbal working memory and visuospatial capabilities [100]. A possible explanation of these deficits is altered brain morphology in BD Met carriers compared to Val/Val. Gray matter volume and the extent of gyrification in Met carriers is significantly lower than in Val/Val individuals, and decreases over the course of illness [20, 103].
In addition to the val66met polymorphism, circulating levels of BDNF have been associated with phases of BD. Multiple studies have shown that BDNF levels are decreased in periods of mania and depression [104-106]. Reports of euthymic BD patients are less consistent, but there do not appear to be marked differences as there are during symptomatic episodes [104-106]. Evidence suggests that BDNF levels decline in euthymic patients over the course of illness, suggesting BDNF levels may reflect disease progression [105]. In relation to cognitive function, there is not the same body of evidence for circulating BDNF levels are there is for BDNF genotype. Though few in number, some studies have reported that circulating BDNF levels may not directly correlate with WCST performance or other executive function tests [26, 107]. One such study also reported a positive correlation between BDNF and verbal fluency performance in both BD and HC participants [26]. However, Dias et al. reported suboptimal matching of groups and the BD group was medically healthy overall, had few episodes, and short illness duration [26]. In both studies, medications may have impacted BDNF levels [26, 107]. These confounds may have contributed to the general lack of association between peripheral BDNF levels and cognitive function in these two studies.

1.4.6 Integrating OS with Other Biomarkers

Though OS markers and BDNF have been independently implicated in BD and its associated executive dysfunction, such markers have also been implicated in other psychiatric disorders such as major depression and schizophrenia [92, 108]. It has not been the case that one biomarker will be able to discriminate between individuals with BD and other psychiatric disorders, or subtypes of BD [109]. Some studies have examined multiple biomarkers, but have only examined pair-wise associations of markers with diagnoses or other outcomes, which might overlook pathological states specific to certain disorders or traits. For example, a study of the inflammatory marker tumor necrosis factor-alpha (TNF-α) and BDNF did not examine how the
interactions between markers related to executive function [16]. However, there is a growing body of research examining relationships between multiple markers and BD, and executive dysfunction.

Studies have shown that OS can be a potent activator of microglial cells, which are the macrophages of the brain [110]. Thus, OS and inflammation may interact through such a mechanism that would not be entirely explained if simply one marker was measured. In the case of BDNF, in addition to its numerous neuroprotective effects it has antioxidant properties, and can reduce OS in the central nervous system [111]. A study of BD, HC, and septic participants found that integrating markers of OS, inflammation, and BDNF could significantly discriminate between HC, euthymic BD, symptomatic BD, and septic states [48]. The same study found that TBARS and BDNF were significantly correlated. Another study has found that TBARS and BDNF levels are negatively correlated in mania, and positively and non-significantly in HC participants [112]. Examining to what extent manic symptoms are driving this correlation would help parse the state and trait-related correlations. A study examining a similar composite score including TBARS, BDNF, and inflammatory markers in a sample of young adults, early in the course of disease, did not find any differences between BD, major depressive disorder (MDD), and HC participants [113]. However, this was a population-based sample not a clinical sample, and illness severity may be lower thus reducing the observed signal. Additionally, in the early stages of such disorders, these markers may be perturbed to a lesser extent and associations with diagnoses or symptoms may be more nuanced.

Research in the field of schizophrenia has discovered similar findings of interactions between peripheral BDNF levels and markers of OS. A study of chronic schizophrenia participants found a negative correlation between BDNF and SOD activity in schizophrenia but
not HC participants [114]. Furthermore, the interaction between BDNF and SOD activity in schizophrenia participants significantly predicted cognitive function.

In addition to evidence from human samples, animal models have shown that OS and BDNF pathways show a substantial overlap and interact with each other. One study examined cortical BDNF expression in rats after inducing OS, and found that the induction of OS directly caused a compensatory increase in BDNF expression that was attenuated by vitamin E administration [115]. OS was induced through chronic hypoxia, which may make the findings more translatable to BD, as altered cerebral blood flow has been implicated in BD and cognitive dysfunction [116]. Given the literature examining interactions between OS and BDNF in adult BD and schizophrenia, and the animal literature supporting a putative mechanism, these factors warrant examination in BD adolescents. Furthermore, these factors have not been examined directly in relation to cognition in adolescent BD, warranting investigation.
2. Materials and Methods

2.1 Study Design

The association between OS and cognition in adolescent BD was assessed using a cross-sectional design to compare serum oxidative stress marker levels with neuropsychological test performance. A screening visit was performed before the main study visit, to gather demographic information and some clinical characteristics. A parent or guardian of the adolescent also participated to provide additional interview-collected and self-reported information. The methods and procedures used in this study have been approved by the Sunnybrook Health Sciences Centre (SHSC) Research Ethics Board (Appendix 1).

2.2 Participant Selection

2.2.1 Participant Recruitment

Thirty adolescents with BD were recruited from the Centre for Youth Bipolar Disorder at SHSC in Toronto, Ontario, Canada. BD participants were diagnosed with BD I, II, or operationally-defined BD – not otherwise specified (NOS) using the Kiddie – Schedule for Affective Disorders and Schizophrenia – Present and Lifetime version (K-SADS-PL). The BD-NOS definition was based on the Course and Outcome of Bipolar Youth (COBY) study [117]. Though BD-NOS diagnoses do not meet the severity criteria of BD-I or II, studies have shown that adolescents with BD-NOS have similar ages of onset, rates of comorbid diagnoses, suicidal ideation and types of manic symptoms to BD-I [117]. Other features of BD-NOS are on a similar continuum of impairment as BD-I. The K-SADS-PL is a semi-structured interview tool intended to obtain current episode and lifetime history of psychiatric disorders [118]. Participants who met diagnostic criteria for BD were then asked to participate in the main study visit. Twenty-five medically and physically HC participants were recruited from the Greater Toronto Area using
advertisements on public transit (TTC) and retail establishments. Both BD and HC participants completed all study procedures. The eligibility criteria for selection are described below. Written, informed consent was obtained from all study participants and a parent or guardian before commencement of study procedures (Appendices 2 and 3).

2.2.2 Inclusion Criteria

For BD participants

- Aged between start of 13th year and end of 21st year
- Primary diagnosis of BD

For HC participants

- Aged between start of 13th year and end of 21st year
- No major or recent psychiatric disorder
- No family history (1st and 2nd degree relatives) of BD or psychosis

2.2.3 Exclusion Criteria

- Has any cardiac condition, auto-immune illness, or inflammatory illness
- Taking any anti-inflammatory, anti-platelet, anti-lipidemic, anti-hypertensive, or hypoglycemic agents
- Has had an infectious illness in the past 14 days
- Not able to provide informed consent
2.3 Study Measures

2.3.1 Physical Measures

Blood Draw

Participants arrived fasting for 10 hours, and a blood-draw via antecubital venipuncture with a 21G butterfly needle (BD Canada, Mississauga, ON, Canada) was performed. All blood-draws were performed by trained study personnel or by phlebotomy staff at the SHSC Blood Collection Centre between 9:00AM and 12:00PM. One gold-topped Serum Separator Tube (SST), two lavender-topped EDTA tubes, and two tiger-topped tubes (BD Canada, Mississauga, ON, Canada) were collected from every participant. The gold tube was sent to the SHSC Clinical Pathology Department for measurement of high sensitivity C-reactive protein, LDL cholesterol, HDL cholesterol, total cholesterol, triglycerides, and glucose levels. The whole-blood-containing lavender tubes were stored at -80°C for future testing. The tiger-topped tubes were left at room-temperature for 15 minutes to allow for clotting, and then centrifuged (Drucker Diagnostics, Port Matilda, PA, USA) at 3000rpm for 15 minutes. The resulting serum phase was pipetted into 0.5mL eppendorf tubes (Eppendorf Canada, Mississauga, ON, Canada) and stored at -80°C.

Serum Analyses

The serum samples were analysed for LPH, 4-HNE, and BDNF levels. Enzyme-linked immunosorbent assays (ELISAs) were used to determine BDNF and 4-HNE (Chemicon, Temecula, CA, USA) and 4-HNE (Cell Biolabs Inc., San Diego, CA, USA) levels. The range of detection of BDNF was 7.8pg/mL – 500pg/mL, with intra-assay variation of 3.7% and inter-assay variation of 8.5%. The detection range for 4-HNE was 1.56 μg/mL - 200 μg/mL. LPH levels were determined using a commercially available ferric thiocyanide assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). The detection range for the LPH assay was 0.25-
5nmol. All samples were analyzed by Dr. Ana Andreazza’s laboratory in a single batch to decrease inter-assay variability.

**Anthropomorphic Data**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Method of Collection</th>
<th>Manufacturer of Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>Wall-mounted stadiometer</td>
<td>Seca Inc., Chino, CA, USA</td>
</tr>
<tr>
<td>Weight</td>
<td>Body Mass Analysis Scale</td>
<td>Conair Consumer Products Inc., Woodbridge, ON, Canada</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>Measuring tape</td>
<td></td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>Electronic sphygmomanometer</td>
<td>Omron Healthcare, Kyoto, Japan</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI score, BMI percentile, and BMI z-score were calculated for participants under the age of 20 using an online pediatric calculator, based on growth charts from the Centre for Disease Control (CDC) [119]. An adjusted BMI score was also obtained using an adjusted weight score to account for the approximate weight of clothes worn by the participant. 1.3kg was removed from the original weight reading if the participant was wearing long pants and a long sleeved shirt, 1.1kg was removed for short pants or short sleeves, and 0.9kg was removed for short sleeves and short pants.
2.3.2 Psychiatric Measures

Main Study Assessment

**Table 2.** Summary of psychiatric measures obtained at each study visit. “I” indicates an interview measure, and “SR” indicates a self-report measure.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Collection Method</th>
<th>Completed by Adolescent</th>
<th>Completed by Parent or Guardian about Adolescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mania &amp; Depression Rating Scales (MRS &amp; DEP-P)</td>
<td>I</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CARDIA Family Medical History</td>
<td>I</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Family History Screen</td>
<td>I</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Adolescent Longitudinal Interval Follow-Up Evaluation (ALIFE) Psychiatric Status Rating (PSR)</td>
<td>I</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>ALIFE Psychosocial Treatment Schedule</td>
<td>I</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>ALIFE Psychotropic/Auxiliary Drugs</td>
<td>I</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Medical History</td>
<td>SR</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
**MRS:** 13-item rating scale (scores range from 0 to 6) regarding the presence and severity of manic symptoms [120]. The 13 scores are combined to create a summary score. This measure is derived from the K-SADS-PL.

**DEP-P:** 21-item rating scale (scores range from 0 to 6) regarding the presence and severity of depressive symptoms [121]. As with the MRS, a summary score is created from these individual items. This measure is derived from the K-SADS-PL.

**CARDIA Family Medical History:** 14 item questionnaire that obtains cardiovascular disease, stroke, and metabolic syndrome history about 1st and 2nd degree relatives [122].

**Family History Screen:** Screening tool used to detect psychiatric disorders in 1st and 2nd degree relatives [123].

**ALIFE PSR:** Part of the Longitudinal Interval Follow-up Evaluation (LIFE), giving a view of week by week changes in psychiatric symptoms across all diagnoses [124]. PSR is completed for the 12 weeks leading up to the main study visit. If a BD subject scored a 4 or more on depression in the two weeks prior to the visit they were classified as depressed. The same cut-off was used for hypomania. If both criteria were met the subject was classified as being in a mixed state.

**Psychosocial Treatment Schedule:** For the same time period as PSR is rated, history about psychosocial treatment (type of treatment and frequency per week) is collected [124].

**Psychotropic/Auxillary Drugs:** Similar to PSR, a 12-week profile of psychototropic drug use is completed via interview [124]. Average dose per week and type of psychotropic is recorded.

**Medical History:** The Western Psychiatric Institute Medical History questionnaire was completed by a parent or guardian of the participant. Questions are asked relating to illnesses, trauma, and pregnancy/labour history [18, 125].
2.3.3 Cognitive Measures

Wechsler Abbreviated Scale of Intelligence (WASI)

The WASI is a brief assessment of general intellectual ability, comprising four main subtests that examine different aspects of intelligence: vocabulary, block design, similarities, and matrix reasoning [126]. All subtests have start and end points determined by age, and discontinuation criteria if performance falls below a specific threshold. Scores on these subtests are converted to age-adjusted t-scores (not directly to IQ scores, in order to increase differentiation between scores) based on the WASI standardization sample of 2,245 healthy US children and adults [126]. These age-adjusted t-scores are then summed and converted to an IQ score. Four main IQ scores are derived from the WASI: performance, verbal, full-4, and full-2. The full-2 score is used as an IQ measure in this study, as the vocabulary and matrix reasoning subtests that make up this score are the most correlated with general intelligence [126].

