Biological and Clinical Correlates of Vascular Risk in Adolescent Bipolar Disorder

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

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ABSTRACT

Early development of cardiovascular disease (CVD) is consistently reported in bipolar disorder (BD). The overall aim of my thesis was to examine potential biomarkers of BD and CVD, with proxies of atherosclerosis. First, method validation of non-invasive standard measures was done to examine if they were associated with traditional cardiovascular risk factors (CVRFs) in adolescents with BD, despite fluctuating disease state, medication(s) and comorbid conditions. Results revealed that carotid intima media thickness (cIMT) and flow-mediated dilation (FMD) are associated with CVRFs in a heterogeneous adolescent clinical sample, and that they may be used in further research in this population as a proxy for CVD. Next, I confirmed that oxidative stress (OS) markers were detectable in the periphery of adolescents with BD, and that OS was associated with CVD risk measures. This supports the hypothesis that there are underlying biological mechanisms that link BD and CVD pathology. Finally, to investigate the early progression of CVD in BD adolescents, we examined pro-inflammatory markers (PIMs), trophic factors and CVRFs, with non-invasive atherosclerosis proxies in psychiatrically healthy adolescents (HC) and adolescents with BD. This case-controlled study allowed us to test for: 1) between group differences at an early stage in disease course; 2) investigate whether potential biomarkers in adult BD are elevated in adolescent BD compared to HC and 3) these biomarkers are associated with atherosclerosis proxies. We found that adolescents with BD had elevated inflammation compared to HC adolescents. Interestingly, there were no between-
group differences in non-invasive vascular measures, indicating that there is perhaps an opportunity for prevention. Lower levels of brain derived neurotrophic factor (BDNF) were associated with worse vascular structure (cIMT) in symptomatic BD adolescents. This suggests that there is possible interplay between biomarkers and vascular risk in BD. Moreover, while inflammation, neurotrophins and OS may play a role in BD symptomology and CVD progression, adolescents with BD do not yet show signs of early atherosclerosis, as determined by proxy ultrasound measures. These findings underscore the importance of behavioral and pharmacological interventions aimed at mitigating cardiovascular risk.
ACKNOWLEDGEMENTS

I have gained invaluable experience working at the Centre for Youth Bipolar Disorder at Sunnybrook Health Sciences Centre and I sincerely thank my supervisor Dr. Ben Goldstein for his support, guidance and expertise throughout my graduate training. Dr. Goldstein, it has been amazing being a part of a clinical research centre that has grown so much over the years. I appreciate the freedom you have given me to contribute meaningfully to the field, collaborate, and forge new avenues for research. You have truly given me the opportunity to test myself and nurtured an enthusiasm to contribute to clinically translatable research. I also thank my co-supervisor, Dr. Krista Lanctôt and my committee members Dr. Roger McIntyre and Dr. Ana Andreazza for their time and guidance throughout my doctoral research. I am grateful to have had such a supportive and honest committee and supervisor. I admire all of you for your thoroughness, attention to detail, tenacity and your emphasis on ‘the big picture’. You have all inspired me to continue doing work that I am passionate about, and you have all set examples of how passionate, dedicated and thoughtful research can contribute meaningfully.

I would also like to thank all of the graduate students, summer students and research staff at the Centre for Youth Bipolar Disorder for the amazing laughs, hard work, support, patience, and memories. You all contributed to my graduate training experience and I am grateful to have been a part of such a great research team.

Also, I would like to thank Omodele Olowoyeye and Gustavo Scola for all of their hard-work, help, and contributions to these research projects.

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<th>Definition</th>
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<tbody>
<tr>
<td>2D</td>
<td>2-dimensional</td>
</tr>
<tr>
<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>ALIFE</td>
<td>Adolescent Longitudinal Interval Follow-up Evaluation</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BD</td>
<td>bipolar disorder</td>
</tr>
<tr>
<td>BD-NOS</td>
<td>bipolar disorder not otherwise specified</td>
</tr>
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<td>BDNF</td>
<td>brain-derived neurotrophic factor</td>
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<tr>
<td>BMI</td>
<td>body-mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>CGAS</td>
<td>Children’s Global Assessment Scale</td>
</tr>
<tr>
<td>cIMT</td>
<td>carotid intima-media thickness</td>
</tr>
<tr>
<td>COBY</td>
<td>Course and Outcome of Bipolar Youth</td>
</tr>
<tr>
<td>CRP</td>
<td>c-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>CVRFs</td>
<td>cardiovascular disease risk factors</td>
</tr>
<tr>
<td>DEP-P</td>
<td>KSADS Depression Rating Scale</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DRS</td>
<td>depression rating scale</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
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<tr>
<td>ED</td>
<td>endothelial dysfunction</td>
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<td>ELISA</td>
<td>enzyme linked immunosorbant assay</td>
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<tr>
<td>ETC</td>
<td>electron transport chain</td>
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<tr>
<td>FFA</td>
<td>free fatty acids</td>
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<tr>
<td>FDR</td>
<td>false discovery rate</td>
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<tr>
<td>FMD</td>
<td>flow-mediated dilation</td>
</tr>
<tr>
<td>gp</td>
<td>g-protein</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione s transferase</td>
</tr>
<tr>
<td>HC</td>
<td>healthy controls</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>INF</td>
<td>inflammation</td>
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<tr>
<td>KSADS</td>
<td>Schedule for Affective Disorders and Schizophrenia for School-Aged Children</td>
</tr>
<tr>
<td>KSADS-PL</td>
<td>KSADS, Present and Lifetime</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>LPH</td>
<td>lipid hydroperoxides</td>
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<tr>
<td>MDD</td>
<td>major depressive disorder</td>
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<tr>
<td>MetS</td>
<td>metabolic syndrome</td>
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<tr>
<td>MRS</td>
<td>mania rating scale</td>
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<tr>
<td>NADH</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NTR</td>
<td>neurotrophin receptor</td>
</tr>
<tr>
<td>OS</td>
<td>oxidative stress</td>
</tr>
<tr>
<td>PC</td>
<td>protein carbonylation</td>
</tr>
<tr>
<td>PIMs</td>
<td>pro-inflammatory markers</td>
</tr>
<tr>
<td>PP</td>
<td>pulse pressure</td>
</tr>
<tr>
<td>PSR</td>
<td>psychiatric status rating</td>
</tr>
<tr>
<td>REB</td>
<td>research ethics board</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RNS</td>
<td>reactive nitrogen species</td>
</tr>
<tr>
<td>SES</td>
<td>social economic status</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>SUD</td>
<td>substance use disorders</td>
</tr>
<tr>
<td>T1DM</td>
<td>type-1 diabetes mellitus</td>
</tr>
<tr>
<td>T2DM</td>
<td>type-2 diabetes mellitus</td>
</tr>
<tr>
<td>TBARS</td>
<td>thiobarbituric reactive substances</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>U.S</td>
<td>United States</td>
</tr>
<tr>
<td>USD</td>
<td>U.S dollars</td>
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</table>
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GENERAL INTRODUCTION

Excessive and premature cardiovascular disease (CVD) mortality in BD has been documented for over 70 years, prior to the use of mood stabilizers and antipsychotics (Goldstein et al., 2015a). Since then, research has highlighted a robust BD-CVD link. BD predicts CVD mortality independently of cardiovascular disease risk factors (CVRFs), even after controlling for symptom severity, medications and metabolic syndrome (MetS) components (Goldstein et al., 2015a, 2015b). Longitudinal studies following BD patients over several years found that new onset CVD occurs 14 years earlier in BD-II, and 17 years earlier in BD-I, compared to adults without mood disorders (Goldstein et al., 2015a, 2015b). Recently, the American Heart Association (AHA) released a scientific statement positioning major depressive disorder (MDD) and bipolar disorder (BD) among adolescents as tier-II moderate risk conditions for accelerated atherosclerosis and early CVD (Goldstein et al., 2015a).

Moreover, the prevalence of mood disorders in adolescents (U.S) is approximately 10%, and the prevalence of current tier-II moderate risk conditions is 5% to less than .05% (Goldstein et al., 2015a). Therefore, MDD and BD are at least 10-times more prevalent than the current moderate-risk conditions combined (Goldstein et al., 2015a). As such, adolescents with BD are a large population at high-risk for accelerated atherosclerosis. It is imperative that early detection methods and a solid understanding of the biology underlying the BD-CVD link are assessed to improve treatment, prevention strategies, and disease monitoring. Studying this link in adolescence provides a window of opportunity for assessing the early limits of detection, and gathering evidence for modifiable risk factors, via pharmacological treatment, lifestyle
alterations, and appropriate screening. Moreover, the vascular-bipolar link may yield insights regarding etiopathology, biomarker discovery, and treatment of BD.

Figure I. Scope of Research Themes to Assess the BD-CVD Link

- Clinical and demographic characteristics
- Symptomatic status or severity
- Biomarkers: INF, OS, BDNF
- CVRFs: dyslipidemia, hyperglycemia, hypertension...

Figure I. Abbreviations: inflammation (INF), oxidative stress (OS), brain-derived neurotrophic factor (BDNF), cardiovascular disease risk factors (CVRFs), bipolar disorder (BD), cardiovascular disease (CVD). This schematic overviews the main areas that will be discussed in depth how each area plays a role in BD pathology, as well as how they relate to CVD development, progression and risk.

Bipolar Disorder: Prevalence, Burden, and Symptomology

BD often arises in adolescence and is continuous with BD in adulthood (Birmaher et al., 2009; Geller, Tillman, Bolhofner, & Zimerman, 2008) BD is characterized by episodes of mania/hypomania, commonly alternating with episodes of major depression [Table I] (Kessler et al., 2009; Osby et al., 2001). In adolescent onset BD, there is an increase in symptom severity, cycling of mood polarity, comorbidity, and suicidality, compared to adult-onset BD (Carter et al., 2003; Goldstein and Levitt, 2006; Leverich et al., 2007; Perlis et al., 2004). Compared to other psychiatric adolescent populations (MDD and ADHD) and psychiatrically healthy adolescents, adolescents with BD have greater functional impairment, and increased number of suicide attempts and hospitalizations (Freeman et al., 2009; Lewinsohn et al., 1995; Peele et al., 2004; Rademacher et al., 2007). Moreover, adolescents with BD spend 55-60% of
the time symptomatic (Birmaher et al., 2009; Geller et al., 2008). Therefore, BD is a persistent
and severe mood disorder that often results in substantial functional impairment, from
adolescence through to adulthood.

**Table 1: Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)
Criteria for Major Depression, Mania, and Hypomania**

<table>
<thead>
<tr>
<th>DSM-5 Criteria for Major Depression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Five or more of the following symptoms (including depressed or irritable mood or loss of interest/pleasure), lasting at least 2 weeks, comprising a change from previous functioning</td>
</tr>
<tr>
<td>*Other symptoms must co-occur with depressed or irritable mood and/or diminished interest/pleasure.</td>
</tr>
<tr>
<td>1. Depressed or irritable mood for most of the day, nearly every day</td>
</tr>
<tr>
<td>2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day</td>
</tr>
<tr>
<td>3. Significant weight loss when not dieting or weight gain (e.g., greater than 5% change in body weight in a month), or decrease or increase in appetite nearly every day</td>
</tr>
<tr>
<td>4. Insomnia or hypersomnia nearly every day</td>
</tr>
<tr>
<td>5. Observable psychomotor agitation or retardation nearly every day</td>
</tr>
<tr>
<td>6. Fatigue or loss of energy nearly every day</td>
</tr>
<tr>
<td>7. Feelings of worthlessness or excessive or inappropriate guilt nearly every day</td>
</tr>
<tr>
<td>8. Diminished ability to think or concentrate, or indecisiveness, nearly every day</td>
</tr>
<tr>
<td>9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation with a specific plan, or a suicide attempt or a specific plan for committing suicide</td>
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<table>
<thead>
<tr>
<th>DSM-5 Diagnostic Criteria for Mania and Hypomania</th>
</tr>
</thead>
<tbody>
<tr>
<td>A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased activity or energy, in addition to 3 (if elated/expansive) or 4 (if only irritable) 4 of the following</td>
</tr>
<tr>
<td>*The other symptoms must co-occur with euphoria and/or irritability.</td>
</tr>
<tr>
<td>1. Inflated self-esteem or grandiosity</td>
</tr>
<tr>
<td>2. Decreased need for sleep</td>
</tr>
<tr>
<td>3. More talkative than usual or pressure to keep talking</td>
</tr>
<tr>
<td>4. Flight of ideas or subjective experience that thoughts are racing</td>
</tr>
<tr>
<td>5. Distractibility</td>
</tr>
<tr>
<td>6. Increase in goal-directed activity or psychomotor agitation</td>
</tr>
<tr>
<td>7. Excessive involvement in pleasurable activities that have a high potential for painful consequences (e.g. excessive spending or sexual indiscretions)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mania</th>
</tr>
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<tbody>
<tr>
<td>• Episode lasts at least 1 week (or any duration if hospitalization is necessary), most of the day, every day.</td>
</tr>
<tr>
<td>• The mood disturbance must be sufficiently</td>
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<table>
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<tr>
<th>Hypomania</th>
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<tbody>
<tr>
<td>• Episode lasts at least 4 days, most of the day, every day.</td>
</tr>
<tr>
<td>• The mood disturbance must be associated with an unambiguous and uncharacteristic</td>
</tr>
</tbody>
</table>
severe either to:
- Cause noticeable functional impairment (i.e. social, academic, work) or
- Necessitate hospitalization to prevent harm to self or others or
- Be associated psychotic features (e.g. disorganized thinking, hallucinations and/or delusions)

change in functioning, and the mood symptoms and change in functioning must be noticeable by others.
- Marked impairment, need for hospitalization, and psychotic features preclude a diagnosis of hypomania.

**Note:** Depression, mania or hypomania thought to be caused by the direct physiological effects of a substance (e.g. illicit drugs/medications), a medical condition, or treatment (e.g. electroconvulsive therapy) disqualify a diagnosis of BD or MDD.

---

**Social and Economic Burden of BD**

The World Health Organization has reported that BD may result in greater disease and economic burden than all forms of cancer, Alzheimer’s disease and epilepsy (Guilbert, 2003; Jin and McCrone, 2015). Similarly, in the U.S the lifetime cost of adult onset BD was estimated at $24 billion USD (Jin and McCrone, 2015). Likewise, adolescent BD confers psychosocial and physiological burden for patients and families, and is a burden on the economic and health-care system (Costello et al., 2003; Freeman et al., 2009; Merikangas et al., 2009; Van Meter et al., 2013).

Ultimately, mood disorders are among the top 5 conditions of highest disease burden, and notably BD was ranked as #2 in adults and #4 in adolescents (Ratnasingham et al., 2013). Adolescent onset BD is burdensome, and is associated with significantly reduced quality of life (Freeman et al., 2009; Miklowitz et al., 2008; Van Meter et al., 2013). Adolescents with BD spend less of the time euthymic, have rapid cycling between polarities and increased risk of suicide attempts compared to adult onset BD (Freeman et al., 2009; Van Meter et al., 2013).

Moreover, those with BD spend more time symptomatic than those with MDD or dysthymia, have reduced care at hospitals, even for non-mood illnesses (Osby et al., 2001). They have increased number of emergency room visits, and take 10 years on average to be...
diagnosed and get proper treatment (Belmaker, 2004; Goldstein et al., 2009a; Hirschfeld et al., 2003; Lish et al., 1994). In Ontario, the years of life lost due to premature mortality in BD is 19 years, with exceedingly high reports of time spent in sub-optimal state of health associated with BD, and overall reduced functioning (Ratnasingham et al., 2013).

**Epidemiological and Population Studies of Cardiovascular Disease and Bipolar Disorder**

The leading cause of death in adults with BD is CVD (Goldstein et al., 2015a; Osby et al., 2001). Recently, a study following subjects over the course of 3 years (National Epidemiologic Survey on Alcohol and Related Conditions), investigated new-onset CVD in adult subjects with BD, compared to those with MDD and HC (Goldstein et al., 2015b). This study determined even after controlling for age, sex, race, tobacco use, hypertension, obesity, as well as, alcohol and drug use disorders, those with BD had significantly greater incidence of new-onset CVD compared to HC, and those with MDD (i.e. the incidence of new-onset CVD in each group: BD-I = 6.3%, BD-II = 5.74%, MDD = 3.98%, HC = 3.70%) (Goldstein et al., 2015b). Notably, those with BD-I developed new-onset CVD approximately 11 years earlier than those with MDD, and 17 years earlier than HC (Goldstein et al., 2015b).

A population-based study with a 20 year follow-up cohort indicated that the elevated mortality in BD is approximately double the mortality rate observed in the general population and that 38% of deaths were caused by CVD in persons with BD (Smith et al., 2013; Westman et al., 2013). In this same population based study it was also found that there were only slight increases in hospital admission rates for cerebrovascular disease, coronary artery disease, and acute myocardial infarction in BD adults, compared to HC (Westman et al., 2013). This indicates that those with BD are at an increased risk for premature mortality due to CVD, and that they are also under-recognized and under-served for these conditions (Smith et al., 2013).
A recent cross-sectional study analyzed a dataset of 1,751,841 patients in primary-care practices in Scotland (United Kingdom) (Smith et al., 2013). They found that even after controlling for age and sex, adults with BD were less likely than HC to have record of CVD, and those with a primary-care record of CVD were less likely to be treated with CVD medications (e.g. statins), and if they were treated, they were more likely to be treated less intensively compared to HC (Smith et al., 2013). Similarly, despite the recent decline in cardiac mortality in Nordic countries, a recent epidemiology paper reported a standardized mortality ratio (SMR) of 2 for CVD in both men and women with BD, with a reduced life expectancy of 11-20 years (Laursen et al., 2013). This under-treatment and under-recognition of CVD and CVD risk in BD may contribute to the excessive and premature mortality due to CVD in BD.

**Cardiovascular Disease Risk Factors and Bipolar Disorder**

Compared to the general population those with BD have higher rates of CVRFs (Fiedorowicz, Palagummi, Forman-Hoffman, Miller, & Haynes, 2008; Vancampfort et al., 2013). A study of 644 BD patients found that 79% were obese or overweight, compared to the prevalence of obesity of 60% in the general population (McElroy et al., 2002). Likewise, an inpatient study of BD patients found that 9.9% had diabetes, which is 3-times greater than the general population (3.3%) (Cassidy et al., 1999). While complex inpatients with BD may have higher rates of CVRFs, several studies have corroborated these findings in population and epidemiological studies. A U.S study found similar findings, with a 5.6% prevalence of hypertension in the control population, compared to 14% in BD patients (Yates and Wallace, 1987). Similar findings have been reported for hyperlipidemia (50% in BD vs. 32 % in the general population), and metabolic syndrome (30-53% in BD vs. 27% national prevalence) (Fagiolini et al., 2005).
Therefore, the occurrence of CVRFs is very prevalent in BD and likely contributes to the elevated CVD risk and mortality in BD. Obesity and other MetS components lead to decreased quality of life and greater burden of mood symptoms (Fiedorowicz et al., 2009). Little is known about CVD risk and CVRFs in adolescents with BD. Understanding the biomarkers associated with CVD and BD will assist in developing effective treatments and therapeutics, and may enhance diagnostics.