Vocabulary: This 42-question section contains word prompts, and the participant must provide a definition of the word. The provided definition is given a score ranging from 0-1 for the first four questions, and 0-2 for the remaining questions. The score is determined through comparison to criteria in the WASI scorebook.

Block Design: This 13-question section requires the participant to create a given design using blocks (2 sides are coloured, 2 sides are uncoloured, and 2 sides are half-coloured). The time taken to complete the design and whether or not it is correct determines the score for that question.

Similarities: This 26-question section provides two words to the participant, and they must determine the similarities between the two words. As with the vocabulary section, their responses are compared to pre-made criteria to determine their score.
Matrix Reasoning: This 35-question section shows a visual pattern on a page, and the participant must choose one of the answers that correctly follow with the pattern.

Cambridge Neuropsychological Test Automated Battery (CANTAB)

The CANTAB Research Suite software (Cambridge Cognition Ltd, Cambridge, UK) was used to administer the test on a touch screen (KEYTEC, Garland, Texas, USA). Some tests required the use of a single-button response, for which a press pad provided by Cambridge Cognition was used that had sufficient sensitivity to record millisecond response times. Every participant completed the same battery with the tests presented in the same order, these tests will be described below in the order that they were presented to the participant. Outcome measures that were used in the analyses below are presented and explained along with these tasks. Measures were chosen based on the recommended output from the CANTAB software and previous reports of IED performance in youth BD [8, 9].

Big/Little Circle (BLC): The BLC task assesses reversal learning, and is used as a training task for the next task in the battery. Two boxes appear on the screen each containing a circle, and for the first 20 trials the participant must select the smaller of the two circles. Afterwards, the participant must then select the larger of the two circles for the last 20 trials [127].

Intra-Dimensional/Extra-Dimensional Shift (IED): The IED task is an executive function task that assesses reversal learning and attentional set-shifting [127]. Four boxes appear on screen, with two of them containing pink shapes. The participant must guess at random the correct shape at first, and then continue selecting the correct shape. After six consecutive correct responses (learning criteria) the criteria changes and a different response will be correct. There are multiple stages to this task, involving more complex discrimination between images, visualized in figure 1. Two main output measures, simple reversal trials and errors, were used in these analyses. Five
secondary output measures were also collected and were corrected for multiple comparisons in subsequent analyses. These secondary measures were, total errors (adjusted for stages completed), total trials (adjusted), completed stage trials, pre-extra-dimensional shift errors (pre-ED errors), and extra-dimensional shift stage errors (EDS errors). A description of all these variables is shown in table 3. This approach is consistent with previous studies of IED performance in BD youth [8, 9]. The CANTAB software provides Z-scores of IED performance that are age and sex-adjusted, compared to historical controls. Z-scores were also averaged for each participant to create a composite Z-score. For raw scores, lower scores indicate better performance, whereas lower Z-scores indicate poorer performance.

Figure 2. Flow chart depicting the progression of stages of the IED task.

Participants must meet learning criteria of 6 consecutive correct responses to progress to the next stage of the task [127]. After 50 incorrect trials at any stage the test is terminated.
Table 3. Descriptions of the IED outcome measures used in this study.

<table>
<thead>
<tr>
<th>IED Measure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple reversal trials</td>
<td>The number of trials taken to complete stage 2 of the IED task. This provides a measure of simple reversal learning.</td>
</tr>
<tr>
<td>Simple reversal errors</td>
<td>The number of errors made during stage 2 of the IED task. This provides a measure of simple reversal learning.</td>
</tr>
<tr>
<td>Total errors (adjusted)</td>
<td>The number of errors made in the IED task, as described above, however 50 errors are added for each stage not attempted. This gives a measure of the participant’s efficiency in completing the task.</td>
</tr>
<tr>
<td>Completed stage trials</td>
<td>The number of trials done on all completed stages of the IED task.</td>
</tr>
<tr>
<td>Total trials (adjusted)</td>
<td>The number of trials done in the entire task, with 25 trials added as an adjustment for each stage not attempted.</td>
</tr>
<tr>
<td>Pre-ED errors</td>
<td>The number of errors made before the ED shift stage. It is the same as the sum of errors in stages 1 to 7.</td>
</tr>
<tr>
<td>ED Shift errors</td>
<td>The number of errors made during the ED shift stage (stage 8).</td>
</tr>
</tbody>
</table>
2.4 Statistical Analyses

Continuous variables were presented as mean ± SD, and categorical variables as count and percentage. One BD participant did not complete the cognitive battery, and was therefore excluded from analyses. Normality was assessed using Shapiro-Wilk tests, with non-parametric statistical tests being used for non-normally distributed variables as appropriate. For some analyses, participants were divided into subgroups based on BDNF levels. Due to a lack of an established BDNF cut-off, a median split (167.7 pg/mL) was used to separate the sample into individuals above the 50th percentile of BDNF (high BDNF subgroup) and those below the 50th percentile (low BDNF subgroup). Continuous variables were compared between groups using independent-samples t-tests and Mann-Whitney U-tests. One-way ANOVA or Kruskal-Wallis H test was used to compare continuous variables when three groups were analyzed. Categorical variables were compared between groups using \( \chi^2 \) tests, or Fischer’s Exact Test for categorical variables with expected counts less than five. Effect sizes were reported as Cohen’s d for continuous variable comparisons, and Cramer’s V for categorical comparisons. Results from the bivariate correlation analysis were further analyzed using General Linear Model (GLM) analysis. Correlation coefficients were compared between BD and HC groups, as well as between BDNF subgroups using Fisher’s Z-test (FZT). Due to a significant difference in age, IQ, and ADHD comorbidity between BD and HC groups, they were controlled for in GLM analyses. A propensity score comprised of these three covariates, as opposed to including them separately, was calculated in order to minimize the loss of degrees of freedom and maximize power in these analyses. Binary logistic regression was used to predict diagnosis (BD or HC group) using the three covariates as terms in the model. A predicted probability (propensity score) between 0 and 1 was calculated for each subject and used in subsequent analyses. For between-group analyses, psychiatric comorbidities were grouped into (1) SUD, (2) ODD or CD, (3) anxiety, and (4)
ADHD. A total score ranging from 0 to 4 was calculated for each subject. Pearson or spearman correlations were used to examine associations between biomarkers and between biomarkers and cognitive function. The Benjamini-Hochberg False Discovery Rate (FDR) method was used to correct for multiple comparisons, with FDR-adjusted p-values expressed as q-values [128]. Statistical significance was defined as p<0.05. With the sample size of 29 BD and 25 HC participants, this study had a statistical power of 0.111 to detect small effects (ES=0.20) between groups, power of 0.436 to detect medium effects (ES=0.50), and a power of 0.820 to detect large effects (ES=0.80). This study was better powered to study correlations within groups, with a power of 0.32 to detect small effects of correlations (ρ=0.30), power of 0.747 to detect medium effects (ρ=0.50), and a power of 0.96 to detect large effects (ρ=0.70). These correlation power calculations were done using the HC sample size to be more conservative, thus the power for each level of effect is larger than stated in the BD group. All statistical tests were performed using SPSS version 22 (IBM Inc, Armonk, New York, USA).
3. Results

3.1 Demographic and Clinical Characteristics of the Sample Population

The BD group was significantly older than the HC group (p=0.005), had significantly higher DEP-P (p<0.001) and MRS scores (p<0.001), significantly lower IQ (p=0.009), and significantly greater BMI (p=0.002), lifetime physical and/or sexual abuse (p=0.032), ADHD (p=0.005), ODD (p=0.006), anxiety (p<0.001), and tobacco use (p=0.033). All differences except for history of abuse remained significant after correcting for multiple comparisons, as seen in table 4. There were no significant differences in IED raw scores, LPH, 4-HNE, or BDNF levels between groups, as shown in tables 5 and 6.

| Table 4. Descriptive characteristics, biomarker levels and cognitive performance of the study population. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Demographic Characteristics | BD Group (n=29) | HC Group (n=25) | p-value | q-value | Effect Size |
| Age (years) | 16.80 ± 1.86 | 15.40 ± 1.68 | 0.007* | 0.016* | 0.787 |
| Male, n (%) | 12 (41%) | 12 (48%) | 0.415 | 0.447 | 0.066 |
| Race (white), n (%) | 27 (93%) | 19 (76%) | 0.270 | 0.315 | 0.131 |
| BMI | 23.82 ± 3.89 | 20.34 ± 3.12 | 0.002* | 0.006* | 0.852 |
| Clinical Characteristics |
| BD-I, n (%) | 6 (21%) |
| BD-II, n (%) | 13 (45%) |
| BD-NOS, n (%) | 10 (34%) |
| KSADS - DEP-P score | 15.28 ± 10.38 | 0.12 ± 0.44 | <0.001* | 0.005* | 1.352 |
| KSADS - MRS score | 10.20 ± 12.54 | 0.20 ± 0.04 | <0.001* | 0.005* | 1.087 |
| Euthymic | 16 (55%) |
| Depressed | 7 (24%) |
Table 4 (continued). Descriptive characteristics, biomarker levels and cognitive performance of the study population.

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>BD Group (n=29)</th>
<th>HC Group (n=25)</th>
<th>p-value</th>
<th>q-value</th>
<th>Effect Size&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypomaniac</td>
<td>2 (7%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed State</td>
<td>4 (14%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illness Duration (years)</td>
<td>2.90 ± 2.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical/Sexual Abuse</td>
<td>5 (17%)</td>
<td>0 (0%)</td>
<td>0.075&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.105</td>
<td>0.263</td>
</tr>
<tr>
<td>ADHD, n (%)</td>
<td>15 (52%)</td>
<td>4 (16%)</td>
<td>0.002&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.006&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.420</td>
</tr>
<tr>
<td>SUD, n (%)</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
<td>0.493</td>
<td>0.493</td>
<td>0.182</td>
</tr>
<tr>
<td>ODD, n (%)</td>
<td>8 (28%)</td>
<td>0 (0%)</td>
<td>0.012&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.021&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.355</td>
</tr>
<tr>
<td>Anxiety, n (%)</td>
<td>17 (59%)</td>
<td>0 (0%)</td>
<td>&lt;0.001&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.005&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.712</td>
</tr>
<tr>
<td>Lifetime Tobacco Use</td>
<td>10 (34%)</td>
<td>2 (7%)</td>
<td>0.031&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.048&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.297</td>
</tr>
<tr>
<td>Current Smoking</td>
<td>4 (14%)</td>
<td>0 (0%)</td>
<td>0.115</td>
<td>0.146</td>
<td>0.267</td>
</tr>
<tr>
<td>IQ&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.46 ± 13.22</td>
<td>112.76 ± 11.95</td>
<td>0.009&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.018</td>
<td>0.122</td>
</tr>
</tbody>
</table>

**Concomitant Medications**

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td>23 (79%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>7 (24%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSRI</td>
<td>4 (14%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>5 (17%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> These variables were non-normally distributed, thus Mann-Whitney U tests were performed.

<sup>b</sup> These variables were normally distributed, thus independent-samples T tests were performed.

<sup>c</sup> Cohen’s d was used for continuous variables and Cramer’s V for categorical variables.

*indicates statistical significance

Variables highlighted in green were used for propensity score calculation.

**Abbreviations:** BD – Bipolar Disorder, HC – Healthy Control, KSADS-PL – Kiddie – Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version, DEP-P – Depression section of KSADS-PL, MRS – Mania Rating Scale of KSADS – PL, ADHD – Attention Deficit Hyperactivity Disorder, SUD – Substance Use Disorder, ODD – Oppositional Defiant Disorder, IQ – Intelligence Quotient, SGA – Second Generation Antipsychotic, SSRI – Selective Serotonin Reuptake Inhibitor
Table 5. Comparison of IED performance raw scores, age and sex-adjusted Z-scores, and biomarker levels between BD and HC groups.

<table>
<thead>
<tr>
<th>IED Raw Score a</th>
<th>BD Group (n=29)</th>
<th>HC Group (n=25)</th>
<th>p value</th>
<th>q value</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple reversal errors</td>
<td>1.55 ± 1.02</td>
<td>1.52 ± 1.01</td>
<td>0.829</td>
<td>0.030</td>
<td>0.030</td>
</tr>
<tr>
<td>Simple reversal trials</td>
<td>8.93 ± 3.95</td>
<td>8.56 ± 3.37</td>
<td>0.788</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Total errors (adjusted)</td>
<td>22.82 ± 17.07</td>
<td>18.32 ± 15.17</td>
<td>0.211</td>
<td>0.277</td>
<td>0.277</td>
</tr>
<tr>
<td>Completed stage trials</td>
<td>74.82 ± 20.63</td>
<td>73.4 ± 16.61</td>
<td>0.510</td>
<td>0.075</td>
<td>0.075</td>
</tr>
<tr>
<td>Total trials (adjusted)</td>
<td>93.79 ± 32.00</td>
<td>83.4 ± 26.24</td>
<td>0.146</td>
<td>0.352</td>
<td>0.352</td>
</tr>
<tr>
<td>Pre-ED errors</td>
<td>6.93 ± 3.13</td>
<td>6.08 ± 2.41</td>
<td>0.513</td>
<td>0.301</td>
<td>0.301</td>
</tr>
<tr>
<td>ED Shift errors</td>
<td>8.65 ± 8.77</td>
<td>7.48 ± 8.94</td>
<td>0.956</td>
<td>0.132</td>
<td>0.132</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IED Z-Score a</th>
<th>BD Group (n=29)</th>
<th>HC Group (n=25)</th>
<th>p value</th>
<th>q value</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total errors (adjusted)</td>
<td>0.36 ± 0.51</td>
<td>0.64 ± 0.44</td>
<td>0.025*</td>
<td>0.075</td>
<td>0.589</td>
</tr>
<tr>
<td>Completed stage trials</td>
<td>-0.09 ± 1.00</td>
<td>-0.08 ± 0.72</td>
<td>0.755</td>
<td>0.755</td>
<td>0.755</td>
</tr>
<tr>
<td>Total trials (adjusted)</td>
<td>0.24 ± 0.58</td>
<td>0.62 ± 0.44</td>
<td>0.016*</td>
<td>0.075</td>
<td>0.724</td>
</tr>
<tr>
<td>Pre-ED errors</td>
<td>0.26 ± 0.36</td>
<td>0.40 ± 0.26</td>
<td>0.139</td>
<td>0.175</td>
<td>0.424</td>
</tr>
<tr>
<td>ED Shift errors</td>
<td>0.18 ± 0.83</td>
<td>0.46 ± 0.78</td>
<td>0.146</td>
<td>0.175</td>
<td>0.343</td>
</tr>
<tr>
<td>Composite Score</td>
<td>0.19 ± 0.44</td>
<td>0.41 ± 0.38</td>
<td>0.047*</td>
<td>0.090</td>
<td>0.550</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPH (μM) b</td>
</tr>
<tr>
<td>4-HNE (fmol/μg) a</td>
</tr>
<tr>
<td>BDNF (pg/mL) a</td>
</tr>
</tbody>
</table>

a These measures were non-normally distributed, thus Mann-Whitney U tests were performed.
b This measure was normally distributed, thus Independent-samples T-tests were performed.
* indicates statistical significance.

Higher raw scores indicate poorer performance, whereas higher Z-scores indicate better performance.

**Abbreviations**: BD – Bipolar Disorder, HC – Healthy Control, ED – Extra-dimensional, IED – Intra-dimensional, Extra-dimensional set-shifting task, LPH – Lipid Hydroperoxides, 4-HNE – 4-Hydroxynonenal, BDNF – Brain-Derived Neurotrophic Factor.
3.2 IED Performance and Biomarker Levels between Groups

3.2.1 Diagnosis

IED performance and biomarker levels were examined between participants diagnosed as BD-I or II, BD-NOS, and HC. As before, no significant differences were seen in the raw scores between groups. The total trials (adjusted) Z-score was significantly different (p=0.043*) between the three groups; BD-I/II participants performed worst, followed by BD-NOS, then HC participants, but did not survive correction for multiple comparisons (q=0.195). There were trends for total errors (adjusted) (p=0.065) and the composite Z-scores (p=0.057), with performance differences showing the same pattern as total trials (adjusted) (BD-I/II < BD-NOS < HC). None of the biomarkers were significantly different between diagnoses.

3.2.2 Symptomatic State

IED performance and biomarker levels were compared across groups based on symptomatic state. BD participants in a depressive, hypomanic, or mixed state were classified as symptomatic (SBD). BD participants in euthymia (EBD) were separated from HC participants with no mood episodes. Table 6 contains the results of these comparisons.

3.2.3 Psychiatric Comorbidity

In the whole group, there were no significant differences in biomarker levels or IED performance between groups based on number of comorbidities. There were no significant differences when these variables were examined within groups.

3.2.4 Current Smoking

No significant differences in IED performance or biomarker levels were seen between current smokers and non-smokers in the whole group or BD group.
Table 6. Comparison of IED performance Z-scores and biomarker levels between symptomatic states of BD and HC participants.

<table>
<thead>
<tr>
<th>IED Z-Score</th>
<th>SBD Group (n=13)</th>
<th>EBD Group (n=16)</th>
<th>HC Group (n=25)</th>
<th>p value</th>
<th>q value</th>
<th>Effect Size a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total errors (adjusted)</strong></td>
<td>0.12 ± 0.54</td>
<td>0.56 ± 0.40</td>
<td>0.64 ± 0.44</td>
<td>0.009*</td>
<td>0.030*</td>
<td>0.94, 0.19,</td>
</tr>
<tr>
<td><strong>Completed stage trials</strong></td>
<td>-0.38 ± 1.34</td>
<td>0.13 ± 0.56</td>
<td>-0.08 ± 0.72</td>
<td>0.592</td>
<td>0.761</td>
<td>0.52, 0.32,</td>
</tr>
<tr>
<td><strong>Total trials (adjusted)</strong></td>
<td>-0.03 ± 0.62</td>
<td>0.46 ± 0.47</td>
<td>0.62 ± 0.44</td>
<td>0.005*</td>
<td>0.030*</td>
<td>0.90, 0.35,</td>
</tr>
<tr>
<td><strong>Pre-ED errors</strong></td>
<td>0.15 ± 0.48</td>
<td>0.35 ± 0.19</td>
<td>0.40 ± 0.26</td>
<td>0.229</td>
<td>0.412</td>
<td>0.57, 0.21,</td>
</tr>
<tr>
<td><strong>ED Shift errors</strong></td>
<td>-0.24 ± 0.92</td>
<td>0.54 ± 0.57</td>
<td>0.46 ± 0.78</td>
<td>0.034*</td>
<td>0.077</td>
<td>1.05, 0.11,</td>
</tr>
<tr>
<td><strong>Composite Score</strong></td>
<td>-0.13 ± 0.59</td>
<td>0.43 ± 0.34</td>
<td>0.46 ± 0.48</td>
<td>0.010*</td>
<td>0.030*</td>
<td>1.20, 0.07,</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biomarker Levels</th>
<th>SBD Group (n=13)</th>
<th>EBD Group (n=16)</th>
<th>HC Group (n=25)</th>
<th>p value</th>
<th>q value</th>
<th>Effect Size a</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPH (μM)</td>
<td>8.06 ± 3.15</td>
<td>8.22 ± 2.73</td>
<td>8.66 ± 3.48</td>
<td>0.831</td>
<td>0.913</td>
<td>0.01</td>
</tr>
<tr>
<td>4-HNE (fmol/μg)</td>
<td>116.26 ± 61.70</td>
<td>104.48 ± 58.63</td>
<td>126.99 ± 60.74</td>
<td>0.340</td>
<td>0.510</td>
<td>0.20, 0.38,</td>
</tr>
<tr>
<td>BDNF (pg/mL)</td>
<td>185.33 ± 72.91</td>
<td>169.35 ± 45.71</td>
<td>181.12 ± 68.99</td>
<td>0.913</td>
<td>0.913</td>
<td>0.27, 0.06,</td>
</tr>
</tbody>
</table>

a Effect size was expressed as Eta squared for LPH, and pair-wise Cohen’s d for the remaining variables. Pair-wise Cohen’s d values are presented as SBD versus EBD, then EBD versus HC, then SBD versus HC.

Kruskal-Wallis H tests were used to compare the three groups on all variables due to non-normality with the exception of LPH which was normal (thus one-way ANOVA was used).

Higher Z-scores indicate better performance.

* indicates statistical significance

**Abbreviations:** BD – Bipolar disorder, ED – Extra-dimensional, IED – Intra-dimensional, Extra-dimensional set-shifting task, LPH – Lipid hydroperoxides, 4-HNE – 4-Hydroxynonenal, BDNF – Brain-derived neurotrophic factor
Total errors (adjusted) (p=0.009), total trials (adjusted) (p=0.005), ED shift errors (p=0.034), and the composite score (p=0.010) were all significantly different between groups. All IED Z-scores except for ED shift errors followed the pattern of symptomatic BD < euthymic BD < HC participants. None of the biomarkers were significantly different between these groups.

3.3 Correlations of OS with BDNF and IED Performance

LPH was significantly positively correlated with BDNF (ρ=0.402, p=0.047) in HC participants, but negatively and non-significantly in BD participants (ρ=-0.165, p=0.382) (figure 3). The correlations between LPH and BDNF were significantly different between the BD and HC groups (Z=2.06, p=0.039). 4-HNE was not significantly correlated with BDNF in any group.

LPH was not significantly correlated with the two main outcome measures. LPH was significantly correlated with completed stage trials in BD participants (ρ=0.462, p=0.012), but did not survive correction for multiple comparisons. 4-HNE and BDNF were not significantly correlated with any IED measure.

Illness duration was not associated with any biomarker or IED performance measure.
There is a significantly positive correlation between BDNF and LPH in the HC group ($\rho=0.402$, $p=0.047$) and a non-significant negative correlation in the BD group ($\rho=-0.165$, $p=0.382$). These correlations are significantly different ($Z=2.06$, $p=0.039$). Spearman correlations were used.

3.4 OS-IED correlations within BDNF subgroups

Upon splitting the sample into participants with high BDNF (16 BD and 11 HC) and low BDNF (13 BD and 14 HC), the correlation analysis completed in section 3.2 was duplicated in these subgroups. Tables 8, 9, and 10 show the correlations in the whole group, BD group, and HC group, respectively. Neither LPH nor 4-HNE was correlated with IED simple reversal trials or errors. 4-HNE was not correlated with any of the secondary output measures.
Table 7. Correlations between LPH and IED performance scores in the whole group, within BDNF subgroups.

| IED Measure b | Low BDNF | | | High BDNF | | |
|---------------|----------|-------------|-------------|
|               | Rho      | p-value     | q-value     | Rho          | p-value     | q-value     |
| Total errors (adjusted) | -0.373 | 0.055      | 0.183       | -0.129       | 0.522      | 0.818       |
| Completed stage trials | -0.068 | 0.736      | 0.818       | 0.462        | 0.015*     | 0.150       |
| Total trials (adjusted) | -0.400 | 0.039*     | 0.183       | -0.090       | 0.654      | 0.818       |
| Pre-ED errors | -0.113 | 0.575      | 0.818       | 0.239        | 0.231      | 0.462       |
| ED Shift errors | -0.327 | 0.096      | 0.240       | -0.019       | 0.925      | 0.925       |

a The median split was performed at 167.7 pg/mL.
b All IED measures were non-normally distributed, thus Spearman correlations were performed.
*indicates statistical significance

Abbreviations: LPH – Lipid Hydroperoxides, IED - Intra-dimensional, Extra-dimensional set-shifting task, BDNF – Brain-Derived Neurotrophic Factor, ED – Extra-dimensional

In low BDNF participants, LPH was significantly negatively correlated (ρ=-0.400, p=0.039) with total trials (adjusted). In high BDNF participants, LPH was significantly positively correlated (ρ=0.462, p=0.015) with IED completed stage trials. The correlation between LPH and IED completed stage trials was significantly different between high and low BDNF participants (Z=1.967, p=0.025), but not the correlation between LPH and IED total trials (adjusted) (Z=1.115, p=0.132). Neither correlation survived correction for multiple comparisons.
Table 8. Correlations between LPH and IED performance scores in the BD group, within BDNF subgroups a.