**Cardiovascular Risk Factors are Correlated with Clinical Psychiatric Characteristics**

CVRFs have also been found to be associated with symptom severity as well as polarity in BD (Fiedorowicz, Coryell, Rice, Warren, & Haynes, 2012; Fiedorowicz et al., 2009). For example, a recent study in adults with BD found that despite controlling for age, sex, and tobacco use, manic and/or hypomanic symptom burden was associated with poor flow-mediated dilation (FMD) (Fiedorowicz et al., 2012). Particularly, these findings indicate that severity of (hypo)manic symptoms may contribute dose-dependently to endothelial dysfunction (ED) in adult BD (Fiedorowicz et al., 2012). Furthermore, while it was found that adults with BD-I had greater than double risk of mortality due to CVD compared to those with BD-II, (hypo)manic symptoms independently predicted mortality due to CVD (controlling for treatment history, diagnosis, age, sex, and CVRFs measured at baseline (Fiedorowicz et al., 2009). Additionally, CVRFs such as elevated fasting glucose and cholesterol have been found to be associated with cognitive impairment in adults with BD (McIntyre et al., 2007). Recent findings in adolescents with BD agree with adult literature, indicating that elevated triglycerides are associated with poor executive function (Naiberg et al., 2016). Likewise, obesity, and several CVRFs have been associated with increased number of suicide attempts in adults with BD (Goldstein, Schaffer, Wang, & Blanco, 2015). Therefore, mood symptom
polarity, severity, as well as diagnosis are associated with CVD and CVRFs. And dysregulated CVRFs can lead to exacerbated clinical features such as poor cognition and suicidality.

The Role of Medication in the Bipolar Disorder – Cardiovascular Disease Link

The concept of symptomatic burden affecting mortality and CVD in BD has been documented historically. For example, in 1853 acute mania and sudden cardiac death was often been described as ‘exhaustion’ (Adland, 1947; Bell, 1853; Derby, 1933; Fiedorowicz et al., 2009). Such that patients having severe mania would have sudden cardiac death, which was presumed to be due to exhausting their energy and heart (Fiedorowicz et al., 2009).

Documented in 1933 (before the advent of modern anti-psychotic and mood stabilizing medications), those with BD were observed to have an increased rate of death with one-third being cardiac in nature (Derby, 1933). This has been reassessed in more recent studies, with vascular disease being the leading cause of excess death in BD, above respiratory, accidents and suicide (Osby et al., 2001; Weiner et al., 2011).

While medications have been robustly associated with CVRFs (e.g. obesity, hyperlipidemia/dyslipidemia), medications have not been shown to directly lead to CVD or CVD mortality in BD (Fiedorowicz et al., 2009). For example, a study following 220 BD/manic patients found an decreased SMR for treated (SMR=1.68) compared to un-treated (SMR= 2.23) (Angst et al., 2002). There are however, clear associations of medications leading to increased CVRFs. For example, lithium-use is associated with increased weight gain and fasting glucose (Hermida et al., 1994; Sachs et al., 2006; Vendsborg et al., 1976). Similarly, use of valproic acid has been shown to be associated with elevated insulin levels and elevated weight gain (Dinesen et al., 1984; Pylvanen et al., 2002). Several second generation antipsychotics (SGAs) have also been documented to lead to hyperlipidemia (Gaulin, Markowitz, Caley, Nesbitt, & Dufresne, 1999; Henderson et al., 2000; Huang & Chen, 2005;
Osser, Najarian, & Dufresne, 1999; Pylvanen et al., 2002; Spivak et al., 1999; Yilmaz, Dosan, Gurgoeze, & Gungor, 2001) insulin resistance and diabetes (Gianfrancesco, White, Wang, & Nasrallah, 2003; Guo et al., 2006; Henderson et al., 2000; Henderson et al., 2005; Lambert, Chou, Chang, Tafesse, & Carson, 2005; Ollendorf, Joyce, & Rucker, 2004; Sernyak, Gulanski, & Rosenheck, 2005), weight gain (Henderson et al., 2000; Simpson, 2005; Volavka et al., 2002; Zipursky et al., 2005) and obesity (Henderson et al., 2000; Simpson, 2005; Volavka et al., 2002; Zipursky et al., 2005). Importantly, (a) evidence from population studies indicate that CVD is highly associated with BD, despite most of the sample being un-medicated, (b) cohort studies of new-onset CVD in BD indicate that despite controlling for medication and CVRFs, adults with BD develop CVD approximately 17 years earlier than HC, and (c) lastly the presence of excessive mortality due to CVD has been noted historically in BD prior to the advent of mood stabilizing and antipsychotic medication (Fiedorowicz et al., 2009; Goldstein, Carnethon, et al., 2015; Goldstein, Schaffer, et al., 2015; Weiner et al., 2011). Therefore, while medications may play a role in the progression of CVD and CVRFs, it is evident that these conditions have a shared pathophysiology that remains to be fully understood. Moreover, non-invasive vascular assessment offers a method of assessing the progression of CVD and CVRFs in adolescents with BD.

Non-invasive Vascular Measures Provide Validated Proxies for Atherosclerosis and CVD Risk:

Examining vascular structure and function in adolescents offers additional boundaries and considerations regarding method of assessment (Urbina et al., 2009). While invasive methods such as angiography and contrast agents may potentially offer other insights, they have a poor risk-benefit ratio for research purposes in adolescents without clinical CVD (Flammer et al., 2012). Non-invasive methods offer predictive and reliable methods for CVD
assessment (Urbina et al., 2009). Non-invasive vascular measures include those that assess vascular function, structure and dynamic properties. Similar to adults, carotid intima media thickness (cIMT) and FMD are associated with hypertension, obesity, dysglycemia, and dyslipidemia (i.e. MetS components) among psychiatrically healthy adolescents and children (Allan, Delaney, Miller, & Spark, 2013; Blacher, Asmar, Djane, London, & Safar, 1999; Chan, Loizzi, Burger, Rutgers, & Monk, 2003; Hodis et al., 1998; Lehmann, Watts, & Gosling, 1992; Nichols, 1990; O’Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002; Urbina et al., 2009; Vaitkevicius et al., 1993).

**Vascular Function:** Ultrasound measurement of FMD provides a proxy measure of endothelial function, as determined by nitric oxide (NO) mediated responsiveness to shear stress of hyperemia (Deanfield et al., 2005; O’Rourke et al., 2002; Urbina et al., 2009). FMD is cross-sectionally linked with known CVRFs and is predictive of future CVD events (Jarvisalo, Ronnemaa, et al., 2002; Ostad et al., 2013; Urbina et al., 2009; Woo et al., 2004b). FMD is used to usually assess macrovascular ED of large conduit arteries (mainly brachial) (Allan et al., 2013).

**Vascular Structure:** Ultrasound measurement of cIMT provides a reliable and validated measure of arterial thickness and vascular structure (Greenland et al., 2000; Riley et al., 1986; Urbina et al., 2009). cIMT measurement in adolescents via 2-dimensional (2D) ultrasound has been shown to be associated with hypertension, obesity, dysglycemia, and dislipidemia in psychiatrically healthy adolescents and children (Iannuzzi et al., 2006; Jarvisalo et al., 2004; Lande et al., 2006; Meyer et al., 2006; Singh et al., 2003; Sorof et al., 2003; Woo et al., 2004a). This mirrors the findings seen in adults with sub-clinical and clinical atherosclerosis, which demonstrates that atherosclerotic plaques, vessel dysfunction and sub-clinical CVD are
detectable even in adolescence (Grobbee & Bots, 1994; Malik et al., 2011; Selvin et al., 2005; Urbina et al., 2009).

**Non-invasive vascular measures are associated with mood symptoms:**

Although previous studies have noted an association between unipolar depression and ED, (Broadley, Korszun, Jones, & Frenneaux, 2002; Broadley et al., 2006; Cooper et al., 2010; Fiedorowicz et al., 2009; Harris, Matthews, Sutton-Tyrrell, & Kuller, 2003; Hemingway et al., 2003; Lavoie, Pelletier, Arsenault, Dupuis, & Bacon, 2010; Pizzi, Manzoli, Mancini, & Costa, 2008; Rajagopalan et al., 2001; Rybakowski, Wykretowicz, Heymann-Szlachcinska, & Wysocki, 2006; Wagner, Tennen, Mansoor, & Abbott, 2006) few studies have examined this association in adolescents or in participants with BD. In a controlled study of young adults, in the absence of conventional CVRFs, MDD was reported to be associated with ED (Cooper et al., 2010; Rajagopalan et al., 2001). Cooper and colleagues noted that in adults (mean age = 36), increased mood disturbance (depressive symptoms, anxiety or anger) were negatively associated with FMD (p <0.05) (Cooper et al., 2010). Similarly, Chen and coworkers noted that cIMT was significantly thicker in elderly patients with late-onset MDD, compared to controls (1.26 +/- 0.3 vs. 1 +/- 0.2 mm; p<0.03) (Chen et al., 2006). There is a paucity of data in this area, which requires further investigation due to the strong associations of these two pathologies (CVD and BD). Investigating FMD and cIMT will yield integrative information on macrovascular function and vascular structure. Likewise, another method of assessing CVD risk and BD pathology is via biomarkers.

**Biomarkers Linking Bipolar Disorder and Cardiovascular Disease**

A biomarker is a biological marker that can be used to monitor disease development and progression, diagnose a condition, inform prognosis and predict/monitor treatment response
A good biomarker is sensitive and specific, and it has been demonstrated to have positive/negative predictive value or accurately classifies a given state/response (Goldstein and Young, 2013). Furthermore, a clinically useful biomarker can inform treatment, prevention, and improve the understanding of a given disease (Goldstein and Young, 2013).

In BD, it is important to understand potential biomarkers relevant to psychiatric characteristics as well as CVD development and risk (Goldstein and Young, 2013). While biomarkers are clinically useful tools that are widely used in many areas of medicine, there are currently no clinical biomarkers for BD. The lack of biomarkers means that BD diagnosis and disease monitoring has been via subjective clinical measures (Goldstein and Young, 2013). Establishing biomarkers for BD in adolescence is particularly important. Firstly, adolescents have not yet been exposed to the impact of the disorder (e.g. stress, medication(s), and symptoms), and measures are less confounded by age, compared to adults with BD (Berk et al., 2011, 2013; Goldstein, Sassi, & Diler, 2012; Goldstein & Young, 2013). Secondly, if biomarkers are validated in adolescent BD, this could provide clinically relevant treatment indications that could reduce CVD risk (Fiedorowicz et al., 2008; Goldstein et al., 2012; Young & Grunze, 2013). For example, a biomarker that could accurately classify diagnosis could prevent unnecessary use of antipsychotic/mood stabilizing medication in youth, thereby preventing the unnecessary risk of metabolic abnormalities and CVRFs.

Although there are several potential mechanisms that may contribute to the prevalence and early onset of CVD in those with BD, we chose to focus on mechanisms that have been most consistently implicated in adults and adolescents with BD, namely oxidative stress (OS), brain derived neurotrophic factor (BDNF), and pro-inflammatory markers (PIMs) (Berk et al.,
Inflammation and Bipolar Disorder

Adults with BD have been shown to have increased frontal cortical inflammation (INF) (Rao et al., 2010). Peripheral levels of PIMs are elevated during mania (Goldstein, Kemp, Soczynska, & McIntyre, 2009; Huang & Lin, 2007; Maes, Bosmans, Calabrese, Smith, & Meltzer, 1995; O’Brien, Scully, Scott, & Dinan, 2006; Ortiz-Dominguez et al., 2007; Tsai, Yang, Kuo, Chen, & Leu, 2001) and depression (Huang & Lin, 2007; Ortiz-Dominguez et al., 2007; Tsai et al., 2001), and notably PIM levels during euthymia have been shown to be similar to that of controls (Brietzke et al., 2009; Cunha et al., 2008; Dickerson, Stallings, Origoni, Boronow, & Yolken, 2007; Ortiz-Dominguez et al., 2007; Tsai et al., 1999). Levels of c-reactive protein (CRP) and interleukin (IL)-6 levels have also been noted to fluctuate during depression and mania (Brietzke et al., 2009; Huang & Lin, 2007). Also, symptomatic severity has been shown to be proportional to PIM levels (Cunha et al., 2008; Maes et al., 1995; Ortiz-Dominguez et al., 2007). While it has been documented that INF is robustly associated with mood symptoms in early-onset BD (Cunha et al., 2008; Guloksuz et al., 2010), this has not been explored in adolescents with BD. Importantly, INF may underlie the association between BD and CVD.

Proinflammatory marker Interleukin-6 is Proatherosclerotic

One of the major PIMs implicated in BD pathology is IL-6. IL-6 acts on target cells via the IL-6 receptor/gp80 and gp130, which are expressed on cell surfaces (Memoli et al., 2007; Taga et al., 1989). Once IL-6 binds to IL-6 receptor, a IL-6*IL-6 receptor complex is formed and gp130 dimerizes (Memoli et al., 2007; Taga et al., 1989). Dimerization of gp130 can then
lead to the activation of Janus-activated kinases that phosphorylate the cytoplasmic portion of gp130 and increase gp130 gene expression (Memoli et al., 2007; Taga et al., 1989).

There is a cycle in which systemic INF can activate adipose tissue to create a proinflammatory and proatherosclerotic state, and increased adiposity also leads to elevated INF (Memoli et al., 2007). This is true for IL-6, with increased IL-6 gene expression in a systemic inflammatory state (Memoli et al., 2007). Moreover, the IL-6 receptor gene single nucleotide polymorphisms (SNPs) have been linked with proatherosclerotic lipid conditions, such as elevated triglycerides and decreased HDL (Chu et al., 2011). In addition, lipid profile, quantity and quality, has been shown to be altered by increased INF (Chu et al., 2011). For example, elevated IL-6 inhibits lipoprotein and monomeric lipase activity, stimulates lipolysis, and can thereby create pro-atherosclerotic conditions (Chu et al., 2011). IL-6 is also involved in the production of triglycerides and facilitates an increase in macrophage uptake of lipids, which can lead to the formation of foam cells (Chu et al., 2011; Yudkin et al., 2000). Furthermore, foam cells continue to express IL-6 once formed, which can further contribute to atherosclerosis progression (Yudkin et al., 2000).

Inflammation is Involved in the Development and Progression of Atherosclerosis and Vascular Impairment

Chronic INF is also linked with vascular ED, atherosclerosis and MetS components (Esteve et al., 2007; Fernández-Real, 2008; Vila and Salaices, 2005). In addition, IL-6 and tumor necrosis factor (TNF)-α have been noted as independent explanatory factors for MetS components (Chan et al., 2002). Notably, ED is thought to be a component in the progression of atherosclerosis (Hansson, 2005). Under normal conditions, PIMs play an important role in response to injury, including vascular endothelial injury. In fact, the vascular endothelium is
involved in the inflammatory response in an atherosclerotic state and releases cytokines in response to vascular injury (Hansson, 2005; Natali et al., 2006; Steinberg and Baron, 2002; Widlansky et al., 2003).

In a proinflammatory state there is FMD impairment, increased vasoconstriction and reductions in endothelium-derived vasodilators, such as NO (Verma et al., 2002). For example, a Framingham offspring study found elevated IL-6 to be associated with ED (Vita et al., 2004). Furthermore, a chronic low-grade pro-inflammatory state facilitates insulin resistance via subclinical ED (Chan et al., 2002).

Similarly, IL-6 has also been shown to be associated with impaired vascular structure. For instance, a recent meta-analysis of 14,832 participants found that in HC, IL-6 levels were associated with cIMT in those with or at risk for CVD (Zhang et al., 2015). Moreover, a reduction of inflammatory cytokines is associated with improvement of endothelial function in obese women (Ziccardi et al., 2002). Likewise, elevated levels of TNF-α are also associated with ED (assessed via FMD) in post-myocardial infarction patients (Kovacs et al., 2006).

**Proinflammatory Markers are Linked with Metabolic Syndrome Components and Cardiovascular Risk Factors**

IL-6 also regulates CRP, the most commonly used biomarker for CVD risk and cardiovascular event assessment (Memoli et al., 2007). Increases in IL-6 can lead to a 1000-fold increase in the production of the primary acute phase reactants, CRP and serum amyloid A (Thorn et al., 2004). Elevated gene expression of both IL-6 and CRP have been found in adipose tissue and cells of patients with chronic inflammatory illnesses (Memoli et al., 2007). Since adipose tissue produces approximately 30% of peripherally circulating IL-6, and this pro-inflammatory production increases with increasing adiposity, adipose tissue directly
increases CRP via IL-6 stimulation (Memoli et al., 2007). IL-6 also has direct effects on plaque formation and stability (Gallistl et al., 2001; Ridker et al., 1997). For example, IL-6 independently contributes to ED, controlling for age, BMI, smoking status, LDL, cholesterol, blood pressure and insulin sensitivity (Esteve et al., 2007).

Moreover, IL-6 and CRP expression is 50 times greater in adipose tissue of patients in an inflammatory state compared to HC, and it is hypothesized that macrophages infiltrating fat tissue represent the principal site of obesity-related cytokine synthesis (Memoli et al., 2007; Weisberg et al., 2003). Taken together, INF plays a principal role in MetS, CVD and BD. Understanding the role of INF in the BD-CVD link is necessary to characterize this shared pathology and advance treatment and prevention strategies. Importantly, INF is thought to be a part of the same pathway as OS, which offers further information regarding the underlying biology of BD and CVD (Berk et al., 2011; Madamanchi, Vendrov, & Runge, 2005).

**Oxidative Stress and Bipolar Disorder**

Several studies have implicated OS in the pathology of psychiatric disorders, including BD (Machado-Vieira, Dietrich, et al., 2007; Steckert, Valvassori, Moretti, Dal-Pizzol, & Quevedo, 2010; Wang, Shao, Sun, & Young, 2009; Yumru et al., 2009). For instance, products of oxidative damage to lipids (i.e. lipid peroxidation) and alterations to antioxidant enzymes have been described in BD patients (Andreazza et al., 2009; Frey et al., 2007; Kuloglu et al., 2002; Scola et al., 2016; Versace et al., 2013) and reactive oxygen species (ROS) play an important role in the pathology of many neuropsychiatric disorders (Berk et al., 2011; Frey et al., 2013a).
**Oxidative Stress and Aerobic Species**

Humans and other aerobic species are susceptible to ROS, superoxide and hydrogen peroxide, which are produced by mitochondria during ATP production via cellular respiration (Chance et al., 1979; Steckert et al., 2010). OS can be defined as an imbalance between antioxidant defense capacity and ROS production, such that elevated ROS can overwhelm cellular defenses and lead to impairment in cellular function (Cochrane, 1991; Steckert et al., 2010). The electron transport chain (ETC) creates an electrochemical proton gradient through complexes and electron donors, which is needed for the synthesis of ATP, however, it also produces ROS at ubiquinone sites of complexes I and III (Adam-Vizi, 2005; Moro et al., 2005).

**High Energy Consumption, Mitochondria and Oxidative Stress: Measuring Damage**

The central nervous system is especially ATP-consuming and is sensitive to alterations in cellular energy metabolism, which can impair function and neuronal plasticity (Mattiasson et al., 2003). In normal non-pathological states, mitochondria produce the majority of ROS, via ETC complexes (Mattiasson et al., 2003). Once in a state of OS, ROS can act to inhibit complexes in the ETC and reduce ATP production (Andreazza, Shao, Wang, & Young, 2010; Steckert et al., 2010). This mitochondrial dysfunction can lead to cellular dysfunction and it has been demonstrated that genes encoding mitochondrial ETC complexes are differentially expressed in BD participants compared to HC (Jou et al., 2009; Konradi et al., 2004; Steckert et al., 2010; Sun et al., 2006). Moreover, increased frequency of DNA damage in patients with BD, relative to controls has been reported, and importantly the frequency of DNA damage is associated with the severity of mood symptoms (Andreazza et al., 2007; Wang, Kreutzer, & Essigmann, 1998; Watt, Routledge, Wild, & Hooper, 2007).
Another assay of OS is via measurement of thiobarbituric reactive substances (TBARS), which are products of lipid peroxidation usually due to ROS reacting with lipids (Poon et al., 2004). These substances can disrupt cellular processes by altering the conformation of proteins and impairing normal cellular lipid signaling processes (Bazan et al., 2005; Poon et al., 2004). BD participants have been shown to have elevated lipid peroxidation via TBARS assessment, as well as elevated NO levels (Poon et al., 2004). In adults with BD, TBARS levels are elevated in acute mania and depression, and have been reported to remain elevated during euthymic states (Andreaazza et al., 2007). Oxidative damage to lipids may therefore be stable throughout the symptomatic course of BD, however conflicting reports have been noted (Andreaazza et al., 2008).