<table>
<thead>
<tr>
<th>IED Measure b</th>
<th>Low BDNF</th>
<th></th>
<th></th>
<th>High BDNF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>p-value</td>
<td>q-value</td>
<td>Rho</td>
<td>p-value</td>
<td>q-value</td>
</tr>
<tr>
<td>Total errors (adjusted)</td>
<td>-0.167</td>
<td>0.586</td>
<td>0.844</td>
<td>-0.153</td>
<td>0.571</td>
<td>0.844</td>
</tr>
<tr>
<td>Completed stage trials</td>
<td>0.094</td>
<td>0.760</td>
<td>0.844</td>
<td><strong>0.755</strong></td>
<td><strong>0.001</strong></td>
<td><strong>0.010</strong></td>
</tr>
<tr>
<td>Total trials (adjusted)</td>
<td>-0.130</td>
<td>0.672</td>
<td>0.844</td>
<td>-0.087</td>
<td>0.748</td>
<td>0.844</td>
</tr>
<tr>
<td>Pre-ED errors</td>
<td>-0.146</td>
<td>0.634</td>
<td>0.844</td>
<td><strong>0.588</strong></td>
<td><strong>0.017</strong></td>
<td>0.085</td>
</tr>
<tr>
<td>ED Shift errors</td>
<td>-0.032</td>
<td>0.917</td>
<td>0.917</td>
<td>-0.119</td>
<td>0.662</td>
<td>0.844</td>
</tr>
</tbody>
</table>

a The median split was performed at 167.7 pg/mL.

b All IED measures were non-normally distributed, thus Spearman correlations were performed.

*indicates statistical significance

Abbreviations: LPH – Lipid Hydroperoxides, IED - Intra-dimensional, Extra-dimensional set-shifting task, BDNF – Brain-Derived Neurotrophic Factor, ED – Extra-dimensional

The correlation between LPH and IED completed stage trials in high BDNF patients was significantly different than in low BDNF participants in the BD group (Z=2.026, p=0.043) and the control group (Z=2.079, p=0.038).
Table 9. Spearman correlations between LPH and IED performance scores in the control group, within BDNF subgroups.

<table>
<thead>
<tr>
<th>IED Measure b</th>
<th>Low BDNF</th>
<th></th>
<th></th>
<th>High BDNF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>p-value</td>
<td>q-value</td>
<td>Rho</td>
<td>p-value</td>
<td>q-value</td>
</tr>
<tr>
<td>Total errors (adjusted)</td>
<td>-0.576</td>
<td>0.031*</td>
<td>0.155</td>
<td>0.200</td>
<td>0.555</td>
<td>0.841</td>
</tr>
<tr>
<td>Completed stage trials</td>
<td>-0.234</td>
<td>0.421</td>
<td>0.841</td>
<td>0.091</td>
<td>0.790</td>
<td>0.878</td>
</tr>
<tr>
<td>Total trials (adjusted)</td>
<td>-0.667</td>
<td>0.009*</td>
<td>0.090</td>
<td>0.182</td>
<td>0.593</td>
<td>0.841</td>
</tr>
<tr>
<td>Pre-ED errors</td>
<td>-0.124</td>
<td>0.673</td>
<td>0.841</td>
<td>0.023</td>
<td>0.946</td>
<td>0.946</td>
</tr>
<tr>
<td>ED Shift errors</td>
<td>-0.520</td>
<td>0.056</td>
<td>0.187</td>
<td>0.322</td>
<td>0.335</td>
<td>0.838</td>
</tr>
</tbody>
</table>

a The median split was performed at 167.7 pg/mL.

b All IED measures were non-normally distributed, thus Spearman correlations were performed.

*indicates statistical significance

**Abbreviations:** LPH – Lipid Hydroperoxides, IED - Intra-dimensional, Extra-dimensional set-shifting task, BDNF – Brain-Derived Neurotrophic Factor, ED – Extra-dimensional

The correlation between LPH and IED total trials in the low BDNF HC participants was significantly different than that in the high BDNF HC participants (Z=2.233, p=0.026), but not the BD group (Z=1.766, p=0.077). Similarly, the correlation between LPH and total trials (adjusted) in the low BDNF HC participants was significantly different than the high BDNF HC participants (Z=2.129, p=0.033), but not BD participants (Z=1.616, p=0.106).

As seen in tables 6-8, there were significant correlations between LPH and completed stage trials in high BDNF patients, and total trials (adjusted) in low BDNF HC participants after
correction for multiple comparisons. These correlations are visualized in figures 4 and 5, respectively.

Figure 4. Correlation between LPH and IED completed stage trials in BD participants.

Low BDNF participants are shown in blue and high BDNF in green. Spearman correlations were used due to the non-normal distribution of IED completed stage trials. There was a significant correlation between LPH levels and completed stage trials in the high BDNF subgroup ($\rho=0.755$, $p=0.001$), but not the low BDNF subgroup ($\rho=0.094$, $p=0.760$).
Low BDNF participants are shown in blue and high BDNF in green. Spearman correlations were used due to the non-normal distribution of IED total trials (adjusted). There was a significant negative correlation between LPH levels and total trials in the low BDNF subgroup ($\rho=-0.667$, $p=0.006$), but not the high BDNF subgroup ($\rho=0.182$, $p=0.593$).

3.5 GLM Analysis

The whole group was analyzed with the model of diagnosis (BD or control), LPH, BDNF, the interaction term between LPH and BDNF, and the aforementioned propensity score. The interaction term was included given the opposite correlations between LPH and BDNF between HC and BD groups. This model significantly explained the variance of total trials.
(adjusted) and is described in table 11. There was a trend for the model when explaining the variance of total errors (adjusted) \((R^2=0.186, F=2.200, p=0.070)\). No other IED measures were predicted by the model in the whole group.

### Table 10. Linear model of predictors of total trials (adjusted) in the whole group.

<table>
<thead>
<tr>
<th>Term</th>
<th>b</th>
<th>SE (b)</th>
<th>β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>176.20 (107.03 – 245.37)</td>
<td>34.401</td>
<td>-0.36 (-0.81 – 0.08)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>19.78 (-3.5 – 39.9)</td>
<td>10.010</td>
<td>0.66 (-0.01 – 1.35)</td>
<td>0.054</td>
</tr>
<tr>
<td>LPH</td>
<td>-12.08 (-20.66 – -3.51)</td>
<td>4.266</td>
<td>-0.36 (-0.64 – -0.09)</td>
<td>0.007*</td>
</tr>
<tr>
<td>BDNF</td>
<td>-0.341 (-0.68 – -0.01)</td>
<td>0.167</td>
<td>0.13 (-0.14 – 0.40)</td>
<td>0.047*</td>
</tr>
<tr>
<td>LPH-BDNF Interaction</td>
<td>0.048 (0.01 – 0.09)</td>
<td>0.021</td>
<td>0.32 (0.042 – 0.60)</td>
<td>0.025*</td>
</tr>
<tr>
<td>Propensity Score</td>
<td>15.62 (-16.00 – 47.24)</td>
<td>15.726</td>
<td>0.17 (-0.14 – 0.51)</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Model \(R^2=0.203\), adjusted \(R^2=0.120\), \(F=2.446\), \(p=0.047\)

b – Unstandardized coefficient, SE (b) – standard error of b, β – standardized coefficient.
95% confidence intervals of the unstandardized and standardized coefficients are in parentheses.

**Abbreviations:** LPH – Lipid Hydroperoxides, BDNF – Brain-Derived Neurotrophic Factor

Individual BDNF groups were then examined, as they were in the bivariate correlation analyses. A model of diagnosis, LPH, and propensity score was used to explain the variance in IED performance. In the high BDNF subgroup, IED completed stage trials was significantly predicted by the model, as shown in table 12. There were trends for total errors (adjusted) \((R^2=0.264, F=2.748, p=0.066)\) and total trials (adjusted) \((R^2=0.264, F=2.757, p=0.065)\) in the
high BDNF subgroup as well. The model did not significantly explain the variance of any other IED measure within BDNF subgroups.

Table 11. Linear model of predictors of completed stage trials in the high BDNF subgroup of the whole group.

<table>
<thead>
<tr>
<th>Term</th>
<th>b</th>
<th>SE (b)</th>
<th>β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>45.62 (18.19 – 73.04)</td>
<td>13.26</td>
<td>-0.42 (-1.12 – 0.27)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>13.97 (-5.59 – 33.53)</td>
<td>9.46</td>
<td>0.75 (0.16 – 0.91)</td>
<td>0.153</td>
</tr>
<tr>
<td>LPH</td>
<td>3.21 (0.98 – 5.44)</td>
<td>1.08</td>
<td>0.54 (-0.71 – 0.32)</td>
<td>0.007*</td>
</tr>
<tr>
<td>Propensity Score</td>
<td>11.60 (-18.82 – 42.02)</td>
<td>14.71</td>
<td>0.20 (-1.79 – 0.29)</td>
<td>0.438</td>
</tr>
</tbody>
</table>

Model R²=0.304, F=3.335, p=0.037

b – Unstandardized coefficient, SE (b) – standard error of b, β – standardized coefficient.
95% confidence intervals of the unstandardized and standardized coefficients are in parentheses.

Abbreviations: LPH – Lipid Hydroperoxides, BDNF – Brain-Derived Neurotrophic Factor
4. Discussion

4.1 Summary of Findings

This study endeavoured to determine if executive functioning was impaired in BD compared to HC participants, if there were differences in OS and BDNF levels, and if these biomarkers were associated with executive function. Given the sample size, the study was best powered to examine correlations between variables. IED performance was impaired across multiple age and sex-adjusted Z-scores in BD participants compared to HC. In contrast, LPH, 4-HNE, and BDNF levels were not significantly different between these two groups. BDNF was significantly positively correlated with LPH in HC participants, but not BD participants, where it was negative and non-significant. These correlations were significantly different between groups as well, which leads to a consistent finding throughout the results, that OS and BDNF are related differently within groups. LPH was associated with poorer IED performance in the BD group, and better performance in the HC group. When examining BDNF subgroups, only high BDNF BD participants showed associations between LPH and poorer IED performance. Conversely, only low BDNF HC participants showed associations between LPH and better IED performance. A GLM of diagnosis, LPH, BDNF, and the interaction between LPH and BDNF significantly predicted IED performance in the whole group, adjusted for demographic covariates, with all terms except diagnosis being significant predictors. Another model of diagnosis, LPH, and age significantly predicted IED performance in high BDNF BD participants, only LPH was a significant predictor.
4.2 Discussion of Findings

Hypothesis 1 predicted that IED performance would be impaired in the BD group, as previous studies have demonstrated [8, 9, 51]. Those studies have used the same executive function paradigm, the IED task, and have repeatedly seen deficits in BD participants. In this study IED performance was significantly poorer in the BD group when compared to HC, supporting hypothesis 1. This serves as a method to “validate” the BD sample, as one of the most consistent findings in adolescent BD cognition studies was replicated. The fact that this finding is continuously replicated suggests that these deficits are not uncommon in adolescent BD. As mentioned, such deficits can cause functional impairment, which can be particularly impactful at a young age where the consequences of failure to succeed in academic or social domains can have an impact on one’s future. Adolescent BD has been shown to be continuous with adult BD, and these executive deficits may persist as well, warranting treatment strategies to alleviate them [59]. Interventions early in the course of illness may have greater rates of success, before years of disease burden and potential neuroprogression result in more lasting impairment.

This study did not support the prediction within hypothesis 2 that OS marker levels would be increased in the BD group compared to HC. However, there were no significant differences in BDNF levels between groups, consistent with previous reports and in partial support of hypothesis 2. No published study has examined OS levels in BD compared to HC adolescents, making this the first such study. One other study, by Versace et al., examined LPH levels in BD adults [80]. Based on the reported effect size (ES=0.68), a sample size of at least 34 participants per group would be required for a statistical power of 0.80 to detect significant LPH differences (based on adult data) [80]. However, prevailing views of BD as neuroprogressive across different stages posit that potential biomarkers of BD may be perturbed to a lesser extent early in the course of illness [34, 129, 130]. In fact, the only other study examining OS markers
in BD adolescents found that LPH levels were significantly lower than previously reported adult data [131]. As such, the lack of significant differences in LPH between the BD and control groups may be a result of these differences being less pronounced at this stage of BD in an adolescent population, but may manifest later in the course of illness.

Levels of 4-HNE were also not significantly different between groups, which is consistent with a previous report of 4-HNE levels in BD adults [80]. Studies examining levels of 4-HNE in post-mortem brain samples have found increased 4-HNE levels in adults with BD in the anterior cingulate cortex as well as the PFC [132, 133]. Both of these regions have been functionally implicated in executive function and in aspects of attentional set-shifting specifically [134, 135]. The anterior cingulate cortex is implicated in making intra-dimensional shifts, i.e. discriminating between similar stimuli within the same attentional set, and the PFC is implicated in making ED shifts between different attentional sets [135]. This suggests that despite a lack of a peripheral signal, 4-HNE may be contributing to decreased executive function, although the present study was not designed to examine such an association. 4-HNE can contribute to increased blood-brain barrier permeability, but levels in the central nervous system may not be sufficient to result in dramatically elevated peripheral levels except in cases of severe damage, such as traumatic brain injury [136]. This may be contributing to the lack of noticeable differences in peripheral 4-HNE levels. Also, since 4-HNE readily reacts to form DNA and protein adducts, it may be doing so at too fast a rate to allow for accumulation of 4-HNE in the circulation [62]. In this sample of BD youth, it may be that the driving force of OS is not sufficient to result in late-stage oxidative damage, and the endogenous antioxidant defenses are able to sufficiently remove early-stage damage.
The relationship between peripheral biomarkers and central nervous system processes is important to understand for the interpretation of such biomarkers. It has been demonstrated that peripheral levels of BDNF in serum are reflective of levels in the cerebrospinal fluid in populations with schizophrenia [137]. Such correlations have also been observed in individuals with Alzheimer’s disease as well [138]. In amyotrophic lateral sclerosis patients, changes in antioxidant enzyme activities were similar in peripheral blood and cerebrospinal fluid measurements [139]. In a study of Parkinson’s disease patients, levels of lipid peroxidation markers in serum and cerebrospinal fluid were elevated [140]. However, such disease states involve overt neurological damage, to an extent not observed in BD, and studies involving the central-peripheral relationship of these biomarkers in BD are warranted. Alternatively, from a clinical standpoint, such biomarkers do not necessarily need to be reflective of mechanistic phenomena if they reliably predict an outcome [141]. If such biomarkers provide predictive value to clinicians to inform decisions, then the mechanism (or lack thereof) may not be as relevant in such cases. Both mechanistic and non-mechanistic studies of biomarkers are important, and such a debate is timely in the literature [141, 142]. Though the current study was not designed in such a way to elucidate definite mechanisms, it provides hypothesis-generating findings for future studies in both mechanistic and non-mechanistic biomarker research.

Though there were no significant differences in OS marker levels between groups, it may be that levels of antioxidant molecules and enzymes are also perturbed in the BD group, which may not necessarily be reflected by LPH and 4-HNE levels. Several studies have examined antioxidant levels in BD participants, and despite some inconsistencies regarding directionality of observations and the polarity of BD participants examined, there appear to be antioxidant alterations in BD [143-145]. Examining OS markers and antioxidants together may provide a more comprehensive description their interactions and how they relate to executive function.
LPH was positively associated with BDNF in the control group, but negatively and non-significantly in the BD group. Although there have not been studies examining the direct relationship between peripheral and central LPH levels, the correlation between LPH and executive function further suggests that these peripheral markers may be reflective of central oxidative processes, as they are associated with behaviour. The positive correlation in HC subjects may be explained based on a study that demonstrated BDNF upregulation in response to oxidative stress [115]. However, further study and replication of this finding in humans is necessary to validate it, as BDNF can also decrease OS levels. The negative correlation is consistent with a recent study of OS and BDNF in schizophrenia [114]. Although serum antioxidant levels were not measured, several studies have shown reductions in antioxidant molecules in BD [19, 81, 82]. It may be that despite similar levels of lipid peroxidation markers, antioxidant molecules are decreased in BD participants. This would then result in an environment of OS which is more severe for the same levels of lipid peroxidation than in HC participants. In such an environment, BDNF levels may be decreased or the baseline levels simply not sufficient to protect against oxidative damage [115]. Given the current lack of understanding of the mechanisms involved in the OS-BDNF interaction, it is difficult to suggest further reasons for these observations. However, regardless of the etiology of these associations, it points to LPH and BDNF together as potential biomarkers of BD, given the opposite correlations between BD and HC participants.

Hypothesis 3 predicted that OS would be associated with poorer IED performance in the BD group. Correlations between OS and IED performance showed a negative association between LPH and performance in BD participants, and positive association between LPH and performance in HC participants, thus supporting this hypothesis. However, these correlations did not survive correction for multiple comparisons. This may mean that more participants are
required to see such correlations when restricting significance with FDR, or that perhaps (as further analyses show) these correlations are specific to certain subgroups of individuals. These results may suggest that OS is contributing to the impaired executive function seen in the BD group, consistent with an emerging body of literature [92]. Furthermore, since correlations between OS and executive function are only seen when examining LPH, it may be that this represents early stage oxidative damage. Early stage OS markers can be degraded by endogenous antioxidant enzymes, and this process occurring may explain the lack of association of cognition with peripheral 4-HNE [80].

The mechanisms behind this correlation are not yet well understood, given the nascent state of the field and difficulty testing mechanistic hypotheses in humans. However, some putative mechanisms can be proposed in relation to the brain’s innate vulnerability to oxidative damage. One such mechanism may be impaired white matter integrity due to oxidative damage. Diffusion tensor imaging can be used to examine white matter tracts through the diffusion of water molecules along or away from these tracts [146]. These tracts are coated in a lipid-rich myelin sheath, a large proportion of which are PUFAs. A recent study found that adults with BD had significantly greater LPH levels than HC, and well as poorer white matter integrity in many pre-frontal localized tracts [80]. Furthermore, LPH was associated with poorer white matter integrity in these same tracts [80]. It has also been shown that poorer integrity of frontal and pre-frontal white matter tracts is associated with impaired executive function [146]. Therefore, it may be that oxidative damage is occurring in these lipid-rich white matter tracts, resulting in perturbed integrity of the tract and possibility neuronal damage, senescence, and death. This would then perturb the function of that tract, presumably regarding executive function.

When examining high and low BDNF subgroups, much stronger correlations between LPH and IED performance were observed. HC participants with low BDNF had stronger positive
correlations between LPH and IED performance, whereas there were stronger negative

correlations between LPH and IED performance in BD participants with high BDNF. It seems
these subgroups within BD and HC participants drive the correlations seen in the entire groups,
as the same tasks are correlated, though to a lesser extent, when not examining the BDNF
subgroups. An interesting point regarding the BDNF subgroups is that they appear to
discriminate in a two-fold manner, in that they reveal significant correlations between-groups as
well as within-groups. This may suggest that BDNF is modulating the association between OS
and IED performance. Furthermore, this potential modulating action of BDNF has different
relationships with cognition in BD participants compared to HC. It may be that the structure of
BDNF or its interaction with its target proteins is altered in BD participants, resulting in altered
antioxidant and neuroprotective properties, or altered expression as a result of OS. This may
explain the association between OS and executive function in high BDNF BD participants
specifically. Alternatively, the regulation of BDNF release may also be altered in BD, potentially
through the val66met polymorphism which impairs BDNF translocation and secretion [147].

Many studies have examined the val66met polymorphism in relation to BD risk and cognitive
function, however mixed results have been found [147]. It may be that subgroups of individuals
have particular gene-gene or gene-environment interactions that result in impaired cognitive
function, such as val66met carriers who have increased OS.

Hypothesis 3 speculated that an interaction between LPH and BDNF may be associated
with executive function, which is supported by the results in the subgroup and GLM analysis.
Correlation analysis revealed that there may be an altered interaction between LPH and BDNF in
BD participants, and this was reinforced in the GLM analysis. Though the subgroup analyses had
limitations due to the dichotomization of a continuous variable, thus losing a large amount of the
BDNF variance, this was not the case in the GLM analysis where BDNF was used as a
continuous variable. Though not independently significant, BD diagnosis also contributed to poorer IED performance. Although underpowered for such an analysis in this study, a three-way interaction between diagnosis, LPH and BDNF would give insight into the extent that the LPH-BDNF interaction differs between groups. The interaction between LPH and BDNF was a significant independent predictor of poorer IED performance in the whole group analysis, warranting analysis with a larger sample. Similar findings with a more statistically robust approach gives further support for the notion that this interaction is playing a role in BD pathology. It is difficult to determine which method of analyzing BDNF is more clinically relevant. The continuous approach is often more statistically robust, but more significance was found using the dichotomization, and is a commonly used practise with biological markers [148]. For instance, biomarkers of cardiovascular health and diabetes are perhaps some of the most clinically established and highly used biomarkers, with distinct clinical cut-offs denoting “normal” and “high” values [149]. Such cut-offs would be useful for BD and many other psychiatric disorders, but require replication of these results and further confirmatory studies to validate the clinical utility of these biomarkers. Given the results of this study, LPH and BDNF are potential biomarkers of executive function in BD, particularly when examined together.

4.3 Limitations

There are several limitations to this study that should be noted. The first one, as has been mentioned above, is the small sample size of this study. The power calculations presented above show that the study was underpowered for most statistical tests performed based on previously reported effect sizes. This may have been a contributing factor to the lack of between-group differences in OS markers. The sample size also limited the inclusion of relevant covariates such as medication status, mood, age, and IQ. Propensity scores were used to correct for these differences in GLM analyses, however it is preferable to have matched groups from the outset.
Additionally, given the heavily skewed distribution of concomitant medications, in that SGAs were taken by almost all BD participants and other medications to a much lesser extent, it was difficult to meaningfully assess the impact of medications on the study outcomes. Larger samples may provide sufficient statistical power to overcome this limitation.

Second, the BD group had varying mood states, with approximately half being euthymic and the other half being depressed, manic, or in a mixed state. Studies have shown that biomarkers and cognitive performance can vary across mood episodes [150]. In this study, mood symptoms were associated with poorer executive functioning, but not any differences in biomarkers. Future studies should aim to either recruit participants of one mood state, or a sufficiently large number of participants to examine between-state differences.

Third, the median split method used for the subgroup analysis may not reflect biologically relevant levels of BDNF or meaningful groups. Though the splitting point is arbitrary, and the approach is data-dependent, there is no established cut-off for “healthy” BDNF levels. Additionally, a large number of significant results emerged upon splitting the groups in this manner, which may indicate that these are relevant subgroups.

Lastly, a methodological limitation of the study is the cross-sectional design. This design precludes any conclusions about the direction of the observed associations.

4.4 Future studies

These preliminary findings offer valuable directions for future research. First, studies with greater sample sizes to replicate these findings should be done in order to confirm these results. This would also allow for examining how medications and the other covariates discussed above relate to cognition.
Second, longitudinal, repeated-measures studies should also be performed, as they will provide valuable insight into the chronology of these associations. These studies are needed to assess the predictive validity of these biomarkers, to see if changes in LPH and BDNF can predict future executive dysfunction. Examining these biomarkers as potential risk factors of BD or markers of the course of illness is a crucial step in translating these results into clinical practise where they can be of use.

Third, examining adolescents at high clinical or familial risk of developing BD may prove particularly fruitful. Offspring of BD parents have a greatly increased risk of developing BD, and prospective monitoring of biomarkers in these adolescents would help in determining the predictive validity of BD of these biomarkers [151]. If these biomarkers are perturbed in high-risk adolescents that go on to develop BD, it may reveal a pathogenic process worthy of pharmacological treatment.

Fourth, predicting treatment response and guiding selection of treatment using biomarkers is also a potential direction of study. Recent biomarker advances in the field of depression have found that inflammatory markers can predict treatment response to antidepressant treatment [152, 153].

Fifth, a potential intermediate phenotype of BD worthy of study is neurobiological measures examined through magnetic resonance imaging. Examining white matter integrity in the tracts associated with the PFC and other executive function-related brain regions may provide greater understanding of how LPH levels relate to executive function. Furthermore, studies examining activation of frontal and pre-frontal brain regions during set-shifting, in relation to OS markers and BDNF may provide further support for the hypothesis that OS and
BDNF, together, relate to executive function. Such studies would also shed light onto the neurobiological underpinnings of BD and the observed executive dysfunction.

Sixth, future studies should examine markers of both oxidative damage and antioxidant levels, in order to determine the overall balance between these two factors of the OS equation. This study only examined markers of oxidative damage, but integrating these results with antioxidant levels would give a much clearer picture of the OS balance. Ratios of OS markers to antioxidants may be useful biomarkers, provide more biological relevance, and may provide more insight into BD pathology.