Reactive nitrogen species (RNS) are another source of cellular OS and damage (Andreaazza et al., 2008). NO is synthesized by nitric oxide synthase (NOS), which is a Ca\(^{2+}\) dependent enzyme (Andreaazza et al., 2008). NO is an important player in nitration and OS due to its ability to react with superoxide free radical to generate peroxynitrite, which can react with thiol groups on amino acids leading to a disturbance of normal protein function (Szabó et al., 2007). Importantly, RNS can also lead to neurotoxic conditions (Stuehr, 1999).

**High Energy Demands and High Oxygen Demands: Metabolic Pathways, Oxidative Stress and the Brain**

Since the brain uses 20 % of total body oxygen and has poor antioxidant capacity, it is particularly sensitive to ROS (Floyd, 1999; Steckert et al., 2010). Postmortem brain sections from participants with BD showed a significant increase of 4-hydroxynonenal levels, a major product of lipid peroxidation, when compared with controls. This suggests that central OS may, in part, contribute to the pathology of BD (Wang et al., 2009). Moreover, *in vivo*
magnetic resonance spectroscopy studies investigating proxy measures of oxidative phosphorylation and phospholipid metabolism gave shown altered levels in the brains of those with BD (Stork and Renshaw, 2005).

Several studies investigating the neurobiological basis of BD have reported neurotrophic pathway dysfunction and abnormal energy metabolism, in those with BD compared to HC (Kato and Kato, 2000; Machado-Vieira et al., 2007b). Increased peripheral lipid peroxidation has been recently reported in blood and in plasma of unmedicated BD subjects, compared to HC and lithium-treated BD subjects (Machado-Vieira et al., 2007b).

Animal models of mania have also investigated the role of OS in mania. d-amphetamine induces hyperactivity in animals and repeated injections in rats has been used as a model of mania (Reddy et al., 1991). In this d-amphetamine regime, rats have increased protein and lipid oxidative damage to their brain (Frey et al., 2006; Reddy et al., 1991). Similarly, in mice d-amphetamine increases protein carbonyl and TBARS levels, which supports the hypothesis that elevated OS is present during a manic-like state (Jayanthi et al., 1998).

**Antioxidant Defense Systems Are Up-regulated in Bipolar Disorder: Compensatory Mechanisms and Medication Effects**

Furthermore, our antioxidant defense system involves the coordinated effects of superoxide dismutase (SOD), catalase (CAT) and glutathione (Kapczinski, Frey, Andreazza, et al., 2008; Reddy et al., 1991). SOD is an enzyme that selectively scavenges superoxide anion radicals by catalyzing the dismutation of the radical to hydrogen peroxide (Andreazza et al., 2008). CAT can then metabolize hydrogen peroxide to produce water and oxygen. However, increased SOD-to-CAT levels can result in an increased level of excess hydrogen peroxide and can lead to OS induced cellular damage (Andreazza et al., 2008). This increase of SOD activity
has been shown to be associated with BD and schizophrenia (Abdalla et al., 1986; Kuloglu et al., 2002). For example, SOD activity was increased during acute symptomatic episodes of BD compared to HC (Kunz et al., 2008). The increase in antioxidant enzymes has been hypothesized to be a compensatory mechanism in response to elevated ROS production occurring during an acute episode (Gsell et al., 1995). Moreover, increased levels of glutathione reductase and glutathione s-transferase (GST) are present in adults with BD, which is hypothesized to be a compensatory mechanism cumulating in abnormal antioxidant defense (Andreazza et al., 2009).

Glutathione has also been shown to play an important role in the neuroprotective antioxidant effects of lithium and valproate (Cui et al., 2007). Chronic lithium treatment may selectively upregulate GST isoenzymes to produce neuroprotective effects against ROS and highlights the role of OS in BD pathology (Bakare et al., 2009; Cui et al., 2007). Similar to lithium, chronic treatment with lamotrigine or olanzapine has been reported to increase GST protein levels and enzyme activity which implicates GST as a vital contributor to mood stabilizing capabilities of these drugs in BD (Bakare et al., 2009).

**Evidence of DNA Damage due to Oxidative Stress in Bipolar Disorder**

DNA damage due to mitochondrial induced OS may also be assessed via telomere shortening (Passos et al., 2007). Telomeres are repetitive sequences at the ends of chromosomes which prevent the loss of important genetic information during replication (Passos et al., 2007). Telomeres shorten during each round of replication and shortening of telomeres is a component of the aging process (Passos et al., 2007). Furthermore, telomere shortening can also be an indicator of cumulative OS and antioxidant capacity (Saretzki and Zglinicki, 2002). The degree of telomere shortening in adults with BD and depression is
indicative of approximately 10 years of accelerated aging, compared to the general population (Simon et al., 2006).

**Oxidative Stress is Involved in Cardiovascular Disease Risk, Development and Progression**

Importantly, OS plays a role in both BD and CVD pathology. Again, those with BD have an increased prevalence of MetS compared to the general population, which can increase the risk for CVD (Fiedorowicz et al., 2008). Elevated OS levels have been shown to be associated with both MetS and BD (Fiedorowicz et al., 2008; Hopps, Noto, Caimi, & Averna, 2010). Moreover, those with MetS have been shown to have activated biochemical pathways that can lead to decreased antioxidant capacity, increased ROS and consequently increased lipid peroxidation (Grattagliano et al., 2008).

**Oxidative Stress and Metabolic Syndrome Components**

MetS is a cluster of pathologies involving insulin resistance, abdominal obesity, dysregulation of adipokine levels and fatty acid metabolism, ED and elevated systemic INF (Grattagliano et al., 2008; Zimmet et al., 2007). MetS is defined by the International Diabetes Federation (IDF) as a group of CVD and type-2 diabetes mellitus risk factors, which includes abdominal obesity, high fasting glucose ($\geq 5.6$mmol/L) and triglyceride levels ($\geq 1.7$mmol/L), as well as high blood pressure (systolic $\geq 130$mmHg; diastolic $\geq 85$mmHg) and low HDL ($< 1.03$mmol/L) (Grattagliano et al., 2008; Zimmet et al., 2007). Classification for having MetS is defined by the IDF as having: greater than or equal to the 90th percentile for waist circumference (i.e. abdominal obesity) and the presence of two or more other MetS components (Grattagliano et al., 2008; Zimmet et al., 2007). OS is linked with all MetS criteria and importantly is associated with the onset of CVD (Grattagliano et al., 2008). For example,
insulin resistance, has been linked with INF, depression, cognitive impairment and OS (Grattagliano et al., 2008).

Cardiovascular Disease Risk Factors & Oxidative Stress: Oxidized Lipids are Pro-atherosclerotic and Contribute to Increased Inflammation

Elevated visceral fat levels lead to deregulation of adipocytes, which can increase leptin and PIMs, and decrease adiponectin secretion (Grattagliano et al., 2008). This altered state can lead to quantitative and qualitative changes in lipids (Grattagliano et al., 2008). For example, elevated serum lipid levels as well as proatherosclerotic lipids, such as small-dense LDL (compared to HDL levels) (Grattagliano et al., 2008). Small-dense LDL molecules are able to migrate through the endothelial layer of the vasculature, and contribute to the development of foam cells (Grattagliano et al., 2008). Oxidized-LDL activates endothelial inflammatory signaling which increases the abundance of local macrophages and INF (Grattagliano et al., 2008). Macrophages consume the oxidized-LDL and form foam cells (Grattagliano et al., 2008). Endothelial cells also produce adhesion molecules in the inflammatory process, which facilitate macrophage migration into the intima layer of the vasculature (Grattagliano et al., 2008). While this response is normal, overabundance of oxidized-LDL and small-dense lipids can lead to chronic INF of the endothelium, foam cell production, and the progression of a proatherosclerotic state (Grattagliano et al., 2008). Greater abdominal adiposity is a source of free fatty acids (FFAs) and INF (Grattagliano et al., 2008). Therefore abdominal obesity is a state of low-grade chronic INF, with studies highlighting elevated levels of CRP and TNF-α in obese subjects (Grattagliano et al., 2008).
Elevated Traditional Cardiovascular Risk Factors Lead to Dysregulated Mitochondrial Fat Breakdown, Elevated Oxidative Stress and Inflammation

Central obesity and MetS both contribute to liver damage, an organ which is vital to metabolic processes (Grattagliano et al., 2008). Intracellular fatty acid trafficking and mitochondrial fat-breakdown via beta-oxidation in the liver are altered in this state due to altered expression of perilipin and adipophilin (Grattagliano et al., 2008). Sources of elevated ROS production due to fat accumulation and insulin resistance occurs through several systems, including via cytochrome p450 2E1 (generates ROS via endogenous ketones metabolism), mitochondrial ETC activity, and peroxisomes (generate hydrogen peroxide) (Grattagliano et al., 2008). Therefore, MetS, OS, INF and mitochondrial dysfunction play a role in metabolism and systemic CVRFs, which may underlie the BD-CVD link.

Moreover, glucose intolerance/ insulin resistance (a MetS component), is associated with OS, INF and CVD. For example, increased blood glucose is associated with endothelium dependent vasodilation (Grattagliano et al., 2008). Post-meal elevation in blood glucose levels, is also associated with elevated OS, again indicating a role of OS in metabolic dysfunction and CVD risk (Grattagliano et al., 2008). Additionally, in the presence of elevated glucose, there is an increase in glucose metabolism which increases the production of nicotinamide adenine dinucleotide (NADH) and flavin adenosine dinucleotide (Grattagliano et al., 2008). This is a compensatory role of a positive feedback mechanism to increase ATP production and decrease glucose levels in the blood (Grattagliano et al., 2008). This alters the mitochondrial proton gradient and leads to a transfer of electrons to oxygen and the production of superoxide at NADH (Grattagliano et al., 2008).

Therefore, blood glucose levels can have a notable impact on metabolic regulation, INF and OS. FFAs and LDL oxidation are other sources of OS (Grattagliano et al., 2008).
Similarly, elevated FFA levels can lead to FFA entry into the Krebs cycle to generate acetyl-CoA and NADH, which again can lead to mitochondrial dysfunction and ROS production (Grattagliano et al., 2008). Elevated ROS production is a major cause of diminished vascular NO levels (Grattagliano et al., 2008). Importantly, this increase in ROS is hypothesized to be due to elevated INF, which is critical in the atherosclerotic process and development of CVD (Grattagliano et al., 2008).

Also, once a cell is in a state of OS, it is less capable of producing BDNF (Hachem, Mothe, & Tator, 2015; Kapczinski, Frey, Andreazza, et al., 2008; Texel & Mattson, 2011). Likewise, a cell with lower levels of BDNF is less able to withstand OS (Hachem et al., 2015; Kapczinski, Frey, Andreazza, et al., 2008; Texel & Mattson, 2011). Thus, levels of OS and BDNF play a role in cell regulation (Hachem et al., 2015; Kapczinski, Frey, Andreazza, et al., 2008). The interplay between OS and BDNF has also been shown to be involved in cognition, and may contribute to cognitive deficits and other symptoms that are characteristic of BD (Hachem et al., 2015; Kapczinski, Frey, Andreazza, et al., 2008; Texel & Mattson, 2011; Wu, Ying, & Gomez-Pinilla, 2004; Zhang et al., 2015). Importantly, in adults with BD, studies have found elevated OS and decreased BDNF (de Oliveira et al., 2009; Frey et al., 2007; Kapczinski, Frey, Kauer-Sant’Anna, & Grassi-Oliveira, 2008; Machado-Vieira, Andreazza, et al., 2007; Manni, Nikolova, Vyagova, Chaldakov, & Aloe, 2005; Versace et al., 2013).

**Brain-derived Neurotrophic Factor**

BDNF is from the nerve growth factor family of neurotrophins (Scola and Andreazza, 2015). Neurotrophins are able to signal neurons as well as other cellular systems to promote growth and differentiation (Huang & Reichardt, 2001; Kaplan & Miller, 2000; Mufson, Kroin, Sendera, & Sobreviela, 1999). Neurotrophin signaling pathways are crucial for cell survival,
regulation and plasticity (Scola and Andreazza, 2015). BDNF promotes cellular signaling and changes by binding to tyrosine kinase receptor and the p75 neurotrophin receptor (NTR) family (Chao, 1994; Frade & Barde, 1998; Hempstead, 2002; Huang & Reichardt, 2003). TrK receptors are high-affinity for the mature forms of neurotrophins, and act to regulate the effects of the nerve growth factor family (Frade & Barde, 1998; Hempstead, 2002; Huang & Reichardt, 2003; Scola & Andreazza, 2015). Alternatively, the neurotrophin p75$^{NTR}$ receptor has a low affinity for mature neurotrophins (Scola and Andreazza, 2015). PreproBDNF is a precursor protein of BDNF and is cleaved into proBDNF by intracellular proteases, then it is converted by extracellular proteases into mature BDNF (Scola and Andreazza, 2015). BDNF induces an effect at tyrosine kinase B and p75$^{NTR}$, with differences in mature and immature BDNF (Frade & Barde, 1998; Hempstead, 2002; Huang & Reichardt, 2003; Poo, 2001). Mature BDNF regulates cells via phosphatidylinositide 3-kinase and nuclear factor-κβ and other cellular signaling proteins responsible for cell survival (Poo, 2001). proBDNF acts via the p75 receptor and binds to activate the apoptotic pathway mediated by c-Jun N-terminal kinases, p53 and Bcl-2-associated X protein (Kaplan and Miller, 2000). Under normal conditions, proBDNF is favorably converted to mature BDNF and the balance between pro-and mature BDNF is regulated centrally through synaptic competition (Barker, 2009; Je et al., 2012)

BDNF is abundant in the brain and also measurable in the periphery (Scola and Andreazza, 2015). Importantly, BDNF is also produced in peripheral tissues, such as in muscle (Matthews et al., 2009), the liver (Cassiman et al., 2001), plasma (Fujimura et al., 2002), and importantly in vasculature (Donovan et al., 2000). Since there are peripheral and central sources of BDNF, serum BDNF levels could be derived from either central or peripheral tissues (Chen & Chang, 2009). BDNF freely crosses the blood-brain barrier and it has been
shown that blood levels of BDNF reflect brain concentrations (Berk, 2009; Elfving et al., 2010; Fernandes et al., 2011; Karege, Schwald, & Cisse, 2002; Klein et al., 2011; Munkholm, Vinberg, & Kessing, 2015; Pan, Banks, Fasold, Bluth, & Kastin, 1998; Sartorius et al., 2009). It is still unclear what role BDNF plays in BD pathology, and there are mixed findings which will be discussed below (Munkholm, Pedersen, Kessing, & Vinberg, 2014).

**Brain-derived Neurotrophic Factor and Bipolar Disorder**

Peripheral measures of BDNF have shown that those with BD have reduced levels compared to HC (Frey et al., 2013a). Additionally, BDNF plasma levels have been shown to be decreased during euthymia, depression, mania and hypomania compared to HC (Munkholm et al., 2014). In a recent meta-analysis, Munkholm and colleagues also note that longer duration of illness is associated with higher levels of BDNF, there are however contradictory findings in studies assessing BDNF in lymphoblasts (Kauer-Sant’Anna et al., 2009; Munkholm et al., 2014; Yatham et al., 2009). There is a major caveat to these findings due to the fact that medication for management of BD increases BDNF and may affect the results (Munkholm et al., 2014). For instance, while participants with subclinical BD symptoms were found to have comparable levels to HC, another study investigating manic, hypomanic, and depressed BD participants showed decreased serum BDNF levels (Cunha et al., 2006; Fernandes et al., 2011; Lin, 2009; Rosa et al., 2014). Likewise, in a recent meta-analyses levels of BDNF were consistently lower in BD patients compared with HC, when mood was not included in the statistical model (Munkholm et al., 2015). If mood state was included in the model, only depressed BD participants had lower levels of BDNF compared to HC participants, with no significant difference in manic and euthymic BD participants compared to HC (Munkholm et al., 2015). The meta-analysis also highlighted that greater symptom severity was also
associated with decreased BDNF levels in BD participants, compared to HC (Munkholm et al., 2015).

Additionally, contradictory findings may also be due to different BDNF sample types have also yielded different findings. For example, lymphoblasts from BD participants have been found to have decreased BDNF levels, as well as decreased hippocampal BDNF mRNA levels, compared to HC (Ray, Shannon Weickert, & Webster, 2014; Tseng et al., 2008). There is also evidence that there may be differential levels of proBDNF and mature BDNF ratios in those with BD compared to HC, however, this has not been extensively investigated (Sodersten et al., 2014). This state of decreased BDNF could disrupt normal cell function and could present as cognitive impairment, which is often observed in participants with BD (Munkholm et al., 2015; Sodersten et al., 2014).

**Brain-derived Neurotrophic Factor Single Nucleotide Polymorphism Val66Met is Associated with Bipolar Disorder**

The BDNF gene has also been investigated for its role in BD. A substitution of a valine to methionine within codon 66 (BDNF Val66Met) is a single nucleotide polymorphism (SNP) which has been shown to lead to a reduction in proBDNF levels (Scola and Andreazza, 2015). Reduced intracellular trafficking of proBDNF in the cortices has been shown to impair the central nervous system (Neves-Pereira et al., 2002; Scola and Andreazza, 2015; Sklar et al., 2002). The Val66Met SNP has also been shown to be associated with several psychiatric diagnoses, such as anxiety disorders and schizophrenia, in addition to BD (Chen et al., 2014; Lang et al., 2005). However large gene association studies have not reported consistent associations between BD and the Val66Met SNP. For instance, while there are candidate gene family based association studies to support Val66Met as a risk locus for BD, some studies have
shown no significant association for Val66Met and BD (Müller et al., 2006; Nakata et al., 2003; Skibinska et al., 2004; Sklar et al., 2002). Also, the association of Val66Met and BD may be more restricted to a sub-phenotype of BD (i.e. rapid cycling BD) (Müller et al., 2006). Despite the discrepant findings in gene association studies, there is support for the role of BDNF in BD pathology, for example, BDNF levels have been shown to return to normal levels after treatment with appropriate medication, for example mood stabilizers in BD (Sen et al., 2008).

**Brain-derived Neurotrophic Factor is Involved in the Development, Regulation and Injury Response of Cardiac and Vascular Tissue**

Notably, BDNF is also involved in the normal development and regulation of cardiac and vascular tissue, as well as in response to injury. BDNF is expressed at similar levels to the brain in the periphery, which is true for heart tissue and in the vasculature (Donovan et al., 2000, 1995; Hiltunen et al., 1996; Nakahashi et al., 2000; Scarisbrick et al., 1993; Timmusk et al., 1993). Animal studies have also highlighted this role of BDNF. For example, BDNF and tyrosine kinase B are selectively expressed post-injury in new vascular smooth muscle cells in the intima layer of adult rodent vessels, as well as, in the human aorta in a pathological state (Donovan et al., 1995). Rodent studies have also reported BDNF to be localized to arteries and capillaries, with adult mice selectively expressing BDNF via endothelial cells in the heart, skeletal muscle and arterial smooth muscle cells (Donovan et al., 2000, 1995; Hiltunen et al., 1996; Scarisbrick et al., 1993).

**Brain-derived Neurotrophic Factor and Cardiovascular Development**

Likewise, BDNF /-/- knock-out animal models highlight the major role of BDNF in normal cardiovascular development (Donovan et al., 2000). BDNF /-/- knock-out pups have
defects and perivascular edema, in which arteriolar and capillary endothelial cells are enlarged and degenerated (Donovan et al., 2000). Likewise, BDNF is required for endothelial cell development in arterioles, capillaries and in the heart, and it is necessary for proper attachment to the basement membrane of vascular structure (Donovan et al., 2000). Without BDNF, vessels are too weak to attach to the basement membrane and are unable to form a robust endothelial cell lining, which results in the detachment of endothelial cells into the vessel lumen (Donovan et al., 2000). Consequently, BDNF deficiency in developing rodents leads to a loss of endothelial cell attachment and initiates apoptotic pathways (Donovan et al., 2000).