Seventh, examining a possible genetic basis for these interactions may be fruitful. BD has very high heritability, suggesting a genetic basis for the disorder. Antioxidant genes and those responsible for OS generation, such as those encoding electron transport chain proteins should be examined to determine BD risk, as well as determine how they relate to executive function. The *BDNF* gene should also be examined, the val66met polymorphism specifically given the extant literature implicating it in BD and executive function. These genetic studies may reveal further subgroups in which biomarkers may be more predictive or treatments more effective.

Lastly, studies into antioxidant interventions as potential treatments for cognitive deficits are warranted. These may prove especially useful when treating subgroups of patients based on OS and BDNF levels. Free radical scavengers such as vitamin E and vitamin C have been examined as potential pro-cognitive treatments in Alzheimer’s disease and neurodegenerative diseases, but with limited efficacy and inconsistent results [85, 154, 155]. N-acetyl cysteine (NAC) has been used in preliminary studies for mood disturbances in BD, and has potential to be a pro-cognitive treatment [156, 157]. NAC is a free radical scavenger, and also replenishes GSH, providing two methods of reducing OS, and has been shown to reduce lipid peroxidation
specifically [158]. Furthermore, NAC has been shown to promote neurogenic pathways, which may be particularly beneficial given the fact that both pathways are perturbed in BD [159]. The relative failure of previous clinical trials of antioxidants may be due to only specific subgroups reacting positively to the treatment, perhaps on the basis of BDNF. BD participants with high BDNF, as identified in this study, may be particularly responsive to these interventions. In addition, these studies involved individuals with overt neurological damage at an old age, and such treatments in neurologically intact, young individuals may prove more effective.
Bibliography


126. Wechsler Abbreviated Scale of Intelligence (WASI) manual. Psychological Corporation: San Antonio, TX.


Appendices

Appendix 1 – REB Approval and Renewal Forms

REB Approval

To: Dr. Benjamin Goldstein
Psychiatry
Room FG 53

From: Dr. Philip Hébert

Date: December 1, 2011

Subject: An Integrative Study of Oxidative Stress, Endothelial Function, Neuropsychological Function and CACNA1C Genotype among Adolescents with Bipolar Disorder

Project Identification Number: 347-2011
Approval Date: December 1, 2011
Expiry Date: December 1, 2012

The Research Ethics Board of Sunnybrook Health Sciences Centre has conducted a Delegated Board review of the research protocol referenced above and approved the involvement of human subjects on the above-captioned date. The quorum for approval did not involve any member associated with this project.

The approval of this study includes the following documents:

- Protocol dated October 14, 2011
- Informed Consent Form for Adolescents 13-19 years of age Version 1 dated October 14, 2011
- Informed Consent Form for Parents of Adolescents 13-19 years of age Version 1 dated October 14, 2011
- Recruitment Poster (received October 17, 2011) (Must submit to Communications & Stakeholder Relations for approval prior to posting.)
- All other study tools received October 17, 2011
  o EndoPAT Product Overview
  o Wechsler Abbreviated Scale of Intelligence
  o Petersen Pubertal Development Scale
  o KSADS-PL Screen Interview
  o K-SADS-PL Diagnostic Interview Version 1.0 dated October 1996
  o K-SADS Mania Rating Scale
  o DUSI
  o Family Medical History
  o Sleep Quality Questionnaire
  o C-GAS Intake Assessment
  o Menstrual History Interview

The Research Ethics Board of Sunnybrook Health Sciences Centre operates in compliance with the Tri-Council Policy Statement 2nd edition, ICH GCP Guidelines, Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Products Regulations, and Part 3 of the Medical Devices Regulations. All Health Canada regulated trials at Sunnybrook are conducted by a Qualified Investigator.

Fully affiliated with the University of Toronto
o Family History Score Sheet – First Degree Relatives
o Family History Score Sheet – Second Degree Relatives
o Medical History
o K-SADS-P Depression Section
o Psychotropic/Auxiliary Drugs/ECT Treatment Schedule
o Psychiatric Status Rating
o WAVE Adults/Adolescents
o Migraine Screener
o Wong-Baker Faces Pain Rating Scale
o Anthropomorphic Data Form
o General Information Sheet

All correspondence with the REB must include the assigned Project Identification Number. The REB requires immediate notification of all internal serious adverse events and significant deviations. Study continuation beyond one year requires submission of a renewal form prior to the expiry date or a study completion report must be received to close the file with the REB.

All REB approved studies may be subject to review by the Sunnybrook Quality Assurance and Education Program and, as Principal Investigator, you are responsible for the ethical conduct of this study. Approval by the Sunnybrook REB entails compliance with current legislation outlined in the Ontario Personal Health Information Protection Act (PHIPA) and all policies and guidelines established by Sunnybrook. All applicable contracts and agreements must be submitted to Sunnybrook Legal Services before this research may be initiated.

Philip C. Hébert, MD PhD FCFPC
Chair, Research Ethics Board

OR

Miriam Shuchman, MD
Vice-Chair, Research Ethics Board
The Renewal Form is an application for continuing ethics approval and must be submitted for review and approval prior to the study's expiry date. Ethics approval expires each subsequent year from the day REB approval was initially granted unless otherwise indicated by the Sunnybrook REB. Failure to submit this form prior to the expiry date signifies that the study does not have REB approval and all research activities must be suspended. Conducting research without REB approval may result in a notice of non-compliance involving corrective action, up to and including, termination of the research study.

**Principal Investigator (PI):** Dr. Benjamin Goldstein

**REB Project Identification Number (PIN):** 347-2011

**Full Study Title:** Oxidative Stress and Endothelial Function as Peripheral Biomarkers of Neurocognition in Adolescent Bipolar Disorder

1. Date of initial Sunnybrook REB approval (dd/mm/yyyy).

   01/12/2011

2. Type of REB review requested. (Final decision rests with the REB Chair.)
   - [x] Delegated Review
   - [ ] Full Board Review

3. Is this an Industry-Sponsored/Supported study?
   - [ ] YES (If YES, complete the table below.)
   - [x] NO (If NO, proceed to question 4.)

<table>
<thead>
<tr>
<th>Invoicing Information for Industry-Sponsored/Supported Studies</th>
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</thead>
<tbody>
<tr>
<td>A fee of $500 Cdn is invoiced for all Industry-Sponsored/Supported Studies applying for continuing ethics approval.</td>
</tr>
</tbody>
</table>

**Invoice to the Following Company:**

**Contact Name:**

**Telephone:**

**E-mail:**

**Street Address:**

**Suite:**

**City:**

**Province/State:**

**Country:**

**Postal/Zip Code:**

4. Is this study open for enrollment at Sunnybrook?  [x] YES  [ ] NO

   If YES, attach a copy of the current Informed Consent Form(s).
If NO, provide reasoning:

### 5. How many participants at Sunnybrook:

<table>
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</tr>
<tr>
<td>Were enrolled</td>
<td>58</td>
</tr>
<tr>
<td>Are currently receiving study treatment/intervention</td>
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</tr>
<tr>
<td>Completed study treatment/intervention &amp; are currently on follow-up</td>
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</tr>
<tr>
<td>Completed study treatment/intervention &amp; follow-up</td>
<td>58</td>
</tr>
<tr>
<td>Withdrew consent</td>
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<tr>
<td>Were planned for inclusion in a chart review (retrospective or prospective)</td>
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</tr>
<tr>
<td>Were included in a chart review (retrospective or prospective)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

### 6. Have all Serious Adverse Events (SAEs) experienced by a Sunnybrook participant been reported to the REB?

☐ YES ☐ NO, will submit immediately ☑ NO SAEs have occurred

### 7. In the opinion of the PI, is there a concern or trend in the SAEs that have occurred with Sunnybrook participants?

☐ YES ☐ NO ☑ NO SAEs have occurred

If YES, provide details and action taken.

### 8. Have all significant protocol deviations/violations been reported to the REB?

☐ YES ☐ NO, will submit immediately ☑ NO significant deviations/violations to report

### 9. Since the last REB approval, is there any new ethical or scientific information outside of a protocol amendment that would be relevant to the continuing review of this study?

☐ YES ☑ NO

If YES, provide details.

### 10. Since the last REB approval, is there any change in the conflict of interest information provided to the REB for any of the investigators, study staff or members of their immediate family?

☐ YES ☑ NO

If YES, provide details.
11. Person completing this form.

<table>
<thead>
<tr>
<th>Title: Ms.</th>
<th>First Name: Melanie</th>
<th>Last Name: Naiberg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dept/Div: Psychiatry</td>
<td>Institution: Sunnybrook Health Sciences Centre</td>
<td></td>
</tr>
<tr>
<td>Full Address: 2075 Bayview Ave</td>
<td>Room Number: F-126</td>
<td></td>
</tr>
<tr>
<td>Telephone: 416-480-6100</td>
<td>Extension: 87572</td>
<td></td>
</tr>
<tr>
<td>E-mail: <a href="mailto:melanie.naiberg@sunnybrook.ca">melanie.naiberg@sunnybrook.ca</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Statement of Principal Investigator (PI).

I assume full responsibility for the scientific and ethical conduct of this study and agree to conduct this study in compliance with the Tri-Council Policy Statement: Ethical Conduct for Research Involving Human Subjects (TCPS), Personal Health Information Protection Act (PHIPA) and any other relevant regulations or guidelines. I certify that all researchers and personnel involved in this study at this institution are appropriately qualified and trained to fulfill their role in this study.

Signature of Principal Investigator: [Signature]
Date (dd.mmm.yyyy): 12 Nov 2019

Research Ethics Office Use Only

The Sunnybrook REB has reviewed the information provided and confirms that this study has obtained ethics approval by way of:

- [X] Delegated Review
- [ ] Full Board Review → Date of Full Board meeting: __________________

This study is only approved for the following period:

Dec 1, 2014 to Dec 1, 2015

Chair/Vice-Chair, Research Ethics Board
Appendix 2 – Consent Form for Adolescents

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Adolescents 13-21 years of age

TITLE OF PROJECT:
Oxidative Stress and Endothelial Function as Peripheral Biomarkers of Neurocognition in Adolescent Bipolar Disorder

PRINCIPAL INVESTIGATOR:
Benjamin I. Goldstein, MD, PhD, FRCPC
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

CO-INVESTIGATORS:
Alan Moody, MD, FRCPC
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

Bradley Strauss, MD, FRCPC
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

L. Trevor Young, MD, PhD, FRCPC
Centre for Addiction and Mental Health
250 College Street
Toronto, Ontario M5T 1R8

SPONSOR:
NARSAD/Brain & Behavior Research Foundation

INFORMED CONSENT
You are being asked to consider taking part in a research study. It is important that you read and understand this document. It describes the purpose, procedures, benefits, risks, discomforts and precautions of the study. It also describes other options that are available to you and your right to withdraw from the study at any time. If this form contains anything you do not understand or would like to know more about, please ask the study doctor or study staff to explain it to you. Upon request, someone may verbally translate this form in your preferred language. You may take as much time as you need to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. You must make sure that all of your questions are answered to your satisfaction before deciding whether or not you will participate in this study.
INTRODUCTION

You are being asked to participate in this research study because you are either being treated for bipolar disorder through the Youth Psychiatry Division of Sunnybrook or because you responded to an advertisement to participate in the study as a psychiatrically healthy participant.

WHAT IS THE USUAL TREATMENT?

Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and waist circumference is also collected. Measuring blood levels of certain biological markers, such as cholesterol and calcium, can be routine practice for some patients.

WHY IS THE STUDY BEING DONE?

This study aims to measure specific biological markers (oxidative stress markers) in the blood among adolescents with bipolar disorder, and to find out whether these markers are associated with their blood vessel functioning (endothelial function) and performance on psychological tests. The levels of the biological markers and their association with changes in blood vessel response and neuropsychological performance will be compared to those of adolescents without bipolar disorder. Furthermore, this study aims to examine how these factors relate to certain genetic markers. These different factors have been studied independently, but have not yet been studied together. By including these factors in the same study, we hope to learn how they relate to one another in adolescents with bipolar disorder and in healthy adolescents.

The overall purpose of this research study is to help the study doctors better understand the biological and psychological processes involved in bipolar disorder, in order to help guide future research on the causes and treatments of bipolar disorder.

WHAT WILL HAPPEN DURING THIS STUDY?