Endothelial cells are the innermost layer of vasculature and are key regulators of vascular homeostasis (Kermani & Hempstead, 2007). They maintain vascular integrity, tone and play a role in inflammatory conditions in pathological states (Kermani & Hempstead, 2007). Under normal conditions the endothelial layer creates an anti-thrombotic state via expression and secretion of various proteins (Robson et al., 1997; Urbich et al., 2002). Moreover, endothelial cells are potential pharmacological targets to potentially alter CVD progression and risk (Kermani & Hempstead, 2007).

**Brain-derived Neurotrophic Factor and Vascular Injury Response**

BDNF is upregulated in response to vascular injury and it has been shown that BDNF regulates vascular development and injury response (Kermani & Hempstead, 2007). For instance, BDNF is necessary for stabilization and development of vasculature, and promotes blood flow and enhanced capillary density under normal and pathological conditions (Kermani & Hempstead, 2007). In addition to neuronal and vascular growth and development, BDNF also plays a role in the regulation of appetite and physical activity (Kaess et al., 2015). For instance, animal studies note that BDNF +/- knock-out mice are obese and have 50% greater food consumption compared to wild-type littermates (Kaess et al., 2015).
**Brain-derived Neurotrophic Factor is Involved in Metabolic Regulation**

Animal models have therefore found that metabolic and cardiovascular dysfunction can be altered via BDNF centrally and peripherally (Lyons et al., 1999). Importantly, BDNF administration decreases food intake and leads to weight loss (Bariohay et al., 2005; Lyons et al., 1999; Rios et al., 2001). In humans, BDNF deficiencies lead to similar characteristics, such as excessive food consumption and childhood onset obesity, as seen in animal models (Gray et al., 2006; Yeo et al., 2004). Chronic administration of BDNF can also improve glucose metabolism in mice and aid in regulating blood pressure (Swardfager et al., 2011).

**Brain-derived Neurotrophic Factor is Involved with Cardiovascular Disease Risk Factors and Cardiovascular Disease Mortality**

BDNF has therefore emerged as a key factor in the pathogenesis of CVD, for instance higher peripheral BDNF levels are associated with lower mortality and CVD related deaths, independent of traditional CVRFs including INF, BMI, activity levels and psychiatric comorbidity (Kaess et al., 2015). A study of gene SNPs and peripheral BDNF levels demonstrated that measures of peripheral BDNF corresponded to a predicted hazard ratio for CVD per T allele (Kaess et al., 2015). Notably, the predicted effect size from the Framingham cohort was comparable to these findings which suggests that the observed BDNF and CVD risk association may be causal (Kaess et al., 2015). Therefore, higher serum BDNF levels are associated with decreased risk for future CVD events and mortality.

Furthermore, BDNF is also a mediator in the survival and growth of the arterial baroreceptor system (Brady et al., 1999). Expression of BDNF is significantly increased in coronary arteries in an atherosclerotic state compared to healthy coronary arteries (Ejiri et al., 2005; Manni et al., 2005). Similarly those with acute coronary syndromes have been shown to have reduced plasma BDNF (Cai et al., 2006; Ejiri et al., 2005; Manni et al., 2005). Despite
reduced BDNF being linked with CVRFs and metabolic diseases, studies have found that increased BDNF levels are associated with CAD (Cai et al., 2006; Ejiri et al., 2005). For instance, elevated blood pressure, LDL, cholesterol, fat-mass, BMI and triglycerides were associated with elevated peripheral BDNF (Golden et al., 2010). This could be due to a protective compensatory action in response to CVRFs and pathology (Golden et al., 2010). For example, obesity is considered a low-level chronic inflammatory state and could lead to elevated BDNF (Golden et al., 2010). Higher inflammation has been seen non-obese participants with abdominal adiposity (Lapice et al., 2009).

In participants with CAD, inflammatory biomarkers such as IL-6 were associated with higher BDNF concentrations (Swardfager et al., 2011). In an inflammatory state monocytes can be stimulated to release more BDNF and therefore increased MetS and CVRFs could in fact lead to elevated BDNF (Kerschensteiner et al., 1999). In a linear regression model controlling for age, sex, antihypertensive use, depression, antidepressant use and serum IL6, better cardiopulmonary fitness was a significant independent predictor of serum BDNF concentrations, reflecting the important link between BDNF and cardiovascular function and disease risk (Swardfager et al., 2011).

**Conclusion**

INF has also been shown to predict CVD, and measurement of inflammatory markers have become a widely used, reliable clinical biomarker for CVD risk (Ridker, 1999; Ridker et al., 1997). Changes in vascular structure and function, as well as inflammatory changes, are present as early as adolescence and are associated with CVD risk proxies in adults (Glowinska-Olszewska et al., 2007; Groner et al., 2006). Moreover, OS has also been repeatedly implicated in CVD risk (Andreazza et al., 2007, 2008). Importantly, INF and OS are closely linked, and are thought to be part of the same cascade (Andreazza et al., 2008). Lipids and proteins are
susceptible to OS, leading to damage to membrane proteins, and tissue high in lipid content. OS contributes to ED, via interactions between ROS and endothelium produced NO (Cai & Harrison, 2000). BDNF is also associated with CVD (Ejiri et al., 2005; Kermani & Hempstead, 2007; Kermani et al., 2005). BDNF reductions may also be implicated in ED and increased vascular endothelial cell apoptosis (Donovan et al., 2000). Interestingly, since vascular endothelial cells can produce BDNF, this association of ED, CVD and BDNF may be bidirectional (Nakahashi et al., 2000). Gaining insight on how these key features are associated is pertinent to understanding BD related CVD risk and neuroprogression. Lastly, there is converging evidence of prolonged INF and increased BMI and central obesity in BD (Fiedorowicz, Palagummi, Forman-Hoffman, Miller, & Haynes, 2008; Frey et al., 2013a). Adolescents with BD had significantly greater waist circumference, BMI, as well as significantly elevated levels of PIMs.

Taken together, CVD is prevalent and premature in those with BD (Goldstein et al., 2015a; Osby et al., 2001). Noninvasive proxy measures of CVD risk (FMD and cIMT) allow for an examination of the biology underlying CVD at the earliest possible stages, and well before the onset of advanced CVD. It is during these early stages that employing biomarkers (PIMs, OS, BDNF) offers the greatest potential value, because the fundamental biology linking BD and CVD is far less confounded by aging and by the impact of decades of illness (including symptom severity, medications and stress) as compared to middle-aged adults with BD. By validating non-invasive CVD measures, and by parsing the biology underlying these measures, among adolescents with BD, we can aid in future treatments and prevention strategies. For example, this approach may lead to the identification of novel biomarker applications, inform novel treatments as well as diagnostic and assessment approaches, and may ultimately diminish the burden of CVD and of mood symptoms in BD. Therefore we
examined this topic in a “real-world” clinical sample of adolescents with BD to validate noninvasive vascular imaging in this population, as a proxy for CVD risk, and to gain further insight on biomarkers linking BD and CVD.
Scope and Hypotheses

Scope

The main underlying theme of our research is bringing together the CVD-BD link through the investigation of potential underlying biological features. Primarily, OS, PIMs, and BDNF have emerged as principal features that are shared between both CVD and BD pathology. Importantly, in order to assess novel biomarkers for their role in vascular structure and function, standard measurements of these CVD risk proxies must be validated in a BD population.

FMD and cIMT are proxy measures for non-invasive CVD risk assessment, and are predictive of future CVD and hard end-points (e.g. myocardial infarction). There is an urgent need for prevention and early detection strategies, due to excessive and premature burden of CVD in BD. No previous studies have investigated non-invasive CVD risk assessment in BD adolescents, which necessitates method validation due to the inherent ‘noise’ of the disorder (cyclic/fluctuating nature of mood symptoms, symptomatic severity, medication/polypharmacy, genetic risk and environmental risk factors).

Furthermore, while there are many reports of increased and premature CVD risk, there have been no studies to investigate whether CVRFs, cIMT and FMD are associated with OS in a BD population. Several studies have linked OS to mood fluctuations, cognitive deficits, brain morphology, symptoms and medication effects in BD, compared to healthy controls and other diseases/disorders. This study will be the first to address whether the increased CVD in BD is related to OS as well, which can inform future avenues for research (underlying mechanism), prevention strategies and treatments.
Lastly, we aim to address the question of whether there are differences in vascular risk and biomarkers in BD, as early as adolescence, compared to psychiatrically HC, and whether there are differences in how these principal biomarkers are associated with non-invasive vascular measures between groups. These primary biomarkers have been implicated in CVD development, progression, and injury response in other disease states (e.g. diabetes mellitus), as well as in BD. It has, however, never been addressed whether these risk factors arise as early as adolescence in BD, compared to HC, and whether there is an interplay between CVRFs and these novel biomarkers. It is unclear why there is excessive and premature CVD disease burden in BD, and this research will aid in understanding the factors that may be involved in the development of hard outcomes (e.g. myocardial infarction, stroke, etc.).

Taken together, this work will (1) explore non-invasive vascular risk measures as well as for their validity and prediction of CVD risk in BD adolescents, (2) investigate key biomarkers in conjunction with non-invasive vascular assessment to help bridge the gap between BD and CVD. Although it is well known that BD is highly linked with premature CVD, these studies for the first time will investigate the potential interplay between biomarkers and CVD risk. This will aid in understanding the underlying mechanisms of CVD development and risk in BD, which may lead to prevention strategies, and treatments improvement that can offer potential for a better prognosis and improved quality of life for adolescents with BD.
**Figure II: Aims and Scope to Assess the BD-CVD Link**

**Aims & Hypotheses**

For studies 1-3 aims and *a priori* hypotheses are noted below. The analyses conducted on each study are from subsets of the same sample of subjects.

**Aims**

**Study 1**: Validate cIMT and FMD as reliable vascular risk proxy measures in adolescents with BD, via (a) assessment of cIMT and FMD measures compared to traditional vascular risk measures.
Study 2: Assessment of putative biomarkers in adolescent BD to gain insight on their role in BD pathology by (a) assessing the potential role of biomarkers in vascular pathology via cIMT and FMD measures, (b) assessing the group differences in biomarkers between symptomatic and asymptomatic bipolar adolescents, compared to healthy controls.

Study 3: Examine cIMT and FMD to determine how the demographic, clinical, and biological correlates of these different measures are associated by (a) comparison of cIMT and FMD in adolescents with BD and HC (b) comparison of demographic, and clinical factors associated with cIMT and FMD.

Hypotheses

Study 1:

(a) Thicker cIMT and lower FMD measures will be associated with greater levels of traditional vascular risk measures in adolescents with BD, as they are in healthy adolescents.

Study 2:

(a) Greater levels of pro-inflammatory markers and oxidative stress as well as lower levels of anti-inflammatory markers and brain-derived neurotrophic factor (BDNF) will be associated with poor vascular structure (thick cIMT) and function (low FMD) (b) Symptomatic and asymptomatic BD adolescents will have higher levels of inflammation, and and lower levels of anti-inflammatory markers and BDNF, compared to healthy controls.

Study 3:

(a) cIMT will be thicker and FMD will be lower in adolescents with BD compared to HC. (b) The variables most strongly associated with poor FMD and cIMT will include: older age, male sex, BD-I subtype, more severe mood symptom burden, use of second-generation antipsychotics and family psychiatric history.
CHAPTER ONE

**Title:** Non-invasive vascular imaging is associated with cardiovascular risk factors among adolescents with bipolar disorder

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Non-invasive vascular imaging is associated with cardiovascular risk factors among adolescents with bipolar disorder

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ABSTRACT

Objective: Cardiovascular disease (CVD) is exceedingly prevalent among adults with bipolar disorder (BD), implicating BD adolescents as a high-risk group for CVD. Non-invasive ultrasound measures of vascular structure (via carotid intima media thickness [cIMT]) and function (via flow mediated dilation [FMD]) predict future CVD and are associated with traditional CVD risk factors among adolescents without mood disorders. This study examined, for the first time, the association of cIMT and FMD with CVD risk factors among adolescents with BD. The presence of multiple potential confounds among adolescents with BD, including various medications and mood states, informs the need to demonstrate whether cIMT and FMD are associated with CVD risk factors in this population specifically.

Methods: Participants were 30 adolescents, 13-19 years old, with BD, without CVD. High-resolution ultrasonography was used to evaluate vascular structure (cIMT) and function (FMD). Analyses examined associations of cIMT and FMD with traditional CVD risk factors.

Results: cIMT was significantly positively associated with systolic blood pressure and waist circumference. FMD was significantly negatively associated with waist circumference, body mass index, triglycerides, and glucose, and positively associated with high-density lipoprotein.

Conclusion: cIMT and FMD are associated with traditional CVD risk factors among adolescents with BD. Irrespective of numerous potential confounds, non-invasive vascular ultrasound approaches may be used as CVD risk proxies among adolescents with BD as they are for other adolescents.

Key Words: carotid intima media thickness, flow-mediated dilation, cardiovascular disease, ultrasound, bipolar disorder, adolescents
Introduction:

Bipolar disorder (BD) is a mood disorder characterized by states of depression and mania and/or hypomania (as distinguished by states of intense elation and/or irritability, in addition to other symptoms) (Kessler et al., 2009; Osby et al., 2001). BD is recurrent, often leads to psychosocial burden, and confers a high risk of suicide. However, the leading cause of mortality in adults with BD is cardiovascular disease (CVD), with standardized mortality ratios of 1.5-2.5 (Osby et al., 2001). Adults with BD have been shown to have CVD approximately 14 years earlier than psychiatrically healthy adults, and 6 years earlier than adults with major depressive disorder (MDD) (Goldstein et al., 2009b). The early onset of CVD in adults with BD indicates that adolescents with BD are a high-risk population for future CVD (Osby et al., 2001; Shah et al., 2009). However, little is known about CVD risk assessment in adolescents with BD.

Non-invasive vascular imaging, examining blood vessel structure as well as function, provides a proxy for CVD risk in adolescents. Ultrasound measurement of carotid intima media thickness (cIMT) provides a reliable and validated measure of vascular structure (Greenland et al., 2000; Riley et al., 1986; Urbina et al., 2009). Ultrasound measurement of flow-mediated dilation (FMD) provides a proxy measure of endothelial function, as determined by nitric oxide (NO) mediated responsiveness to shear stress of hyperemia (Broadley et al., 2005; O’Rourke et al., 2002; Urbina et al., 2009). A recent scientific statement from the American Heart Association provided support for non-invasive vascular imaging, on the basis of a growing scientific literature, which demonstrates that these measures are cross-sectionally linked with traditional cardiovascular risk factors (Jarvisalo, Ronnemaa, et al., 2002; Jourdan et al., 2005; Shah et al., 2009; Urbina et al., 2009; Woo et al., 2004b). Similar to adults, cIMT and FMD are associated with hypertension, obesity, dysglycemia, and dyslipidemia among
psychiatrically healthy adolescents and children (Chao et al., 2003; Hodis et al., 1998; Kelly & Steinberger, 2008; Urbina et al., 2009).

Several studies have examined vascular imaging in relation to major depressive disorder or depression symptoms among adolescents (Dietz and Matthews, 2011; Osika et al., 2011; Rajagopalan et al., 2001). However, these studies have focused on whether mood is associated with cIMT and/or FMD. No previous study has examined cIMT and/or FMD among adolescents with BD. Similarly, no previous study has focused on whether cIMT and FMD are associated with traditional CVD risk factors among adolescents with mood disorders. This is problematic because it is not yet known whether cIMT and FMD are associated with standard CVD risk factors among adolescents with BD, as they are in the general population of adolescents. This remains an open question, given the presence of multiple potential confounding factors that could interfere with signal detection. For example, psychotropic medications, particularly second-generation or atypical antipsychotics, have been associated with weight gain, dyslipidemia and impaired blood glucose regulation (Correll, 2007; Dietz and Matthews, 2011; Elovainio et al., 2005; Goldstein et al., 2011; Tomfohr et al., 2011). Use of tobacco, which is common among youth with BD, is another potential confounder (Goldstein et al., 2008). Similarly, stress related to the severe mood states of depression and mania may impact vascular function and structure (Miller, Freedland, Carney, Stetler, & Banks, 2003; Miller, Rohleder, & Cole, 2009; Rybakowski et al., 2006). We therefore examined this topic in a “real-world” clinical sample of adolescents with BD, in order to determine whether non-invasive vascular imaging is a valid proxy for CVD risk in this population.
Methods:

All procedures were approved by the Research Ethics Board (REB), and are in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants (parents and/or guardians and adolescents) prior to study procedures. English-speaking subjects were recruited consecutively between 2010 and 2013. Volunteer or community service hours were provided for study participation, for the hours participated.

Sample

Subjects (N=30, 13-19 years old) were English-speaking adolescents recruited via the Centre for Youth Bipolar Disorder at Sunnybrook Health Sciences Centre, a tertiary care hospital in Toronto, Ontario. All subjects were without any existing cardiac condition (e.g. congenital heart disease), autoimmune illness or inflammatory illness, or currently taking medication for any of the noted illnesses (e.g. anti-platelet, anti-inflammatory, or anti-hypertensive medication). Subjects were excluded if they had an infectious illness within the past 2 weeks, or were unable to provide informed assent/consent (i.e. due to developmental delay or psychosis). Diagnoses of BD type I (BD-I), type II (BD-II) or not otherwise specified (BD-NOS) were confirmed via gold-standard semi-structured diagnostic interviews as outlined below. Information about eligible participants who declined participation was not systematically recorded. Several potential participants declined due to the requirement of a blood draw and/or the lack of financial compensation. No potential participants declined due to the requirements (described below) to fast or to abstain from tobacco, alcohol, and drugs.

Assessment

Interviews and questionnaires

Diagnoses were derived from semi-structured diagnostic interviews using the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime
version (K-SADS-PL) (Kaufman et al., 1997). Subjects met Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria for BD-I, BD-II, or operationalized criteria for BD-NOS, that was primary (not induced by substance use, medications, or a medical condition).

Parents were interviewed about their adolescents, and adolescents were directly interviewed. Diagnoses were based on consensus ratings incorporating all available data. Diagnoses were confirmed by a consensus conference with a licensed child-adolescent psychiatrist following the interview.

Basic demographic information was obtained. Socio-economic status (SES) was ascertained using the 4-factor Hollingshead Scale (Hollingshead, 1975). Information regarding subjects’ comorbid diagnoses (e.g., anxiety disorders, conduct disorder) and clinical characteristics (e.g., psychosis, physical abuse, sexual abuse) were discerned from the K-SADS-PL. Subjects were considered to have a lifetime substance use disorder (SUD) if they met DSM-IV criteria for abuse or dependence of alcohol or any drug other than nicotine. Lifetime exposure to psychotropic medications was ascertained categorically in the treatment history section of the K-SADS-PL and from a medical history questionnaire.

**Biological and Physical measures**

All participants completed ultrasound procedures, followed by blood-draw and clinical diagnostic interviews, starting between 8 and 10 am. Participants were instructed to fast 10 hours prior to assessment (no food or beverage other than water), as well as not to smoke tobacco, consume alcoholic beverages, or illicit drugs for 24 hours prior to the procedures.

Ultrasound (Phillips, iU22) procedures were carried out by a trained sonographer, and used to evaluate FMD and cIMT. Blood pressure was taken prior to initial ultrasound procedures while recumbent and after resting quietly for at least 10 minutes, and electrodes were placed for a 3-lead electrocardiogram (ECG). All sessions began with cIMT
measurements, with the subject lying comfortably in a supine position, with their neck extended and head at a 45-degree angle away from the side being examined. Two-dimensional Doppler imaging, with a high-frequency (10 MHz) linear-array transducer was used for all imaging procedures. Multiple scanning sites were analyzed: common cIMT (the region 1 cm proximal to carotid dilatation), bulb IMT (the region between carotid artery flow divider and the carotid dilatation) and internal common cIMT (the 1 cm long artery region distal to the carotid flow divider), for both the right and left sides (Agrotou et al., 2013; Urbina et al., 2009). Duplicate scans were performed to ensure reliability.