Study Visit 1

You will be asked to take part in a screening interview to see if you are eligible to participate in this study. This interview is the initial part of Visit 1 and will consist of questions about you regarding specific medical illnesses and medications that might interfere with the assessment of the factors listed above, and it will take about 10-15 minutes. If you do not have these specific illnesses or take these specific medications, you will be asked to complete a psychiatric interview and to answer questions regarding your medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street.

If you meet the study criteria for being a participant with bipolar disorder or a control participant, you will be asked to return for a 2nd study visit.

Study Visit 2

The first part of this visit involves measuring your blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index fingers of your hands while you are lying on your back. The EndoPAT will gather information for 10 minutes while you are resting. Then a blood pressure cuff will be tightly inflated on your arm for 5
minutes so that it prevents blood flow. The ultrasound will again gather information for 10 minutes after the blood pressure cuff is released.

Next, a small amount (about 3 tablespoonfuls or 6 tubes) of blood will be taken from a vein in your arm using a needle after an overnight fast (i.e. no food or beverages other than water 10 hours prior to the blood draw). The blood draw will take 15 – 60 minutes. In addition, your height, weight, waist circumference and blood pressure will be measured.

Lastly, you will take part in a series of 5 brief computerized tests. A research assistant will guide you through the steps of completing this task. You will be instructed to press a touch-sensitive computer screen, similar to a game-like interface, in response to different images presented on the screen. This task will take approximately 45 minutes to complete.

Your parent can accompany you to the endothelial assessment, blood draw, and computerized tasks, and can wait for you just outside the testing room. However, because the procedures must be the same for all participants, parents may not be inside the testing room.

The psychiatric interviews will take approximately 1-4 hours and the blood work, endothelial assessment and computerized tests will take approximately 2-3 hours. The total duration of study procedures may therefore take up to 7 hours.

Visit 2 will be scheduled as soon as possible after Visit 1, but may occur up to 1 month later if necessary.

<table>
<thead>
<tr>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL TIME:</strong></td>
<td>Approximately 3 hours</td>
</tr>
<tr>
<td>1 – 4 hours</td>
<td></td>
</tr>
<tr>
<td>Informed Consent = 45 minutes</td>
<td>Blood vessel assessment = 40 minutes</td>
</tr>
<tr>
<td>Screening = 10 – 15 minutes</td>
<td>Blood draw = 15 – 60 minutes</td>
</tr>
<tr>
<td>Psychiatric Interview / complete self – report forms = 3 hours</td>
<td>Physical measurements = 10 minutes</td>
</tr>
<tr>
<td></td>
<td>Computerized tests = 45 minutes</td>
</tr>
</tbody>
</table>

Endothelial assessment device (EndoPAT) that will be used:
HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
It is expected that about 120 adolescents and their parents will take part in this study at Sunnybrook. The length of this study for participants is, at most, 2 months. The entire study is expected to take about 36 months to complete and the results should be known in 1 year following the completion of study procedures.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?
Participants are required to complete two study visits, both of which will be held at Sunnybrook Health Sciences Centre in the Centre for Excellence in Youth Bipolar (CEYB). The visits consist of a (1) screening phase and (2) testing phase, which will take up to 7 hours to complete. Although participation in this study is entirely voluntary, you are responsible for completing the full procedure for each visit. If you choose not to complete any of the study requirements, you will not be able to participate in the study.

Screening
The screening visit will take up to 4 hours to complete. The results from the assessments completed at this visit will determine if you are eligible to participate in the study. Depending on the results, there is a chance that you will not be eligible to participate. If selected to participate, you will be asked to return to Sunnybrook within the next month for a second visit.

At the screening visit, study staff will complete an interview with you assessing your medical and medication history, eating habits, physical activity, life events, and use of any drugs or alcohol. You will also be asked to complete self-report questionnaires assessing similar content. All of these measures are part of regular care for bipolar disorder within CEYB. In addition, an intelligence test will be completed with the interviewer.

Testing
The testing visit will take up to 3 hours to complete. During this visit, study staff will first measure your blood vessel functioning using an imaging device. Next, about 3 tablespoonfuls of blood will be taken from a vein in your arm using a needle to check for certain biological markers. The collection of blood is a necessary part of this study. If, as a result of your participation in this study, any new clinically important information about your health is obtained, you will be given the opportunity to decide whether you wish to be made aware of that information. Your height, weight, waist circumference and blood pressure will also be measured. Lastly, you will complete a series of 5 brief computerized tests. Both the endothelial and
computer assessment are not part of regular care for bipolar disorder, and, although blood work can be part of patient care, the biological markers being examined in this study are not.

**Duration of Storage of Information**

All blood samples will be stored at Sunnybrook Health Sciences Centre in an access-restricted freezer space. Your individual results of biological and genetic markers, and other results pertaining to endothelial function and psychological test performance will not be reported to you because, at this point in time, these are research measurements, and they do not currently have any clear relevance to your medical health. You and your family doctor will be informed, however, of any abnormal findings regarding your blood sugar and/or cholesterol.

Any samples obtained from you will be destroyed once analysis is complete. If the research study is extended beyond this time, you will be asked once again to give consent to extend the storage period for a specified amount of time. If you cannot be reached, your samples will be destroyed at that time.

**Limits to Sharing Information with Collaborators and Laboratories**

The blood samples obtained from you will not be used for any other investigations outside of this study (i.e. for the purpose of investigating bipolar disorder). The information may be sent for specific testing to the laboratories of collaborators with Dr. Goldstein’s team; however information will not be shared with any individuals who are not involved in this study.

**WHAT ARE THE POTENTIAL RISKS AND/OR DISCOMFORTS OF PARTICIPATING IN THIS STUDY?**

It is possible that you may experience mild discomfort, bruising during or, rarely, infection as a result of the blood draw. You are also asked to start fasting 10 hours prior to the scheduled blood draw. The blood draw may not take place until noon the following day so total time fasting may be up to 13 hours. You may also experience discomfort during the 5 minutes that the blood pressure cuff is tightly inflated during the endothelial assessment or for a few minutes afterward. This discomfort is expected (Selamet Tierney et al. *Journal of Pediatrics* 2009;154:901-5). There are no known additional risks associated with the blood pressure cuff inflation procedure. You may discontinue any of the procedures at any time. Participants in this study may experience emotional discomfort when completing the psychiatric interviews and questionnaires. You may refuse to answer any question/s, and may stop the interview/follow-up at any time if you experience discomfort or for any other reason. Participants may contact the study doctor (Dr. Benjamin Goldstein) or study staff during business hours with any questions or concerns regarding risks or discomforts. The telephone number for this purpose is: 416-480-5328.

**WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS STUDY?**

There are no direct benefits from participation in this study. However, this study relies on your participation in order to explore bipolar disorder among adolescents, which will broaden understandings of the illness and may eventually lead to novel assessment, prevention and treatment strategies. Findings from this study may therefore benefit future individuals or families affected with or at risk for bipolar disorder.
CAN PARTICIPATION IN THIS STUDY END EARLY?

The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:

- You are unable or unwilling to follow the study procedures
- If you are disruptive to the study

If you are removed from this study, the investigator(s) will discuss the reasons with you.

You can also choose to end your participation at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical treatment or health care. There will be no penalty or loss of benefits to which you are otherwise entitled. If you withdraw voluntarily from the study, you are encouraged to contact: Dr. Benjamin Goldstein at 416-480-5328; 2075 Bayview Avenue, Toronto, Ontario, M4N 3M5. If you withdraw consent to participate after beginning the study, the data collected up to that time point will be used.

WHAT ARE THE COSTS FOR PARTICIPATING IN THIS STUDY?

There is no cost for participation.

WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?

If you become sick or injured as a direct result of your participation in this study, your medical care will be provided. Financial compensation for such things as discomfort due to injury is not routinely available. By signing this consent form, you do not give up any of your legal rights.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?

Parents will be compensated $25 per study visit for travel expenses and parking. Adolescents will be compensated $20 for completing study screening procedures. Eligible participants will also receive $70 at the completion of Visit 2.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

In order to verify that the study is being conducted correctly, the Sunnybrook Research Ethics Board will be allowed to inspect participants’ personal records held by the study doctor. All study data will be stored for a period of at least 5 years. All reasonable measures to protect the confidentiality of participants’ study records and their identity will be taken to the extent permitted by the applicable laws and/or regulations, and will not be made publicly available. The results of this study may be presented at meetings or in publications; however, participant’s identity will not be disclosed.
DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?

The study doctors do not have any conflicts of interest regarding this study.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

1. You have the right to have this form and all information concerning this study and your rights as a participant explained to you and, if you wish, translated into your preferred language, before you make any decision.

2. By signing this consent form, you do not give up any of your legal rights.

3. You have the right to receive a copy of this signed and dated informed consent form before participating in this study.

4. You have the right to be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff. This may include new information about the risks and benefits of being a participant in this study.

5. You have the right to access, review and request changes to your personal health information.

6. You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of the results of this study, please contact: Dr. Benjamin Goldstein, 416-480-5328.

If you have any questions about this study, you are encouraged to contact the principal investigator for this study: Dr. Benjamin Goldstein at 416-480-5328.

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Brian Murrany, Chair of the Sunnybrook Research Ethics Board at 416-480-6100, ext. 88144.
Oxidative Stress and Endothelial Function as Peripheral Biomarkers of Neurocognition in Adolescent Bipolar Disorder

Name of Participant: _____________________________________________

Participant:

By signing this form, I confirm that:

- This research has been fully explained to me and all of my questions answered to my satisfaction
- I understand the requirements of participating in this research study
- I have been informed of the risks and benefits, if any, of participating in this research study
- I have been informed of any alternatives to participating in this research study
- I have been informed of the rights of research participants
- I have read each page of this form
- I understand that my medical records will be accessed for the purpose of this study
- I understand that I, and my family doctor, will be notified of abnormal findings in my blood sugar or cholesterol.
- I have agreed to participate in this research study
- I understand that my family doctor may be informed of my participation in this research study
- This informed consent document may be placed in my medical records

__________________________________________  _________________________________  ___________
Name of Adolescent (print)                  Signature                                                          Date
Assistance Declaration

Was the participant assisted during the consent process? □ Yes □ No

☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.

☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

___________________________      _________________________     ___________
Name of Person Assisting (print)   Signature                          Date

Person Obtaining Consent

By signing this form, I confirm that:

• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

___________________________      _________________________________     ___________
Name of Person Obtaining Consent (print)   Signature                          Date
Appendix 3 – Consent Form for Parent/Guardian

CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Parents of Adolescents 13-21 years of age

TITLE OF PROJECT:
Oxidative Stress and Endothelial Function as Peripheral Biomarkers of Neurocognition in Adolescent Bipolar Disorder

PRINCIPAL INVESTIGATOR:
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Sunnybrook Health Sciences Centre
2075 Bayview Avenue
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Bradley Strauss, MD, FRCPC
Sunnybrook Health Sciences Centre
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

L. Trevor Young, MD, PhD, FRCPC
Centre for Addiction and Mental Health
250 College Street
Toronto, Ontario M5T 1R8

SPONSOR:
NARSAD/Brain & Behavior Research Foundation

INFORMED CONSENT

Your adolescent is being asked to consider taking part in a research study. As part of the study, you will be asked to answer questions and fill out questionnaires about your adolescent. It is important that you read and understand this document. It describes the purpose, procedures, benefits, risks, discomforts and precautions of the study. It also describes other options that are available to your adolescent and his/her right to withdraw from the study at any time. If this form contains anything you do not understand or would like to know more about, please ask the study doctor or study staff to explain it to you. Upon request, someone may verbally translate this form in your preferred language. You may take as much time as you need to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. You must make sure that all of your questions are answered to your satisfaction before deciding whether or not you will participate in this study.
INTRODUCTION

Your adolescent is being asked to participate in this research study because he/she is either being treated for bipolar disorder through the Youth Psychiatry Division of Sunnybrook or because he/she responded to an advertisement to participate in the study as a psychiatrically healthy participant.

WHAT IS THE USUAL TREATMENT?
Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and waist circumference is also collected. Measuring blood levels of certain biological markers, such as cholesterol and calcium, can be routine practice for some patients.

WHY IS THE STUDY BEING DONE?
This study aims to measure specific biological markers (oxidative stress markers) in the blood among adolescents with bipolar disorder, and to find out whether these markers are associated with their blood vessel functioning (endothelial function) and performance on psychological tests. The levels of the biological markers and their association with changes in blood vessel response and neuropsychological performance will be compared to those of adolescents without bipolar disorder. Furthermore, this study aims to examine how these factors relate to certain genetic markers. These different factors have been studied independently, but have not yet been studied together. By including these factors in the same study, we hope to learn how they relate to one another in adolescents with bipolar disorder and in healthy adolescents.

The overall purpose of this research study is to help the study doctors better understand the biological and psychological processes involved in bipolar disorder, in order to help guide future research on the causes and treatments of bipolar disorder.

WHAT WILL HAPPEN DURING THIS STUDY?

Study Visit 1
You will be asked to take part in a screening interview to see if you and your adolescent are eligible to participate in this study. This interview is the initial part of Visit 1 and will consist of questions about your adolescent regarding specific medical illnesses and medications that might interfere with the assessment of the factors listed above, and it will take about 10-15 minutes. If your adolescent does not have these specific illnesses or take these specific medications, you will be asked to complete a psychiatric interview regarding your adolescent and to answer questions regarding his/her medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs.

If your adolescent meets the study criteria for being a participant with bipolar disorder or a control participant, you will both be asked to return for a 2nd study visit.

Study Visit 2
The first part of this visit involves measuring your adolescent’s blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index
fingers of your adolescent’s hands while he/she is lying on his/her back. The EndoPAT will gather information for 10 minutes while your adolescent is resting. Then a blood pressure cuff will be tightly inflated on his/her arm for 5 minutes so that it prevents blood flow. The ultrasound will again gather information for 10 minutes after the blood pressure cuff is released.

Next, a small amount (about 3 tablespoonfuls or 6 tubes) of blood will be taken from a vein in your adolescent’s arm using a needle after an overnight fast (i.e. no food or beverages other than water 10 hours prior to the blood draw). The blood draw will take 15 – 60 minutes. In addition, your adolescent’s height, weight, waist circumference and blood pressure will be measured.

Lastly, your adolescent will take part in a series of 5 brief computerized tests. A research assistant will guide him/her through the steps of completing this task. He/she will be instructed to press a touch-sensitive computer screen, similar to a game-like interface, in response to different images presented on the screen. This task will take approximately 45 minutes to complete.

You can accompany your adolescent to the endothelial assessment, blood draw, and computerized tasks, and can wait for him/her just outside the testing room. However, because the procedures must be the same for all participants, you may not be inside the testing room.

The psychiatric interviews will take approximately 1-4 hours and the blood work, endothelial assessment and computerized tests will take approximately 2-3 hours. The total duration of study procedures may therefore take up to 7 hours.

Visit 2 will be scheduled as soon as possible after Visit 1, but may occur up to 1 month later if necessary.

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL TIME:</strong></td>
<td>1 – 4 hours</td>
<td>Approximately 3 hours</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>= 45 minutes</td>
<td>Blood vessel assessment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= 40 minutes</td>
</tr>
<tr>
<td>Screening</td>
<td>= 10 – 15 minutes</td>
<td>Blood draw</td>
</tr>
<tr>
<td></td>
<td></td>
<td>= 15 – 60 minutes</td>
</tr>
<tr>
<td>Psychiatric</td>
<td></td>
<td>Physical measurements</td>
</tr>
<tr>
<td>Interview /</td>
<td></td>
<td>= 10 minutes</td>
</tr>
<tr>
<td>complete self –</td>
<td></td>
<td></td>
</tr>
<tr>
<td>report forms</td>
<td></td>
<td>Computerized tests</td>
</tr>
<tr>
<td></td>
<td>= 3 hours</td>
<td>= 45 minutes</td>
</tr>
</tbody>
</table>

Endothelial assessment device (EndoPAT) that will be used:
HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
It is expected that about 120 adolescents and their parents will take part in this study at Sunnybrook. The length of this study for participants is, at most, 2 months. The entire study is expected to take about 36 months to complete and the results should be known in 1 year following the completion of study procedures.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?
Participants are required to complete two study visits, both of which will be held at Sunnybrook Health Sciences Centre in the Centre for Excellence in Youth Bipolar (CEYB). The visits consist of a (1) screening phase and (2) testing phase, which will take up to 7 hours to complete. Although participation in this study is entirely voluntary, your adolescent is responsible for completing the full procedure for each visit. If your adolescent chooses not to complete any of the study requirements, he/she will not be able to participate in the study.

**Screening**
The screening visit will take up to 4 hours to complete. The results from the assessments completed at this visit will determine if your adolescent is eligible to participate in the study. Depending on the results, there is a chance that your adolescent will not be eligible to participate. If selected to participate, you will both be asked to return to Sunnybrook within the next month for a second visit.

At the screening visit, study staff will complete an interview with you and your adolescent assessing your adolescent’s medical and medication history, eating habits, physical activity, life events, and use of any drugs or alcohol. You and your adolescent will also be asked to complete questionnaires assessing similar content. All of these measures are part of regular care for bipolar disorder within CEYB. In addition, your adolescent will complete an intelligence test with the interviewer.

**Testing**
The testing visit will take up to 3 hours to complete. During this visit, study staff will first measure your adolescent’s blood vessel functioning using an imaging device. Next, about 3 tablespoonfuls of blood will be taken from a vein in your adolescent’s arm using a needle to check for certain biological markers. The collection of blood is a necessary part of this study. If, as a result of your adolescent’s participation in this study, any new clinically important information about his/her health is obtained, he/she will be given the opportunity to decide whether he/she wishes to be made aware of that information. Your adolescent’s height, weight,
waist circumference and blood pressure will also be measured. Lastly, your adolescent will complete a series of 5 brief computerized tests. Both the endothelial and computer assessment are not part of regular care for bipolar disorder, and, although blood work can be part of patient care, the biological markers being examined in this study are not.

**Duration of Storage of Information**

All blood samples will be stored at Sunnybrook Health Sciences Centre in an access-restricted freezer space. Your adolescent’s individual results of biological and genetic markers, and other results pertaining to endothelial function and psychological test performance will not be reported to him/her because, at this point in time, these are research measurements, and they do not currently have any clear relevance to his/her medical health. Your adolescent and his/her family doctor will be informed, however, of any abnormal findings regarding his/her blood sugar and/or cholesterol.

Any samples obtained from your adolescent will be destroyed once analysis is complete. If the research study is extended beyond this time, your adolescent will be asked once again to give consent to extend the storage period for a specified amount of time. If he/she cannot be reached, his/her samples will be destroyed at that time.

**Limits to Sharing Information with Collaborators and Laboratories**

The blood samples obtained from your adolescent will not be used for any other investigations outside of this study (i.e. for the purpose of investigating bipolar disorder). The information may be sent for specific testing to the laboratories of collaborators with Dr. Goldstein’s team; however information will not be shared with any individuals who are not involved in this study.

**WHAT ARE THE POTENTIAL RISKS AND/OR DISCOMFORTS OF PARTICIPATING IN THIS STUDY?**

It is possible that your adolescent may experience mild discomfort, bruising during or, rarely, infection as a result of the blood draw. Your adolescent is also asked to start fasting 10 hours prior to the scheduled blood draw. The blood draw may not take place until noon the following day so total time fasting may be up to 13 hours. Your adolescent may also experience discomfort during the 5 minutes that the blood pressure cuff is tightly inflated during the endothelial assessment or for a few minutes afterward. This discomfort is expected (Selamet Tierney et al. *Journal of Pediatrics* 2009;154:901-5). There are no known additional risks associated with the blood pressure cuff inflation procedure. Your adolescent may discontinue any of the procedures at any time. Participants in this study may experience emotional discomfort when completing the psychiatric interviews and questionnaires. You may refuse to answer any question/s, and may stop the interview/follow-up at any time if you experience discomfort or for any other reason. Participants may contact the study doctor (Dr. Benjamin Goldstein) or study staff during business hours with any questions or concerns regarding risks or discomforts. The telephone number for this purpose is: 416-480-5328.

**WHAT ARE THE POTENTIAL BENEFITS OF PARTICIPATING IN THIS STUDY?**

There are no direct benefits from participation in this study. However, this study relies on you and your adolescent’s participation in order to explore bipolar disorder among adolescents, which will broaden understandings of the illness and may eventually lead to novel assessment, prevention and treatment
strategies. Findings from this study may therefore benefit future individuals or families affected with or at risk for bipolar disorder.

CAN PARTICIPATION IN THIS STUDY END EARLY?

The investigator(s) may decide to remove your adolescent from this study without his/her consent for any of the following reasons:

- He/she is unable or unwilling to follow the study procedures
- He/she is disruptive to the study

If your adolescent is removed from this study, the investigator(s) will discuss the reasons with him/her.

You can also choose to end your participation at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical treatment or health care. There will be no penalty or loss of benefits to which you are otherwise entitled. If you withdraw voluntarily from the study, you are encouraged to contact: Dr. Benjamin Goldstein at 416-480-5328; 2075 Bayview Avenue, Toronto, Ontario, M4N 3M5. If you withdraw consent to participate after beginning the study, the data collected up to that time point will be used.

WHAT ARE THE COSTS FOR PARTICIPATING IN THIS STUDY?

There is no cost for participation.

WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?

If you become sick or injured as a direct result of your participation in this study, your medical care will be provided. Financial compensation for such things as discomfort due to injury is not routinely available. By signing this consent form, you do not give up any of your legal rights.

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?

Parents will be compensated $25 per study visit for travel expenses and parking. Adolescents will be compensated $20 for completing study screening procedures. Eligible participants will also receive $70 at the completion of Visit 2.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

In order to verify that the study is being conducted correctly, the Sunnybrook Research Ethics Board will be allowed to inspect participants’ personal records held by the study doctor. All study data will be stored
for a period of at least 5 years. All reasonable measures to protect the confidentiality of participants’ study records and their identity will be taken to the extent permitted by the applicable laws and/or regulations, and will not be made publicly available. The results of this study may be presented at meetings or in publications; however, participant’s identity will not be disclosed.

DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?

The study doctors do not have any conflicts of interest regarding this study.

WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

7. You have the right to have this form and all information concerning this study and your rights as a participant explained to you and, if you wish, translated into your preferred language, before you make any decision.

8. By signing this consent form, you do not give up any of your legal rights.

9. You have the right to receive a copy of this signed and dated informed consent form before participating in this study.

10. You have the right to be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff. This may include new information about the risks and benefits of being a participant in this study.

11. You have the right to access, review and request changes to your personal health information.

12. You have the right to be informed of the results of this study once the entire study is complete. If you would like to be informed of the results of this study, please contact: Dr. Benjamin Goldstein, 416-480-5328.

If you have any questions about this study, you are encouraged to contact the principal investigator for this study: Dr. Benjamin Goldstein at 416-480-5328.

The Sunnybrook Research Ethics Board has reviewed this study. If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Brian Murray, Chair of the Sunnybrook Research Ethics Board at 416-480-6100, ext. 88144
Oxidative Stress and Endothelial Function as Peripheral Biomarkers of Neurocognition in Adolescent Bipolar Disorder

Name of Participant: ________________________________

Parent:

By signing this form, I confirm that:

• This research has been fully explained to me and all of my questions answered to my satisfaction
• I understand the requirements of participating in this research study
• I have been informed of the risks and benefits, if any, of participating in this research study
• I have been informed of any alternatives to participating in this research study
• I have been informed of the rights of research participants
• I have read each page of this form
• I understand that my adolescent’s medical records will be accessed for the purpose of this study
• I understand that my adolescent and his/her family doctor will be notified of abnormal findings in my adolescent’s blood sugar or cholesterol.
• I have agreed to participate in this research study
• I understand that my adolescent’s family doctor may be informed of his/her participation in this research study
• This informed consent document may be placed in my adolescent’s medical records

_________________________  ________________________________  ___________
Name of Parent (print)  Signature  Date
Assistance Declaration

Was the participant assisted during the consent process? □ Yes   □ No

☐ The consent form was read to the participant/substitute decision-maker, and the person signing below attests that the study was accurately explained to, and apparently understood by, the participant/substitute decision-maker.

☐ The person signing below acted as a translator for the participant/substitute decision-maker during the consent process. He/she attests that they have accurately translated the information for the participant/substitute decision-maker, and believe that that participant/substitute decision-maker has understood the information translated.

___________________________      _________________________________     ___________
Name of Person Assisting (print)    Signature                                                        Date

Person Obtaining Consent

By signing this form, I confirm that:

• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

___________________________      _________________________________     ___________
Name of Person Obtaining Consent    Signature                                                        Date