FMD was assessed following cIMT measurements. Subjects remained in a comfortable supine position, with their right arm positioned closest to the sonographer. A stable stand was used to hold the transducer steady while conducting brachial artery measurements. FMD imaging was conducted with a simultaneously obtained electrocardiogram (ECG) recording. Prior to conducting resting brachial artery resting measurements, a blood pressure cuff was gently placed below the antecubital fossa (lower arm placement). The blood pressure cuff was then inflated to 50 mmHg above systolic for 5 minutes. Following deflation, images were recorded at 60 seconds and then at intervals of 30 seconds for 5 minutes. FMD was defined as a percentage-change in arterial diameter in response to reactive hyperemia, compared with baseline. FMD measurements were analyzed at different time points (FMD response over time and time to peak FMD) to address the temporal fluctuations in brachial artery response to hyperemia, and gain greater insight on endothelial functioning. Following all ultrasound measurements, a final blood-pressure reading was taken on the contralateral arm.

Following ultrasound procedures, trained phlebotomists conducted blood-draws. Blood was collected between 9 and 11 am, following an over-night fast (10 hours), for analysis of glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density
lipoprotein (LDL). The exact time of blood-draw was noted for each subject. Duplicate measures of subject weight and height were taken to the nearest 0.5 cm and 0.1 kg, respectively. Adjustments were made to account for clothing (the following were subtracted from weight: 1.4 kg for long pants and long shirt/sweatshirt, 1.1 kg for short pants or short-sleeves, and 0.9 kg for short pants and short sleeves), and used to calculate adjusted body mass index (BMI). Subjects were asked to hold their arms up and away from their trunk and bend sideways, to find the location of their waist. Waist circumference was measured using a flexible tape measure, with subjects standing up straight. Anthropomorphic measurements were taken prior to study interviews.

**Statistical Analyses**

Descriptive statistics were calculated for all relevant variables. Independent samples t-tests were examined for categorical (e.g. exposure to lithium) variables and bivariate correlation analyses (Pearson’s correlation coefficient) were completed for all continuous variables (e.g. age). In this preliminary study, intended to generate heuristics for future studies, we did not correct for multiple testing. Statistical analyses were performed using SPSS 20 for Windows (SPSS Inc, Chicago IL, USA).

**Results:**

Descriptive statistics are presented in Table I.

*Carotid Intima Media Thickness*

Of the six assessed regions of the carotid artery, the left and right bulb cIMT measurements were the only regional measurements significantly associated with CVD risk factors. Left bulb cIMT was significantly associated with systolic blood pressure ($r=0.38$, $p=0.04$) and right bulb cIMT was significantly associated with waist circumference ($r=0.39$, $p=0.04$). Maximum cIMT was significantly greater in males compared to females ($t = 2.310$, $p=0.04$).
p=0.029). Mean and maximum cIMT measurements were also significantly associated with age (r =0.39 and 0.47; p =0.04, and 0.01, respectively). Compared to previously reported data from healthy children and adolescents (mean=0.413, SD=0.057; data aggregated by Urbina et al., 2009), adolescents with BD had significantly thicker common cIMT measurements (t=6.6, p<0.0001; mean=0.463, SD=0.054) (Urbina et al., 2009). SES was not significantly associated with cIMT or standard cardiovascular risk factors. Although the small number of minority participants (N=2) precluded analyses, visual inspection of the data confirmed that they were not outliers.

**Flow-Mediated Dilation**

FMD was significantly associated with each of the CVD risk factors examined. Lower (poorer) FMD was significantly correlated with higher BMI, waist circumference, and fasting glucose and triglyceride levels, and lower HDL levels (as seen in Table II). Glucose was significantly negatively associated with FMD at multiple time-points, with correlations ranging from -0.40 to -0.57. BMI was also significantly negatively associated with FMD at multiple time-points, with correlations ranging from -0.39 to -0.44. FMD was also associated with triglyceride levels at 180 seconds (-0.41), waist circumference at 240 seconds (-0.43), and HDL at 240 seconds (0.39). Smoking was significantly negatively associated with maximum FMD (t = -2.418, p=0.023) and FMD at 180 seconds (t = -2.872, p=0.008). FMD response over time was analyzed for comparison with previously-reported findings (Jarvisalo et al., 2002b). The mean time to peak FMD was 63.1±22.6 seconds. Other clinical and demographic characteristics were not associated with FMD.

**Discussion & Conclusions:**

This is the first study to our knowledge that examines the association between non-invasive vascular imaging and cardiovascular risk factors among adolescents with BD, a group
that is at increased risk for premature CVD. Measures of cIMT and/or FMD were significantly associated with each of the traditional cardiovascular risk factors.

There have been inconsistent findings with systolic blood pressure associations with cIMT in youth (Lande et al., 2006; Sass et al., 1998; Sorof et al., 2003). Lande and colleagues found no significant association between cIMT and systolic or diastolic blood pressure, and only noted significant association with ambulatory blood pressure measurements, in hypertensive and weight matched controls. In a small study of healthy children and adolescents, cIMT was not significantly associated with blood pressure (Sorof et al., 2003). In agreement with previous findings in healthy adolescents, there was no significant association with diastolic blood pressure and cIMT (Jourdan et al., 2005). Our findings are from a relatively similar sized sample and note strong association with systolic blood pressure, which is consistent with previous findings (Jourdan et al., 2005; Sass et al., 1998).

For preliminary illustrative purposes, when comparing the mean cIMT for adolescents with BD, to healthy children and adolescents (as per data aggregated by Urbina et al., 2009), those with BD had significantly thicker common cIMT measurements (t=6.6, p<0.0001) (Urbina et al., 2009). In healthy adolescents (aged 11.5-21 years) cIMT measurement, from the far wall of the common carotid artery had a mean value of 0.413mm (Urbina et al., 2009). The mean cIMT value of our sample of adolescents (aged 13-19) with BD, from the far wall of the carotid artery (averaged left and right side measurements of the common cIMT) was 0.463mm. Future studies incorporating a psychiatrically healthy control population will further evaluate this difference directly.

The 2:1 ratio of female to male participants in the study sample is consistent with clinical and epidemiological (Canadian and U.S.) data from adolescent BD samples (Birmaher et al., 2009; Kozloff et al., 2010; Lewinsohn et al., 1995). There were no significant sex
differences in cIMT measurements among adolescents with BD, inconsistent with previous
findings (Jourdan et al., 2005; Sass et al., 1998; Urbina et al., 2009). Sass and colleagues noted
that sex differences were not significant in healthy children and adolescents until 18 years of
age, noting that beyond 18 years of age, healthy adult men show greater arterial thickening
(Sass et al., 1998). Previous studies have also noted marginally significant or non-significant
associations with cIMT and age (Urbina et al., 2009). Studies of both hypertensive and healthy
children and adolescents showed no significant association of age and cIMT (Lande et al.,
2006; Sass et al., 1998; Sorof et al., 2003). Jourdan and colleagues observed a small but
significant positive association of age and cIMT (r=0.15), in healthy children and adolescents
(Jourdan et al., 2005). Our data show a significant, strong association (r=0.39 to 0.47) between
age, and both mean and maximum cIMT measurements, as seen in adult populations (Stein,
2004; Stein et al., 2004a, 2004b). One can tentatively speculate that this preliminary finding
may suggest premature vascular aging among adolescents with BD. Future studies examining
this hypothesis directly using a control group are warranted.

Lower FMD at different time points was significantly negatively associated with BMI,
triglycerides, fasting glucose, waist circumference and positively associated with HDL
cholesterol levels. This was consistent with adolescents without BD (Jarvisalo et al., 2004;
Woo et al., 2004a, 2004b). Woo and colleagues (2004) reported that obese youth (n=36; 9-12
years old) had lower FMD compared to healthy non-obese controls (n=36) (Woo et al., 2004a).
Importantly, it was also noted that greater BMI was significantly negatively associated with
FMD in all subjects (n=72) (Woo et al., 2004a). This is consistent with our sample of
adolescents with BD, among whom BMI was significantly negatively associated with FMD.

Similarly, LDL cholesterol and higher levels of fasting glucose have been associated
with impaired FMD in adolescents without BD (Jarvisalo et al., 2004). Jarvisalo and colleagues
studied 45 children and adolescents (7-14 years old), with type 1 diabetes mellitus (T1DM), found that peak FMD was decreased in adolescents with T1DM compared to healthy controls (Jarvisalo et al., 2004). Also, among adolescents with T1DM, FMD was negatively associated with LDL levels (Jarvisalo et al., 2004). While we did not find significant association of LDL and FMD, we did find a significant association with HDL levels. We are not aware of previous findings regarding association between HDL and FMD among adolescents, although this association has been shown to be significant in elderly populations (Yeboah et al., 2008).

There was no significant effect of age or gender on mean or maximum FMD, time to peak FMD response or any FMD time point measurements, which is consistent with healthy adolescents (Jarvisalo et al., 2004).

FMD response over time was analyzed to examine for a shift in the FMD response curve, as Jarvisalo and colleagues reported a shift in the time to peak FMD response in healthy children and adolescents with greater waist circumference (Jarvisalo et al., 2002b). Time to peak FMD was not significantly correlated with any traditional CVD risk factors. However, the mean time to peak FMD in the current study (63.1±22.6 seconds) was earlier than that reported in healthy children (mean time to peak FMD was reported as 70 seconds in a sample of 105 healthy children). As with other findings noted above, this difference will be examined in future studies incorporating a comparison group of non-BD adolescents.

Several limitations should be acknowledged when interpreting these findings. First, the study employed a cross-sectional methodology, which cannot inform our understanding of the direction of the observed associations. Longitudinal analyses would also allow for further characterization of FMD response over time, and atherosclerotic progression in adolescents with BD. Second, although similar in size to several previous studies of non-BD adolescents, the sample size is modest. Larger samples are needed to detect associations with smaller effect
sizes. Third, no control group was included. Future studies will include adolescents without mood disorders in order to directly examine for any diagnosis-related differences in the associations between the variables examined in this study. Fourth, this study included a heterogeneous clinical sample, and the observed associations may be undermined by confounding variables (e.g. different medications, symptoms, stressors). However, we specifically set out to examine whether cIMT and FMD are associated with cardiovascular risk factors in a “real-life” clinical sample of adolescents with BD, as this is the group in which we sought to validate these techniques. Future studies will examine for the magnitude of the associations of these variables with FMD and cIMT. A prospective longitudinal study investigating treatment response and non-invasive measurement of cardiovascular risk may also give insight into the potential development and progression of vascular dysfunction in this population. Future studies may also wish to investigate less operator-dependent modalities of vascular measurement, which may decrease inter-operator variability. Despite the presence of these confounding variables, cIMT and FMD still showed multiple significant associations with traditional cardiovascular risk factors.

In conclusion, present findings suggest that non-invasive vascular imaging approaches, specifically cIMT and FMD, may be used as CVD risk proxies among adolescents with BD, as they are for other adolescents. Although the presence of multiple potentially confounding variables may have limited the number and magnitude of present findings, we nonetheless found evidence that the fundamental association between these vascular imaging measures and CVD risk factors remains detectable. Future directions include larger-scale, controlled studies with repeated measures of vascular imaging measures and mood symptom severity, as well as studies focusing on salient biomarkers relevant to both CVD and BD, such as inflammation, oxidative stress, and growth factors (Berk et al., 2011; Goldstein, Fagiolini, et al., 2009a).
Acknowledgements
Funding was provided by the Heart and Stroke Foundation (Ontario).

Ethical Standards
All procedures were approved by the Research Ethics Board (REB), and are in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants (parents and/or guardians and adolescents) prior to study procedures.

Conflicts of Interest
Dr. Goldstein has been a consultant for BMS, and has received honoraria from Purdue Pharma.
### Table 1: Descriptive statistics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%) or Mean +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical and Demographic Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20/30 (66.7%)</td>
</tr>
<tr>
<td>BD-I</td>
<td>12/30 (40%)</td>
</tr>
<tr>
<td>BD-II</td>
<td>12/30 (40%)</td>
</tr>
<tr>
<td>BD-NOS</td>
<td>6/30 (20%)</td>
</tr>
<tr>
<td>Age (yrs.)</td>
<td>17.1 +/- 1.61</td>
</tr>
<tr>
<td><strong>CVD Risk Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>112.63 +/- 9.79</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>68.86 +/- 8.89</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.63 +/- 4.78</td>
</tr>
<tr>
<td>BMI Percentile</td>
<td>71.45 +/- 25.95</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>80.7 +/- 10.12</td>
</tr>
<tr>
<td>Waist circumference percentile</td>
<td>2.64 +/- 1.00</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.24 +/- .94</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.93 +/- .38</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.33 +/- .71</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.48 +/- .48</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.66 +/- 0.59</td>
</tr>
<tr>
<td>Current smoking</td>
<td>4/30 (13.3 %)</td>
</tr>
<tr>
<td><strong>Non-invasive Imaging</strong></td>
<td></td>
</tr>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.44 +/- 0.04</td>
</tr>
<tr>
<td>Maximum cIMT (mm)</td>
<td>0.53 +/- 0.07</td>
</tr>
<tr>
<td>Maximum Brachial artery FMD (%)</td>
<td>5.85 +/- 2.82</td>
</tr>
<tr>
<td>Time to Peak FMD (s)</td>
<td>63.1 +/- 22.6</td>
</tr>
</tbody>
</table>

**Table 1.** List of abbreviations: Bipolar disorder (BD), Not otherwise specified (NOS), Body mass index (BMI), Low density lipoprotein (LDL), High density lipoprotein (HDL), Carotid intima media thickness (cIMT), Flow mediated dilation (FMD). Waist circumference percentile is presented as coded groups (scores presented range from 1-5, representing percentile ranges from 10th – 24th, 25th – 49th, 50th -74th, 75th – 89th, and >90th, respectively).
Table 2: FMD response over time – Associations with MetS components

<table>
<thead>
<tr>
<th>FMD time-points</th>
<th>MetS components</th>
<th>Pearson’s correlation (r)</th>
<th>Statistic (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Fasting Glucose</td>
<td>-0.45</td>
<td>0.017</td>
</tr>
<tr>
<td>150</td>
<td>Fasting Glucose</td>
<td>-0.44</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.39</td>
<td>0.039</td>
</tr>
<tr>
<td>180</td>
<td>Triglycerides</td>
<td>-0.41</td>
<td>0.030</td>
</tr>
<tr>
<td>210</td>
<td>Fasting Glucose</td>
<td>-0.57</td>
<td>0.001</td>
</tr>
<tr>
<td>240</td>
<td>Waist circumference</td>
<td>-0.43</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.44</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>HDL</td>
<td>0.39</td>
<td>0.040</td>
</tr>
<tr>
<td>270</td>
<td>Fasting Glucose</td>
<td>-0.40</td>
<td>0.036</td>
</tr>
<tr>
<td>300</td>
<td>Fasting Glucose</td>
<td>-0.49</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Table 2. Flow mediated dilation (FMD) time-points significantly associated with metabolic syndrome (MetS) components.
Statement of Significance and Impact

CVD is exceedingly prevalent among adults with BD, implicating BD adolescents as a high-risk group for CVD. Non-invasive ultrasound measures of vascular structure (cIMT) and function (FMD) predict future CVD and are associated with traditional CVRFs among adolescents without mood disorders. The presence of multiple potential confounds among adolescents with BD, including various medications and mood states, informs the need to demonstrate whether cIMT and FMD are associated with CVRFs in this population specifically. This study examined, for the first time, the association of cIMT and FMD with CVRFs among adolescents with BD, addressing Aim 1.

cIMT and FMD were chosen to assess vascular structure and function because they have been shown to reliably predict future CVD in healthy and at-risk child and adult populations, and offer a non-invasive method (Urbina et al., 2009). The use of invasive methods in adolescent research, in those who currently do not have clinical CVD, for example, angiography, could offer other insights, however are not feasible due to their poor risk-to-benefit ratio (Hessel, Adams, & Abrams, 1981; Urbina et al., 2009). The traditional CVRFs [e.g. fasting glucose, triglycerides, LDL, HDL, BMI, and blood pressure] were investigated for their widely reported role in CVD development and risk in both pediatric and adult populations (Fiedorowicz et al., 2008; Groner et al., 2006; Lakka et al., 2002; Levitan et al., 2008; Zimmet et al., 2007). The methods chosen are reliable, and feasible to measure in a clinical research setting.

To statistically assess whether the non-invasive atherosclerotic proxies were associated with traditional CVRFs in this heterogeneous adolescent BD sample, we first conducted Pearson’s correlations. This allowed us to assess if each of the factors were individually
correlated with each non-invasive measure, which is a typical report for literature examining these vascular measures (Jarvisalo et al., 2004; Urbina et al., 2009). For example, Jarvisalo and colleagues assessed LDL and FMD in a healthy pediatric population and found that elevated LDL was correlated with poor FMD (Jarvisalo et al., 2002b). To compare between-group effects, independent samples t-tests were used. For instance, to investigate if there was a significant difference in vascular measures and CVRFs between males and females, smokers and non-smokers, and those using lithium vs. no current lithium use.

First we aimed to assess if cIMT was significantly correlated with traditional CVRFs in adolescents with BD. This study demonstrated that despite the heterogeneity of the group (e.g. different affective states and medication use), there are clear strong correlations between cIMT and CVRFs in adolescents with BD. There have been inconsistent findings with systolic blood pressure associations with cIMT in youth (Rybakowski et al., 2006; Sass et al., 1998; Shah et al., 2009). For example, cIMT and systolic or diastolic blood pressure have not been shown to be significantly associated, in hypertensive and weight-matched youth controls. Our findings show a significant association with systolic blood pressure, which is consistent with previous findings (Greenland et al., 2000; Sass et al., 1998). Jourdan and colleagues observed a small but significant positive association of age and cIMT (r=0.15), in healthy children and adolescents (Jourdan et al., 2005). We also found a significant, strong association (r=0.39 to 0.47) between age, and both mean and maximum cIMT measurements, as seen in adult populations (Stein, Fraizer, et al., 2004; Tomfohr et al., 2011; Urbina et al., 2009). Since this has not been reported in populations below the age of 18, one can tentatively speculate that this finding may suggest premature vascular aging among adolescents with BD (Hatch et al., 2014).

We then examined FMD for association with CVRFs. As expected, our results show that poor FMD was significantly negatively associated with greater BMI, triglycerides, fasting
glucose, waist circumference and positively associated with lower HDL (Hatch et al., 2014). This is consistent with adolescents without BD (Woo et al., 2004a, 2004b). For example, greater BMI is significantly negatively associated with FMD in obese and non-obese adolescents (Hatch et al., 2014; Meyer et al., 2006; Woo et al., 2004b). Similarly, higher LDL and fasting glucose are associated with poor FMD in adolescents without BD (Woo et al., 2004a).

The findings of this study suggest that non-invasive vascular imaging approaches, specifically cIMT and FMD, may be used as CVD risk proxies among adolescents with BD, as they are for other adolescents. Despite the presence of multiple potentially confounding variables, we found evidence that the fundamental association between these vascular imaging measures and CVRFs remains detectable. Validating clinically relevant measures that offer feasible (e.g. lower cost and greater access) and low-risk/non-invasive avenues to measure CVD risk is particularly important for the adolescent BD population, as they are a high risk population for premature CVD development and mortality. This also offers insight for future studies, and indicates that these methods may be used to assess vascular risk in this population.
CHAPTER TWO

Title: Cardiovascular and Psychiatric Characteristics associated with Oxidative Stress Markers among Adolescents with Bipolar Disorder

Authors: Jessica Hatch, Ana Andreazza, Omodele Olowoyeye, Gislane Tezza Rezin, Alan Moody, Benjamin I. Goldstein

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Work performed by the student: All methods described in the manuscript were completed by the student, excluding the ultrasound procedures (completed by Omodele Olowoyeye). Assessment of oxidative stress markers was jointly run with Gislane Tezza Reszin.
Cardiovascular and Psychiatric Characteristics associated with Oxidative Stress Markers among Adolescents with Bipolar Disorder

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ABSTRACT

Introduction: Two knowledge gaps in the field of bipolar disorder (BD) research relate to the absence of clinically validated biomarkers and the limited understanding of the biology underlying the excessive and premature burden of cardiovascular disease (CVD). Oxidative stress has been implicated as a potential biomarker in both BD and CVD. Objective: To examine psychiatric and cardiovascular characteristics associated with peripheral oxidative stress markers among adolescents with BD, who are at high risk for CVD. Methods: Participants were 30 adolescents, 13-19 years old, with BD and without CVD. Ultrasonography was used to evaluate vascular function and structure. Traditional CVD risk factors were also measured. Psychiatric assessments were conducted via semi-structured interview. Serum levels of oxidative stress (lipid hydroperoxides (LPH) and protein carbonylation (PC)) were assayed. Results: Compared to published data on adults with BD, adolescents had significantly lower levels of LPH and PC ($t_{52}(11.34), p<0.0001; t_{58}(29.68), p<0.0001$, respectively). Thicker mean and maximum carotid intima media thickness was associated with greater levels of LPH ($r=.455, p=.015; r=.620, p<0.0001$, respectively). LPH was associated with diastolic blood pressure ($r=-.488, p=0.008$) and pulse pressure ($r=.543, p=0.003$). Mood symptoms and medication were not significantly associated with oxidative stress. Conclusion: Adolescents with BD have lower levels of oxidative stress compared to adults with BD, supporting prevailing illness staging theories for BD. Oxidative stress is robustly associated with a proxy measure of atherosclerosis and may explain in part the increased risk of CVD in BD. Replication of these findings in larger samples including healthy controls is warranted.

Keywords: adolescent, atherosclerosis, bipolar disorder, cardiovascular, oxidative stress
Introduction

Bipolar disorder (BD), prevalent in 2-5% of the general population, is characterized by states of depression and mania and/or hypomania (states of extreme elation and/or irritability, among other symptoms) (Kessler et al., 2009). To date, there are no definitive biomarkers despite a pressing need for objective markers that can be employed in assessment, prognosis, treatment selection, and determination of treatment response (Frey et al., 2013).

Oxidative stress has been implicated as a leading putative biomarker in BD (Andreazza et al., 2007; Berk et al., 2011; Frey et al., 2013; Kapczinski et al., 2008). Oxidative stress comprises a disruption in oxidant and anti-oxidant balance, in which increased oxidant levels may lead to physiological damage (Andreazza et al., 2009). Reactive oxygen species (ROS) generation is ongoing in normal physiological conditions; however the imbalance in the redox regulation can lead to irreversible cellular damage (Dalle-Donne et al., 2006).

It has been demonstrated that patients with BD have significantly different levels of antioxidant enzymes, lipid peroxidation and nitric oxide (NO) levels (Gergerlioglu et al., 2007), compared to patients without BD (Andreazza et al., 2009; Andreazza et al., 2008; Brown et al., 2014). Aberrant oxidative stress levels may therefore contribute to the pathophysiology of BD (Andreazza et al., 2008). Notably, oxidative stress levels may comprise state markers of disease activity among adults with BD (Andreazza et al., 2008; Siwek et al., 2013). Recent meta-analyses on oxidative stress in adults with BD have highlighted the alterations in oxidative stress and antioxidant enzymes in BD, compared to psychiatrically healthy adults (Andreazza et al., 2008; Brown et al., 2014). Brown and colleagues reported a meta-analysis on 971 participants with BD and 886 healthy controls, concluding that there is a clear role of oxidative stress in BD pathology, with a particularly robust association with elevated levels of lipid peroxidation (Brown et al., 2014).
Previous literature has also noted that BD can be described in terms of stages of disease course based not only on psychiatric symptomology, but also via white matter integrity, inflammation and oxidative stress (Andreazza et al., 2009; Berk et al., 2011; Goldstein & Young, 2013; Kauer-Sant’Anna et al., 2009). It has been highlighted that in the early stages of BD major antioxidant systems are not elevated compared to control patients; however, in the later stages in the course of BD, there is a significant increase in antioxidant levels (Andreazza et al., 2009). It may therefore be the case that in the earlier stages in the course of BD, there has not yet been a mechanism to compensate for the higher levels of oxidative stress, and that this compensatory action may take place too late in the course of illness (Andreazza et al., 2009). Oxidative stress has therefore been postulated to be a marker of neuroprogression for those with BD, which would provide much needed information for staging purposes, and perhaps better prevention strategies and personalized care (Berk et al., 2011).

BD pathology comprises not only aberrant mood patterns, but has been associated with increased risk of cardiovascular disease (CVD) (Osby et al., 2001). CVD is excessive and premature in adults with BD suggesting that adolescents with BD are at a high risk for future CVD (Goldstein et al., 2009a). Likewise, oxidative stress has also been shown to be involved in the aging of vasculature and the development and progression of endothelial dysfunction, atherosclerosis, and CVD (Fyhrquist et al., 2013; Hopps et al., 2010; Madamanchi et al., 2005; Schnabel and Blankenberg, 2007). In CVD, oxidative stress is potentially increased in multiple pathways, including but not limited to: shear stress on vasculature, NO impairment, apoptosis and cellular senescence (Fyhrquist et al., 2013). Oxidative stress contributes to endothelial dysfunction, whereby vascular endothelial damage can result from interactions between ROS and vascular endothelial produced NO (Cai & Harrison, 2000).
Non-invasive imaging measures of vascular structure and function have been previously validated among non-BD adolescents, as demonstrated by the association of these measures with standard cardiovascular risk factors (Chan et al., 2003; Hatch et al., 2014; Hodis et al., 1998; Kelly & Steinberger, 2008; Urbina et al., 2009). We recently demonstrated that, despite multiple potential confounds such as variation in medications and other clinical characteristics, non-invasive vascular imaging measures are associated with traditional cardiovascular risk factors among adolescents with BD as well (Hatch et al., 2014).

While oxidative stress has been established as a leading candidate biomarker in BD pathology among adults, there is a paucity of data on this topic among adolescents with BD. A recent magnetic resonance spectroscopy study found that adolescents and young adults with BD do not differ significantly from controls with regard to brain glutathione (Godlewska et al., 2014; Lagopoulos et al., 2013). However, no prior study has examined serum oxidative stress markers in this population. Similarly, no previous study of any age group has examined whether oxidative stress is associated with vascular function and structure in BD. We therefore set out to conduct a preliminary study examining the psychiatric and cardiovascular characteristics that may be associated with peripheral oxidative stress among adolescents with BD.

**Methods**

All procedures were approved by the Research Ethics Board (REB), and are in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all participants (parents and/or guardians and adolescents) prior to study procedures.
\textbf{Sample}

English-speaking adolescents (N=30, 13-19 years old) were recruited from the Centre for Youth Bipolar Disorder, Sunnybrook Health Sciences Centre (Toronto, Ontario). Participants were excluded if they had an infectious illness within the past 14 days, or were unable to provide informed consent (i.e. due to developmental delay or psychosis). Participants were free from any cardiac condition, autoimmune, infectious, or inflammatory illness, and were not taking medication for any of the noted conditions.

\textbf{Assessment}

\textit{Interviews}

The Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime version (K-SADS-PL), a semi-structured diagnostic interview, was utilized for diagnostic purposes (Kaufman et al., 1997). DSM-IV criteria were confirmed for bipolar subtype (BD type I (BD-I) and BD type II (BD-II), and BD not otherwise specified (BD-NOS) was defined using operationalized criteria from the Course and Outcome of Bipolar Youth (COBY) study (Birmaher et al., 2006). Participants were interviewed directly, and parents/guardians were separately interviewed about the participant. A child-adolescent psychiatrist confirmed diagnoses.

\textit{Ultrasound and Physical assessments}

Participants completed study procedures starting with diagnostic interviews on visit one. Visit two began with ultrasound, followed by blood-draw and symptom assessments, with appointments starting between eight and ten a.m. Participants fasted ten hours prior to appointment start time (no food or drink, except for water). All participants were also instructed not to use illicit drugs, smoke tobacco or consume alcohol for 24 hours prior to the appointment. Adherence to fasting and abstinence from drugs was assessed via interview.
A sonographer carried out ultrasound (Phillips, iU22) procedures to measure flow-mediated dilation (FMD) and carotid intima media thickness (cIMT). FMD assesses macrovascular function, while cIMT assesses macrovascular structure. Imaging procedures utilized two-dimensional Doppler imaging, with a high-frequency (10 MHz) linear-array transducer, with duplicate scans performed for reliability. Blood pressure and electrodes were placed for a three-lead electrocardiogram (ECG), prior to FMD and cIMT measurement. Procedures began with participants reclining and rested for at least ten minutes. cIMT was measured with the subject recumbent, neck extended and head at a 45-degree angle, contralateral to the side being examined. The common cIMT, bulb IMT, and internal common cIMT (regions as described in Urbina et al., 2009) (Urbina et al., 2009) for right and left sides, were investigated (Agrotou et al., 2013; Urbina et al., 2009). Blood pressure was measured using a stethoscope, sphygmomanometer and adjustable cuff, as per standard procedures. Pulse pressure (PP) was calculated as the difference between systolic blood pressure and diastolic blood pressure.

Participants remained recumbent with their right arm adjacent to the sonographer for FMD assessment. A stand was used to hold the transducer steady during FMD measurement, which was taken concurrently with an ECG recording. Lower-arm placement of the blood pressure cuff was used, with the cuff inflated to 50 mmHg above systolic for five minutes. Following cuff deflation, FMD was recorded for five minutes, and analyzed at 30-second intervals. As in previous studies, FMD was calculated as a percentage-change in brachial artery diameter compared with baseline measurements. A second blood-pressure reading was taken on the contralateral arm following ultrasound procedures.

Blood-draw was carried out between nine and eleven a.m. (exact time was recorded). Fasting glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-
density lipoprotein (LDL) were assessed. Anthropomorphic measurements were assessed next. Subject weight and height were recorded (to the nearest 0.5 cm and 0.1 kg, respectively), with duplicate measures taken for reliability purposes. To account for clothing, adjusted body mass index (BMI) was calculated (subtracting from measured weight: 1.4 kg for long pants and long-sleeves/sweatshirt, 1.1 kg for short pants or short-sleeves, and 0.9 kg for short pants and short sleeves) and waist circumference was measured using a flexible tape measure (to the nearest 0.5 cm).

Assays

Commercial enzyme linked immunosorbant assay (ELISA) kits, were used to quantitatively assess lipid hydroperoxides (LPH) (Cayman Chemical, #705003) and protein carbonylation (PC) (Cell BioLabs). LPH was assessed following standard procedures and company kit directions, as noted in previous literature (Versace et al., 2013). LPH was extracted from serum samples via addition of cold chloroform (1mL) and ‘LPH Assay Extract R’ (500µL), per serum sample (500µL). Sample tubes were then centrifuged (1500 x g, at 0 degrees Celsius) for five minutes, to isolate the chloroform-LPH extract layer. Following extraction, 450µL of (2:1) chloroform-methanol mixture and 50µL of chromogen mixture were added to tubes, per 500µL of chloroform extract. Following an incubation (five minutes) at room temperature, samples were loaded into 96-well glass plates and absorbance read at 500nm. To determine the amount of lipid peroxidation in samples, the observed absorbance of the samples were compared with a standard hydroperoxide curve.

A standard Bradford assay was conducted to assess the protein content of the samples compared to bovine serum albumin (BSA) standard, via colometric analysis. Samples were diluted to 10µg/mL and incubated in a 96-well protein binding plate at 4°C overnight to detect
PC levels – according to manufacture procedures (Millipore, #S7250). Absorbance was measured at 450nm, with PC content recorded in nmol/mg (Andreazza et al., 2009).

**Statistical Analyses**

Descriptive statistics were calculated for all relevant variables. Shapiro-Wilk test was used to test for normality in our sample data. Independent samples t-tests and bivariate correlation analyses (Pearson’s correlation coefficient) were completed as appropriate. LPH and PC levels of our adolescent BD sample were compared to recent findings in adults with BD, derived from the lab of co-author Dr. Andreazza using the same techniques and protocol (Andreazza et al., 2009; Versace et al., 2013). Means, standard deviations and sample sizes were used to examine between age-group differences via standard t-tests, based on data from published manuscripts (Andreazza et al., 2009; Versace et al., 2013). Within the adolescent BD sample, a linear regression was used to examine the association between peripheral oxidative stress markers (LPH and PC) and medication class, controlling for age and sex. We used a false discovery rate (FDR) to correct for multiple comparisons, with maximum α set to 0.05 and with all tests pooled for the most conservative calculation (Strimmer, 2008). FDR adjusted p-values are reported in results. Statistical analyses were performed using SPSS 21 for Windows (SPSS Inc., Chicago IL, USA).

**Results**

Descriptive statistics are presented in Table I. Compared to previously published data on adults with BD, adolescents had significantly lower levels of LPH and PC, as seen in Table II. Mood symptom scores, duration of illness, and age of onset were not significantly associated with LPH or PC levels, as seen in Table III. Assessment of current PSR ratings for depression in this sample is indicative of minimal-mild sub-threshold symptoms (mean= 2.1;
SD =1.34). However scores ranged from asymptomatic (PSR score =1; 50% of the sample) to meeting full diagnostic criteria for depression (PSR score = 5; 10% of the sample), in agreement with our KSADS depression section ratings reported (Table 1). No participants met criteria for mania, and current hypomania PSR scores indicated minimal-mild sub-threshold symptoms (mean=1.5; SD=1.08) ranging from 1 (asymptomatic; 76.7 % of the sample) to 5 (full hypomanic criteria met; 3.3% of the sample), in agreement with our KSADS mania rating scale scores (Table I). There was, however, a significant association of LPH and greater global functioning (Table III). Levels of oxidative stress markers did not differ for adolescents on different medication classes, as seen in Table IV (data was statistically assessed as noted in adult comparison literature in Andreazza et al., 2009) (Andreazza et al., 2009). Although not statistically significant, there was a similar pattern for both LPH and PC, whereby levels were highest in BD-I, followed by BD-II, and lowest in BD-NOS. Effect sizes for differences between group subtypes and LPH levels varied from a moderate effect size of .45 for BD-I vs. BD-II and .44 for BD-II vs. BD-NOS, with the larger effect size of .68 for BD-I vs. BD-NOS. The same trend was observed for PC levels. Effect sizes for differences between group subtypes and PC levels varied from a moderate effect size of .42 for BD-I vs. BD-II and .44 for BD-II vs. BD-NOS, with a large effect size of .92 for BD-I vs. BD-NOS. These findings, although preliminary, provide heuristics that can inform future research on the topic of differences in oxidative stress across BD subtypes among adolescents.

Thicker mean and maximum cIMT measurements were associated with greater levels of LPH (r=0.455, p=0.015; r=0.620, p<0.0001, respectively). PC levels were not significantly associated with cIMT (mean cIMT, r=−0.343, p=0.74; maximum cIMT, r=0.236, p=0.227), and both LPH and PC levels were not significantly associated with FMD measurements (r=−0.080, p=0.687; r=0.292, p=0.132, respectively).
HDL ($r=-0.453$, $p=0.015$) was significantly associated with PC levels, however, PC was not significantly associated with LDL ($r=-0.174$, $p=0.385$). LPH levels were significantly associated with diastolic blood pressure ($r=-0.488$, $p=0.008$) and PP ($r=0.543$, $p=0.003$), but not systolic blood pressure ($r=0.203$, $p=0.292$), HDL or LDL ($r=0.043$, $p=0.829$; $r=0.068$, $p=0.738$, respectively). PC was not significantly associated with systolic blood pressure, diastolic blood pressure or PP ($r=0.161$, $p=0.403$; $r=-0.081$, $p=0.683$; $r=0.293$, $p=0.222$, respectively). PP was not significantly different in this adolescent BD population compared to PP reported for healthy young adults ($t_{2174} = 1.38$, $p=0.166$), and children ($t_{515}=1.55$, $p=0.121$) (153, 154). PC and LPH levels were not significantly associated with fasting glucose levels ($r=-0.197$, $p=0.305$; $r=-0.161$, $p=0.412$, respectively), triglycerides ($r=-0.239$, $p=0.211$; $r=-0.157$, $p=0.415$, respectively), BMI ($r=0.132$, $p=0.502$; $r=0.163$, $p=0.407$, respectively), or waist circumference measurements ($r=0.217$, $p=0.258$; $r=0.311$, $p=0.100$, respectively). The associations of LPH levels and maximum cIMT, as well as LPH and PP were significant despite FDR correction (respectively; $p=0.001$, and $p=0.004$).

**Discussion**

This is the first study to our knowledge that examines peripheral levels of oxidative stress markers among adolescents with BD, and the first study in any age group of BD that examines the association of oxidative stress markers with non-invasive vascular imaging or traditional cardiovascular risk factors. The primary findings of this study are that oxidative stress markers are significantly lower than those reported among adults, and that lipid oxidation is significantly positively associated with evidence of early atherosclerosis. Associations between oxidative stress markers and standard cardiovascular risk factors (i.e. blood pressure) were mixed. In contrast to prior studies of oxidative stress among adults with BD, and in contrast to our prior study of inflammatory markers in a similar-sized sample of
adolescents with BD, there were no significant associations between oxidative stress markers and mood symptom severity. There was a non-significant pattern across BD subtypes, with levels of LPH and PC highest in BD-I, followed by BD-II, and lowest in BD-NOS.

These findings are constrained by several limitations. This study was limited in sample size, and did not include healthy controls. In addition, the cross-sectional design of the study prevents us from examining directional hypotheses. Future studies investigating the course and staging of BD and CVD are underway and will examine these areas in further detail. However, despite these limitations, this is the first study to date regarding peripheral measures of oxidative stress among adolescents with BD, and it provides important preliminary findings and heuristics to guide further investigation.

In our sample, adolescents had significantly lower levels of oxidative stress compared to those previously reported among adults, which may signify an earlier stage in illness course. The prevailing illness staging theory for BD encompasses the effects of both neuroprogression and allostatic load (Berk et al., 2011). Allostatic load is the physiological change of a system in response to the cumulative exposure of a combination of stressful life events, genetic determinants, and environmental factors (Berk et al., 2011; Kapczinski et al., 2008). Thus a later stage of BD represents years of neuroprogression and exposure to a cumulative effect of allostatic load, which may subserve the association of oxidative stress, mood severity and functioning (Berk et al., 2011). There are likely multiple reasons for the lower levels of oxidative stress in our sample versus prior studies of adults (Andreaazza et al., 2009; Versace et al., 2013). For instance, oxidative stress levels increase with age, and are associated with the normal aging process factors, such as telomere shortening (Stadtman, 2004). While age may play a factor, adults with BD show significantly elevated levels of oxidative stress compared to age-matched healthy controls, and it is expected that disease state and trait play a significant
role in the observed peripheral oxidative stress marker levels (Andreazza et al., 2009; Versace et al., 2013). Furthermore, while these findings may represent an earlier stage of BD, in which the oxidant-antioxidant system has not yet been overwhelmed and oxidative stress factor levels are still considerably low (Andreazza et al., 2009; Andreazza et al., 2008), it is worth underscoring that the mean duration of BD in our sample was longer than in previous early-stage adult BD findings. This suggests that both age and illness duration contribute to oxidative stress disturbance.

Additionally, oxidative stress and cardiovascular risk factors were measured to assess whether oxidative stress at this early stage was detectable and associated with a validated proxy measure of atherosclerosis and CVD risk. Our results indicate that greater cIMT measurements were associated with greater oxidative stress, as measured via serum LPH levels. Similarly, a recent study investigating atherosclerosis and oxidative stress in chronic haemodialysis patients noted that subjects with higher TBARS levels, a measure of oxidative stress, and lower levels of antioxidants (e.g. glutathione peroxidase, superoxide dismutase and vitamin E), had significantly increased cIMT (Dursun et al., 2008).

Vascular function has also been shown to be associated with oxidative stress. Schabel and colleagues noted that elevated asymmetric dimethylarginine, a peripheral marker of oxidative stress, was associated with impaired FMD (Schnabel and Blankenberg, 2007). Similarly, an article highlighting the pediatric precursors of adult CVD noted that oxidative stress can lead to endothelial damage and impaired vascular reactivity (Groner et al., 2006). It was therefore of interest as to whether these oxidative stress factors would be associated with impaired vascular function in an adolescent BD population, especially considering the high prevalence of CVD in patients with BD. In our sample we did not observe a significant association of LPH or PC oxidative stress factors with FMD. Although this may be due to the
lower levels of oxidative stress markers compared to adults or those at a later stage of illness, to date there have not been studies examining the link between oxidative stress and vascular structure or function among adults with BD.

This study yielded significant findings regarding the link between oxidative stress and CVD risk factors; greater peripheral levels of oxidative stress (i.e. PC) was significantly associated with lower levels of HDL. Traditional CVD risk factors, including HDL, BMI, LDL, glucose, as well as high diastolic and systolic blood pressure, have all been shown to be associated with oxidative stress (Keaney et al., 2003; Madamanchi et al., 2005).

The current study found that greater levels of LPH are associated with pulse pressure, a proxy measure of vascular impairment. Mean arterial pressure and pulse pressure may be more closely related to vascular stiffness and distensibility than diastolic or systolic blood pressure (Sesso et al., 2000). Recent studies on healthy subjects have reported pulse pressure means (36.3-43.6; SD=12) (Hlaing et al., 2004) for children (n=487), ages 8-16, and pulse pressure means (44-48; SD=10) for young adults (ages 24-29; n=2146) (Raitakari et al., 2009). Raitakari and colleagues noted that pulse pressure measured in adolescence was significantly related to thicker cIMT in adulthood (Raitakari et al., 2009). Thus, the association of greater peripheral oxidative stress and pulse pressure in this sample may be indicative of future vascular impairment in adulthood. Present findings therefore provide preliminary support for the hypothesis that oxidative stress may contribute in part to the excessive and premature burden of CVD in BD (Goldstein et al., 2009a; Osby et al., 2001).

Interestingly, we did not see any significant associations between oxidative stress and metabolic syndrome components (i.e. waist circumference, LDL, HDL, and fasting glucose levels) or BMI. Obesity is a risk factor for vascular impairment and cardiovascular disease, and is associated with depression and BD (Goldstein et al., 2011). It is also important to note that
pharmacological treatment may modify the association between obesity and oxidative stress. For instance, in a recent study of adolescents with BD, obesity was associated with greater levels of inflammation and with mood-stabilizing medication; however there was an inverse association between inflammatory levels and mood-stabilizing medication (Goldstein et al., 2011). It is therefore possible that medication is modifying the association between oxidative stress levels and metabolic syndrome components.

Sex has also been implicated as a contributor to variation in oxidative stress levels (Bengesser et al., 2014; Reininghaus et al., 2015). While there were no sex differences in LPH, there were higher levels of PC in males compared to females (as seen in Table III). It is possible that estrogen levels may have played a role in this difference. Estrogen has been implicated as a protective factor against vascular impairment as well as potentially oxidative stress (Frey & Dias, 2014; Karim, Hodis, Stanczyk, Lobo, & Mack, 2008). Further research regarding the role of estrogen and its potential association with oxidative stress and vascular impairment in this population is needed.

Lastly, mood symptom severity, duration of illness, medication, global functioning and age of onset of BD and other clinical psychiatric factors were also examined. In prior studies of adults with BD, oxidative stress has been associated with symptom severity and functional impairment (Andreazza et al., 2007, 2009; Andreazza et al., 2008; Dalle-Donne et al., 2006; Gergerlioglu et al., 2007) We therefore sought to examine clinical psychiatric factors (duration of illness, depression, mania and functioning scores, as well as BD subtype and medication), in addition to comparisons with previous literature on adults with BD. Unlike previous adult findings, LPH and PC were not significantly associated with psychiatric clinical characteristics (Andreazza et al., 2009; Versace et al., 2013). Counter-intuitively, better global functioning was significantly associated with higher levels of LPH (as seen in Table III). Similarly, in
contrast to previous findings, we found no effect of medication, which may be due to the small sample size and heterogeneous medication regimens. PSR scores denoting symptomatic patients (depression or hypomania) were observed in this sample; however, due to the small sample of symptomatic patients compared to non-symptomatic patients, we were unable to further assess these group differences in relation to vascular or oxidative stress factors.

Moreover, this level of symptomatic severity is similar to that observed in the prior studies of adults with BD to which we have compared the current sample of adolescents (Andreazza et al., 2009; Versace et al., 2013). Additional studies will be warranted in order to determine whether these findings are spurious, owing to small sample size, or related to developmental differences in the neurobiology of BD.

In summary, oxidative stress factors in adolescents with BD are substantially lower than those reported among adults with BD and are associated with traditional and novel measures of CVD risk. This study implicates oxidative stress as a potential factor underlying premature atherosclerotic progression in this population. If future studies confirm this finding, particularly using prospective methodology, it would suggest the value of studying antioxidants as preventive interventions to attempt to mitigate the excessive cardiovascular risk among adolescents with BD. The fact that oxidative stress was associated with CVD risk but not with mood symptoms suggests the possibility that the vascular impairment and CVD risk implications occur at lower levels of oxidative stress and/or precede the association between oxidative stress and mood. Larger studies on this topic will be needed to determine whether the pattern of decreasing oxidative stress from BD-I to BD-II and BD-NOS is meaningful and/or representative of a staging phenomenon. Future studies examining other clinical psychiatric factors, such as cognition are warranted. Similarly, examination of genetics may provide more information regarding subgroups among whom the association between oxidative stress and
mood is enhanced. Finally, longitudinal studies examining oxidative stress, psychiatric symptoms, and structural and functional vascular changes in adolescents with BD may bolster our understanding regarding staging, neuroprogression, and somatoprogession (i.e. accumulation of medical burden) in adolescent BD.

Table I. Demographic and clinical characteristics of adolescents and adults with bipolar disorder

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adolescents (n=30)</th>
<th>Early Stage Adults (n=30)†</th>
<th>Late Stage Adults; (n=30)†</th>
<th>Adults (n=24)††</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex (%)</td>
<td>33.3</td>
<td>43.4 (13.0)</td>
<td>30.0 (9.0)</td>
<td>33.3</td>
</tr>
<tr>
<td>Age, yr</td>
<td>17.1(1.6)</td>
<td>22.4 (3.9)</td>
<td>41.1 (8.4)</td>
<td>33.2 (7.7)</td>
</tr>
<tr>
<td>Age at onset of illness, yr</td>
<td>13.1(3.6)</td>
<td>20.2 (4.2)</td>
<td>27.2 (7.4)</td>
<td>19.4 (6.2)</td>
</tr>
<tr>
<td>Duration of illness, yr</td>
<td>3.9(3.2)</td>
<td>2.1 (2.9)</td>
<td>13.9 (5.1)</td>
<td>13.8 (7.4)</td>
</tr>
<tr>
<td>Mania score *</td>
<td>8.6(10.8)</td>
<td>1.5 (2.8)</td>
<td>3.6 (4.1)</td>
<td>2.3 (2.3)</td>
</tr>
<tr>
<td>Depression score**</td>
<td>12.6(11.9)</td>
<td>3.8 (7.1)</td>
<td>9.2 (6.0)</td>
<td>8.2 (5.9)</td>
</tr>
<tr>
<td>Functioning score ***</td>
<td>65.5(14.2)</td>
<td>63.3 (12.5)</td>
<td>61.4 (17.5)</td>
<td>---</td>
</tr>
<tr>
<td>Medication (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood Stabilizer</td>
<td>32</td>
<td>83.3</td>
<td>86.7</td>
<td>75</td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>70</td>
<td>76.7</td>
<td>60.0</td>
<td>58.3</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>20</td>
<td>10</td>
<td>20.0</td>
<td>50</td>
</tr>
<tr>
<td>BD Subtype (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD I</td>
<td>40</td>
<td></td>
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<tr>
<td>BD II</td>
<td>40</td>
<td></td>
<td></td>
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<tr>
<td>BD-NOS</td>
<td>20</td>
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</tbody>
</table>

* Young Mania Rating Scale for adults, KSADS MRS for adolescents
** Hamilton Rating Scale for Depression for adults, KSADS DEP-P for adolescents
*** Global Assessment of Functioning for adults, CGAS for adolescents
† Andreazza et al., 2009
†† Versace et al., 2014
Table II. Lipid hydroperoxides and protein carbonylation levels among adolescents and adults with bipolar disorder

<table>
<thead>
<tr>
<th>Oxidative Stress marker (nmol/mg)</th>
<th>Adolescents</th>
<th></th>
<th></th>
<th>Adults</th>
<th></th>
<th></th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Lipid hydroperoxides</td>
<td>30</td>
<td>4.69</td>
<td>2.85</td>
<td>24</td>
<td>17.3</td>
<td>5.2</td>
<td>$t_{25}(11.34), p&lt;0.0001^{††}$</td>
</tr>
<tr>
<td>Protein carbonyl content</td>
<td>30</td>
<td>2.22</td>
<td>0.91</td>
<td>30</td>
<td>105.5</td>
<td>19.0</td>
<td>$t_{58}(29.68), p&lt;0.0001^{†}$</td>
</tr>
</tbody>
</table>

$^{††}$ Versace et al., 2014

$^{†}$ Andreazza et al., 2009
Table III. Clinical variables and oxidative stress markers among adolescents with bipolar disorder

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD) unless otherwise indicated</th>
<th>Oxidative Stress Marker ; Pearson’s correlational coefficient (r) or Independent samples T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>LPH</strong></td>
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<tr>
<td>Male sex (%)</td>
<td>33.3</td>
<td>Male:5.9 (3.9)</td>
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<tr>
<td>Age, yr</td>
<td>17.1(1.6)</td>
<td>.280</td>
</tr>
<tr>
<td>Age at onset of illness, yr</td>
<td>13.1(3.6)</td>
<td>.274</td>
</tr>
<tr>
<td>Duration of illness, yr</td>
<td>3.9(3.2)</td>
<td>-.248</td>
</tr>
<tr>
<td>Mania score</td>
<td>8.6(10.8)</td>
<td>-.102</td>
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<tr>
<td>Current Depression score</td>
<td>12.6 (11.9)</td>
<td>-.33</td>
</tr>
<tr>
<td>Current Functioning score</td>
<td>65.5 (14.2)</td>
<td>.410</td>
</tr>
<tr>
<td>First or Second degree family history of BD (%)</td>
<td>50</td>
<td>No:4.2 (2.2)</td>
</tr>
<tr>
<td>BD Subtype (%)</td>
<td></td>
<td>BD-I</td>
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<td></td>
<td></td>
<td>BD-II</td>
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<td></td>
<td></td>
<td>BD-NOS</td>
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</tbody>
</table>

† Equal variances not assumed – Levene’s test for equality of variances significant

* P<0.05
Table IV. Medication Class and oxidative stress markers among adolescents with bipolar disorder

<table>
<thead>
<tr>
<th>Medication</th>
<th>Oxidative Stress Biomarkers (n=30)</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Carbonyl content; nmol/mg</td>
<td>Mean</td>
<td>(SD)</td>
<td>β</td>
<td>t</td>
<td>p value</td>
<td>Lipid hydroperoxidases content; nmol/mg</td>
<td>Mean</td>
<td>(SD)</td>
<td>β</td>
<td>t</td>
<td>p value</td>
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<tr>
<td><strong>Antipsychotic</strong></td>
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<tr>
<td>Yes</td>
<td>2.2</td>
<td>.87</td>
<td>.053</td>
<td>.152</td>
<td>.880</td>
<td>4.19</td>
<td>.929</td>
<td>-.012</td>
<td>-.056</td>
<td>.956</td>
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<tr>
<td>No</td>
<td>2.3</td>
<td>1.1</td>
<td></td>
<td></td>
<td></td>
<td>4.22</td>
<td>.846</td>
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<td><strong>Antidepressant</strong></td>
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**Statement of Significance and Impact**

Two knowledge gaps in the field of BD research relate to the absence of clinically validated biomarkers and the limited understanding of the biology underlying the excessive and premature burden of CVD. OS has been implicated as a potential biomarker in both BD and CVD. In this study we aimed to assess whether there is an association between peripheral OS and non-invasive atherosclerosis proxies (cIMT and FMD) (Aim 2), as well as clinical characteristics (Aim 3), in adolescents with BD.

LPH and PC were chosen as the main OS factors to assess due to consistent reports in the literature of their association with BD symptomology and progression in adults. For instance, a meta-analysis on adult participants with BD and healthy controls, concluded that OS is fundamentally apart of BD pathology, with a particularly robust association with elevated levels of lipid peroxidation (Brown, Andreazza, & Young, 2014). The non-invasive atherosclerotic proxies cIMT and FMD were chosen due to our experience with these methods, reliable findings, as well as due to being well reported and validated standard measures of CVD risk (Hatch et al., 2014; Urbina et al., 2009).

Independent samples t-tests and bivariate correlation analyses (Pearson’s correlation coefficient) were completed as appropriate. For example, LPH and PC levels of our adolescent BD sample were compared via independent samples t-test to recent findings in adults with BD, to assess between group differences in OS levels. Means, standard deviations and sample sizes were used to examine between age-group differences via standard t-tests, based on data from published manuscripts (Andreazza et al., 2009; Versace et al., 2013). Within the adolescent BD sample, a linear regression was used to examine the association between peripheral OS (LPH and PC) and medication class, controlling for age and sex. FDR was used to correct for multiple comparisons, with maximum α set to 0.05 and with all tests
pooled for the most conservative calculation (Benjamini, 2010; Benjamini and Hochberg, 1995).

First, we wanted to examine if there were detectable levels of OS in adolescents with BD, and whether OS markers were associated with CVRFs in this population. This study demonstrated that there are detectable levels of LPH and PC in adolescents with BD, and that these levels are lower than what is observed in adults with BD, when compared to previous literature. Previous studies have shown that adults with BD have elevated levels of OS, particularly lipid peroxidation. Here we show that while adolescents with BD have detectable levels of OS, it is significantly lower than what is observed in the adult population. This is in agreement with the theory of neuroprogression and allostatic load, which is to say that with each stressor, year of illness, and symptomatic episode there is a ‘loading’ which leads to the observed elevation in OS (among other measures), in adults compared to adolescents with BD. Importantly, we found that elevated OS was significantly associated with CVRFs, as well as non-invasive measures of vascular structure (cIMT), even after controlling for age and sex. The findings of this study demonstrate that OS is associated with CVD risk in adolescents with BD, suggesting that OS may potentially underlie the BD-CVD link. Additionally, since OS was detectable in adolescents with BD, it also suggests that this may be used as a potential marker as early as adolescence.

Furthermore, OS has emerged as a potential clinically useful biomarker that can inform treatment response, monitor disease progression and assess risk (Goldstein and Young, 2013). In BD, it is important to understand potential biomarkers relevant to psychiatric characteristics as well as CVD development and risk, such as OS (Goldstein and Young, 2013). There are no current clinical biomarkers for BD, and therefore subjective clinical measures are the method of assessment (Goldstein and Young, 2013). This study supports OS as a biomarker for BD,
that is relevant to the BD-CVD link. Future research examining OS in adolescent BD are warranted. For instance, longitudinal studies examining the fluctuations in OS over-time, symptomatic polarity, and in high-risk populations would be useful in determining the role of OS in disease progression and diagnosis. Therefore, biomarkers and noninvasive vascular measures provide a means of assessing disease progression and CVD risk. Investigating biomarkers relevant to both CVD and BD can provide clinically relevant insights, that have the potential to improve treatment monitoring, inform novel therapeutics and improve patient quality of life.
CHAPTER THREE

Title: Inflammatory Markers and Brain-Derived Neurotrophic Factor as Potential Bridges Linking Bipolar Disorder and Cardiovascular Risk among Adolescents

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Work performed by the student: Gustavo Scola completed biomarker assays, and Omodele Olowoyeye completed ultrasound procedures. All other methods, analyses and manuscript write up were completed by the student.
Inflammatory Markers and Brain-Derived Neurotrophic Factor as Potential Bridges Linking Bipolar Disorder and Cardiovascular Risk among Adolescents

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ABSTRACT

Objective: Bipolar disorder (BD) is associated with increased rates of cardiovascular disease (CVD). Brain-derived neurotrophic factor (BDNF) and inflammatory markers are leading biomarkers in BD. We examined whether these biomarkers underlie the link between BD and CVD proxies among adolescents with bipolar spectrum disorders. Methods: Subjects were 60 adolescents, 13-19 years old (40 with BD and 20 healthy control (HC)). Semi-structured interviews determined diagnoses based on DSM-IV. Serum was assayed for BDNF, interleukin (IL)-6, and tumor necrosis factor (TNF)-α. Carotid intima media thickness (cIMT) and flow-mediated dilation (FMD) were assessed using ultrasound. Procedures were conducted at a subspecialty clinic (January 2011-May 2014). Results: BD adolescents had significantly greater waist circumference (BD: 81.72(11.67), HC: 75.64 (8.63); U=547.5, p=.021), BMI (BD: 25.5(5.29), HC: 21.76 (3.43); U=608.5, p<.0001), pulse pressure (BD: 42.31(10.57), HC: 33.84 (6.69); U=561.5, p<.001) and IL-6 (BD: 8.93(7.71), HC: 4.96 (6.38); U=516, p<.0001) vs. HC. BD-I (n= 14) and BD-II (n= 16) subjects had greater IL-6 vs. HC (F²,52=7.96, p=.001). Controlling for BMI and age did not alter these findings. IL-6 was higher in symptomatic (n=19) and asymptomatic BD (n=21) vs. HC (F²,52=7.96, p=.001). In symptomatic BD, lower BDNF was associated with greater mean cIMT (ρ=-.507, p=.037). Conclusion: This study found evidence of increased inflammation among adolescents with BD. While present findings suggest a potential interplay between symptomatic status, biomarkers, and atherosclerosis proxies, there were no significant differences in cIMT or FMD in BD adolescents compared to HC. This may indicate that there is potential opportunity for CVD prevention strategies in BD adolescents.

Keywords: carotid intima-media thickness, flow-mediated dilation, inflammation, BDNF, bipolar disorder, adolescent
Introduction

A recent (2015) statement from the American Heart Association (AHA) positioned bipolar disorder (BD) and major depressive disorder (MDD) among youth as tier II moderate-risk conditions associated with accelerated atherosclerosis and early cardiovascular diseases (CVD) (Goldstein et al., 2015a). Risk of new onset cardiovascular disease in BD is about 3 times greater than in psychiatrically healthy adults, and there is a 1.5 – 2.5-fold increase in mortality due to a cardiovascular event, compared to the general population (Osby et al., 2001; Weeke et al., 1987). Beyond the increased mortality risk, adults with BD develop CVD 17 years younger than adults without mood disorders (Goldstein et al., 2015b). Importantly, mortality due to CVD is predicted by symptomatic burden in adults with BD, independent of BD diagnosis and treatments, and traditional CVD risk factors (CVRFs) (Fiedorowicz et al., 2009). Reasons for excessive and premature CVD in BD are poorly understood.

Investigating non-invasive structural and functional proxies for atherosclerotic risk in adolescents with BD allows the opportunity to understand the biology underlying the BD-CVD link in a group with limited exposure to the symptoms and treatments of BD compared to adults. Carotid intima media thickness (cIMT), a structural measure of atherosclerotic risk, and flow-mediated dilation (FMD), a nitric oxide (NO)-mediated functional measure of atherosclerotic risk, are important non-invasive proxies for CVD risk. This is particularly useful in youth, when “hard” endpoints such as myocardial infarction are rare (Urbina et al., 2009). Even in adolescents with BD, who present with various medications, comorbidities, and mood states, cIMT and FMD are associated with traditional CVRFs such as decreased high-density lipoproteins (HDL), and elevated fasting glucose, triglycerides, and greater waist circumference (Hatch et al., 2014). Thus, similar to healthy control (HC) adolescents and adolescents with CVD-risk diseases (e.g. Type I diabetes mellitus; T2DM), cIMT and FMD
are valid CVD risk proxies for adolescents with BD (Hatch et al., 2014; Jarvisalo et al., 2004; Jarvisalo, Putto-Laurila, et al., 2002; Urbina et al., 2009).

The question arises as to what biological processes that may underlie the BD-CVD association. Inflammation is a process that has been robustly associated with both BD and CVD. In adults with BD, peripheral levels of pro-inflammatory markers (PIMs) are elevated during mania (Brietzke et al., 2009; Huang & Lin, 2007; Maes et al., 1995; O’Brien et al., 2006; Ortiz-Dominguez et al., 2007; Tsai et al., 2001) and depression (Brietzke et al., 2009; O’Brien et al., 2006; Ortiz-Dominguez et al., 2007), and return to a similar level as observed in HC subjects during euthymia (Brietzke et al., 2009; Cunha et al., 2008; Dickerson et al., 2007; Guloksuz et al., 2010; Tsai et al., 1999). Although there are few data regarding inflammation among adolescents with BD, a prior study found that 40% of adolescents with BD had levels of high-sensitivity c-reactive protein (CRP) that reflect increased CVD risk in adults (Goldstein et al., 2011). Notably, inflammation has been shown to predict CVD in the general population, and the evaluation of inflammatory markers has become integrated in the clinical evaluation of CVD (Ridker, 1999; Ridker et al., 2000a, 1997; Urbinati et al., 2009). CRP, interleukin (IL)-6 and tumor necrosis factor (TNF)-α have been shown to be associated with CVRFs, pro-atherosclerotic diseases/states (e.g. Metabolic syndrome (MetS) and T2DM), as well as CVD outcomes and mortality (Esposito et al., 2004; Ridker et al., 2000b, 1997). Moreover, changes in vascular structure and function, as well as inflammation, are present as early as adolescence and are associated with CVD risk proxies in adults (Glowinska-Olszewska et al., 2007; Groner et al., 2006). Inflammation may therefore subserve the BD-CVD link (Frey et al., 2013b; Goldstein, Carnethon, et al., 2015; Goldstein & Young, 2013; Mitchell & Goldstein, 2014).
Trophic factors comprise another putative link between BD and CVD. Neurotrophins are proteins that centrally and peripherally regulate cellular processes (Huang & Reichardt, 2001; Kaplan & Miller, 2000; Mufson et al., 1999; Scola & Andreazza, 2015). Several studies have noted reduced brain-derived neurotrophic factor (BDNF) levels during mania and depression (Andreazza & Young, 2013; Berk et al., 2011; Brown et al., 2014; Cunha et al., 2006; de Oliveira et al., 2009; Simões Fernandes et al., 2009; Frey et al., 2013b; Lin, 2009; Machado-Vieira, Dietrich, et al., 2007). Reduced BDNF levels are implicated in vascular endothelial dysfunction (ED) and are associated with CVD (Donovan et al., 2000; Ejiri et al., 2005; Kermani & Hempstead, 2007; Kermani et al., 2005). Despite the theoretical link, prior studies have not examined whether BDNF is related to CVD risk in BD.

Given the prior literature that BD is associated with increased inflammatory and decreased trophic markers, along with prior literature supporting similar association in CVD, we set out to examine the association of these markers with CVD proxies in adolescents with BD who are at increased risk for early CVD. In this study we compared BD and HC adolescents with regard to non-invasive atherosclerosis proxies (cIMT, FMD) and putative biomarkers (PIMs and BDNF), with consideration of the role of BD subtypes and symptomatic status.

**Methods**

Study procedures are Research Ethics Board (REB) approved and are in accordance with the Helsinki Declaration of 1975. Prior to study procedures, written informed consent was obtained from all adolescent participants and their guardians, ensuring that all participants were aware of potential risks involved in participating.
Sample

Adolescents (N=60, 13-19 years old) were recruited from a subspecialty clinical research program, focusing on adolescent BD, at a tertiary academic health sciences centre. HC adolescents were recruited via advertisements posted in community centres, public transit and local papers. All participants were English-speaking, and were excluded from the study if they had an infectious illness within the past 14 days, were unable to provide informed consent (i.e. due to developmental delay or psychosis), or if they had/were taking medication for a cardiac or inflammatory condition (any cardiac condition, autoimmune, infectious, or inflammatory illness). HC were excluded if they or a 1st or 2nd degree family member had MDD, BD, psychosis and/or schizophrenia, or had current drug dependence/abuse. Conditions such as ADHD and anxiety disorders were not excluded.

Assessment

Interviews

The Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime version (K-SADS-PL), a semi-structured diagnostic interview, was used to determine diagnoses (Kaufman et al., 1997). A child-adolescent psychiatrist confirmed diagnoses (B.G.). DSM-IV criteria were used for bipolar I disorder (BD-I) and bipolar II disorder (BD-II); BD not otherwise specified (BD-NOS) was defined using operationalized criteria from the Course and Outcome of Bipolar Youth (COBY) study, via information gathered in the Mania Rating Scale (MRS) and Depression Rating Scale (DRS) in the K-SADS-PL (Birmaher et al., 2009). Symptomatic status was determined from Psychiatric Status Ratings of three or greater for hypomania or depression on the Adolescent Longitudinal Interval Follow-up Evaluation (ALIFE) (Goldstein, Birmaher, et al., 2009; Leon et al., 2000;
Miklowitz et al., 2007). PSR scores during the week of the study procedures were used to define symptomatic groups. Symptomatic was defined as a PSR score of three or more on either depression or hypomania ratings (Goldstein, Birmaher, et al., 2009; Leon et al., 2000; Miklowitz et al., 2007). Asymptomatic was defined as a PSR score of less than three (i.e. 2 or 1) for both depression and hypomania ratings (Goldstein, Birmaher, et al., 2009; Leon et al., 2000; Miklowitz et al., 2007). PSR scores of 1 or 2 indicate euthymia/within normal fluctuations of mood (i.e. 1 or 2 symptoms may be present, but for substantially lower severity e.g. “Feeling better – but not yet back to usual self”, and brief duration (less than 1 day) (Goldstein, Birmaher, et al., 2009; Leon et al., 2000; Miklowitz et al., 2007). Socio-economic status (SES) was determined using the 4-factor Hollingshead Scale (Hollingshead, 1975).

Lifetime exposure to psychotropic medications was collected from the treatment history section and medical history questionnaire of the K-SADS-PL. Family history of psychiatric illnesses was also obtained via the Family History Screen in the KSADS-PL. Participants were interviewed directly, and parents/guardians were separately interviewed about the participant, family history and social status measures.

**Vascular imaging**

2-dimensional Doppler ultrasound (Phillips, iU22), with a high-frequency (10 MHz) linear-array transducer, was used for FMD and cIMT imaging procedures, with duplicate far-wall scans performed for reliability. Ultrasound procedures began with participants lying down and rested for at least 10 minutes. cIMT was measured with the subject lying down with their neck extended and head at a 45-degree angle away from side being examined. Three regions of the carotid artery were assessed for left and right sides (common cIMT, bulb IMT, and internal common cIMT; regions as described in Urbina et al., 2009) (Agrotou et al., 2013; Urbina et al., 2009). FMD measurements were conducted immediately following cIMT measures, with the
subject remaining in a recumbent position. Brachial artery FMD of the right arm of each participant was assessed with concurrent 3-lead electrocardiogram recordings. Lower-arm placement of the blood pressure cuff was used, with cuff inflation to 50 mmHg above systolic blood pressure for five minutes. A stand was used to hold the transducer steady on the participant’s brachial artery. Post-cuff deflation FMD was recorded for 5 minutes and analyzed as the average FMD post-deflation and the maximum FMD post-deflation. FMD was calculated as a percentage-change in brachial artery diameter post-deflation compared with baseline measurements.

**Phlebotomy and anthropomorphic measurements**

Participants fasted (no food or drink, except for water) 10 hours prior to blood draw, which was completed between 9 and 11 am. All participants were instructed not to use illicit drugs, smoke tobacco or consume alcohol for 24 hours prior to the appointment. Adherence to fasting and abstinence from drugs was assessed via interview and self-report. Fasting glucose, triglycerides, total cholesterol, HDL and low-density lipoprotein (LDL) were assessed. Blood samples were centrifuged at 3000 rpm for 15 minutes, and serum was collected and stored at -80°C.

Duplicate subject weight and height measurements were recorded to the nearest 0.5 cm and 0.1 kg, respectively. Weight adjustments were made to account for clothing (1.4 kg for long pants and long shirt/sweatshirt, 1.1 kg for short pants or short-sleeves, and 0.9 kg for short pants and short sleeves, were subtracted from weight), and used to calculate adjusted body mass index (BMI). Subjects were instructed to raise their arms up and away from their trunk, and bend sideways, to find the location of their waist. Waist circumference was measured using a flexible tape measure, with subjects standing up straight.
Blood pressure was assessed pre- and post-ultrasound procedures; a 10-minute rest period was given before each blood pressure measurement. Pulse pressure (PP) was calculated as the difference between systolic blood pressure and diastolic blood pressure. Ultrasound procedures were conducted as noted in previous literature and in accordance with AHA recommendations (Hatch et al., 2014; Hatch et al., 2015; Urbina et al., 2009).

**Assays**

Serum was assessed for cytokine levels IL-6 and TNF-α, using company kit procedures for the Human cytokine magnetic bead panel (HCYTOMAG-60K; Miliplex Map, EMD Millipore, Germany). BDNF, was assessed following company kit instructions for Sandwich ELISA procedures (CYT306; ChemiKine, EMF Millipore, Germany).

**Statistical Analyses**

Descriptive statistics were calculated for all relevant variables. Shapiro-Wilk test confirmed that our biomarker data is not normally distributed. Non-parametric analyses (Mann-Whitney U) and bivariate correlation analyses (Spearman’s correlation coefficient), general linear models and linear regressions (model inclusion p<.20) were completed as appropriate. Log transformed IL-6 was used in linear models. Statistical analyses were performed using SPSS 22 for Windows (SPSS Inc, Chicago IL, USA). False discovery rate (FDR) was used to correct for multiple comparisons, with maximum acceptable FDR α set to 0.05 (Benjamini, 2010).

**Results**

Demographic and clinical characteristics are presented in Table I. Adolescents with BD were significantly older than HC adolescents. Sensitivity analyses compared the impact of removing the youngest and oldest individuals from analyses, since these values were near outliers (>2SD). There was no significant difference when groups were age-matched (extreme
aged values removed). Therefore, for sample size considerations, the whole group was used in analyses. The BD group consisted of 14 subjects with BD-I, 16 subjects with BD-II and 10 subjects with BD-NOS. Within the BD sample, 19 were symptomatic (5 were hypomanic; of which 4 had PSR 5 or 6, and 14 were depressed; of which 7 had PSR 5 or 6) and 21 were asymptomatic.

BD adolescents had significantly greater waist circumference, BMI, pulse pressure and IL-6 levels, compared to HC. IL-6 remained significantly greater in BD adolescents after controlling for age and BMI in linear regressions. Traditional CVRFs (i.e. waist circumference, blood pressure, BMI, cholesterol, HDL, LDL, fasting glucose and triglycerides) were not significantly associated with PIMs or BDNF in BD adolescents. There were no significant differences between groups in cIMT or FMD measures.

In a general linear model with IL-6 as the dependent variable, we found significant differences between BD-subtypes and HC subjects ($F_{3,51}=5.29$, $p=.003$). Post-hoc comparisons confirmed that BD-I and BD-II subgroups, but not BD-NOS, each had greater levels of IL-6 compared to HC ($p<.05$) (Figure 1a). There was a significant linear association of IL-6 increasing across groups (HC to BD-I subtypes; $\rho=.476$, $p=.0002$).

Similarly, a general linear model with symptomatic status (symptomatic BD vs. asymptomatic BD vs. HC groups), revealed significant differences in the dependent variable, IL-6 ($F_{2,52}=7.96$, $p=.001$). Post-hoc comparisons confirmed that symptomatic BD adolescents have the highest IL-6 levels and HC adolescents have the lowest levels ($p<.05$) (Figure 1b). There was a significant linear association, with increasing IL-6 across HC, asymptomatic and symptomatic groups ($\rho=.443$, $p=.0007$). All general linear models and linear associations between BD subtypes and symptomatic status remained significant after controlling for multiple comparisons ($p<.05$).
TNF-α analyses were undertaken after removing 4 outliers (≥2 standard deviations from the mean; 3 BD-II [71.37, 56.96, 107.31] and 1 HC [351.76]). TNF-α was significantly greater in adolescents with BD (11.38±10.28, n=36), compared to HC (4.37±2.65, n=15; t_{(2,49)}=2.59, p=.012), which is the opposite direction when outliers are included. TNF-α remained significantly greater in BD adolescents after controlling for age and BMI in linear regressions. A general linear model of BD-I vs. BD-II vs. BD-NOS vs. HC revealed significant differences in TNF-α between groups (F_{3,47}=3.01, p=.039), with significant differences only between BD-I and HC groups (p=.026; post-hoc Bonferroni) (Figure IIa). There was a significant linear association of TNF-α decreasing across groups (BD-I to HC; ρ=-.330, p=.018).

A general linear model of symptomatic BD vs. asymptomatic BD vs. HC groups also revealed significant differences in TNF-α between groups (F_{2,48}=4.64, p=.014), with post-hoc analyses showing that symptomatic BD were significantly different from HC (p=.011) (Figure IIb). There was a significant linear association of TNF-α decreasing across groups (Symptomatic BD to HC; ρ=-.409, p=.003). All TNF-α subsample analyses remained significant after correction for multiple corrections (p<.05). Approximately half of the BD subjects were taking anti-psychotics. Binary logistic regressions were completed to assess medication effects on inflammatory markers. No significant differences were observed between BD medication groups.

In linear regression models, IL-6 and TNF-α are elevated in BD, and there was no significant between group (BD vs. HC) differences in BDNF. Adding age, BMI, or age and BMI to the linear regression for BDNF did not change non-significant findings. Including age and BMI individually and together as covariates for TNF-α and IL-6 models also did not change reported findings. When holding age constant IL-6 is still significantly elevated in BD.
compared to HC (T=3.35, p=.002), and when holding age and BMI constant, the IL-6 finding remains significant (T=3.28, p=.002). Similarly, for TNF-α, when holding age constant (T=3.09, p=.003), as well as age and BMI constant (T=2.7, p=.009), TNF-α remains significantly higher in BD adolescents compared to HC.

Lastly, there was evidence for a relationship between biomarkers and vascular imaging measures among symptomatic BD adolescents. Lower BDNF levels were associated with significantly thicker mean cIMT among symptomatic BD adolescents (ρ=−.507, p=.037; n=18), but not asymptomatic BD (ρ=.117, p=.614; n=21) or HC (ρ=.404, p=.121; n=16). Maximum cIMT was not significantly associated with BDNF in symptomatic BD subjects (ρ=−.446, p=.073). Similarly maximum and mean FMD were not significantly associated with symptomatic BD (ρ=.174, p=.504; ρ=.321, p=.198, respectively). In HC and asymptomatic BD, BDNF was not significantly associated with FMD or cIMT measures.

Discussion

This study brings together two core themes in BD, namely biomarkers and cardiovascular risk. We found that adolescents with BD had higher levels of IL-6 compared to HC, and that this difference was observed in BD-I and BD-II but not BD-NOS. Moreover, between-group comparison based on symptomatic status found that IL-6 was highest in symptomatic BD, followed by asymptomatic BD, followed by HC. Similarly, TNF-α was elevated in BD compared to HC, specifically BD-I compared to HC, and was significantly higher in symptomatic BD compared to HC. Finally, among symptomatic BD adolescents, but not asymptomatic BD adolescents or HC, lower BDNF levels were associated with significantly thicker mean cIMT. While preliminary, these findings highlight an area that future work can build upon to potentially aid in early detection and classification of CVD risk.
IL-6 has been shown to be associated with both cIMT and FMD in those with/or at risk for CVD and in healthy populations (Esteve et al., 2007; Zhang et al., 2015). Moreover, IL-6 has been demonstrated to regulate and modulate CRP, a widely used biomarker for CVD risk and cardiovascular events (Memoli et al., 2007). Therefore, while we did not assess CRP in this study, IL-6 is associated with both cIMT and FMD, modulates CRP, and has been shown to be elevated in CVD and BD (Brietzke et al., 2009; Ridker et al., 2000b). Similarly, TNF-α has been shown to be associated with pro-atherogenic alterations in chronic inflammatory states, and is associated with thicker cIMT (Kablak-Ziembicka et al., 2011; Popa et al., 2007). Elevated TNF-α has also been shown to be associated with poor FMD and ED in patients with rheumatoid arthritis, T2DM, and those post-myocardial infarction (Bosello et al., 2008; Gonzalez-Juanatey et al., 2012; Kovacs et al., 2006; Nystrom et al., 2006). Interestingly, anti-TNF-α treatment in those with rheumatoid arthritis lead to improvements in FMD (Bosello et al., 2008; Gonzalez-Juanatey et al., 2012).

It is uncertain why the vascular ultrasound measures were not significantly different between BD and HC groups, or why inflammatory markers were not significantly associated with vascular measures. Although the vascular ultrasound measures are operator dependent, and it is theoretically possible that this underlies the lack of detecting between-group differences, we previously confirmed that impaired cIMT and FMD are associated with increased levels of traditional CVRFs (e.g. elevated BMI and cholesterol), supporting the validity of these measures (Hatch et al., 2014). One can speculate that while cIMT and FMD are already linked with CVRFs, the duration of illness among these adolescents with BD has not been sufficient to impart vascular impairment. This indicates that there is potential for prevention of accelerated atherosclerotic development in BD during adolescence. Moreover, most mood-stabilizing and antidepressant medications are known to have anti-
inflammatory effects, as well as pro-neurotrophic effects, such that one cannot rule out the possibility that medications contributed to the negative finding (Berk et al., 2011; Fiedorowicz, Linder, & Sodhi, 2014; Goldstein, Kemp, et al., 2009). Future studies with larger sample sizes would enable the assessment of atherosclerosis proxies and the role of inflammation, with the inclusion of important covariates such as symptomatic status and determination of potential interactions.

In contrast to the current findings, a recent small study of adolescents (9-20 years of age) with BD-I ($n=16$), adolescents at familial risk for developing BD ($n=15$) and HC ($n=13$) found no significant differences in inflammatory marker levels between groups (Scola et al., 2016). Prior studies have found that inflammatory markers are related to symptoms and other clinical characteristics in BD, but did not include an HC comparison group (Goldstein et al., 2015a, 2011).

These findings are constrained by several limitations. First, this study was not powered to detect small effect sizes; a larger sample would have provided greater power to detect differences and to include more comprehensive covariate analysis. Second, the cross-sectional design of the study prevents us from examining directional hypotheses. Third, the groups were not well matched for age or BMI. However, results remained unchanged in sensitivity analyses that were restricted to age-matched groups, and between-group differences in TNF-$\alpha$ and IL-6 were independent of BMI and age in linear regressions. Fourth, all BD participants were taking psychiatric medication; as such, it was not possible to parse medication effects from diagnosis effect. Despite these limitations, this is the first study to assess the BD-CVD link, integrating non-invasive atherosclerosis proxies and peripheral biomarkers in a case-control study, and provides important preliminary results to guide further investigation.

Additional studies are needed in order to better understand the directionality of the
observed findings. Longitudinal studies in particular are needed to understand how inflammation and BDNF fluctuate with symptomatic episodes, and in turn to understand how such fluctuation may correspond with atherosclerosis and CVD over time. Although prior studies of adults have found that symptom burden in BD is relevant to CVD risk, those studies have not included novel PIMs and/or neurotrophins (Fiedorowicz, 2014; Fiedorowicz et al., 2009; Stillman, Moser, Fiedorowicz, Robinson, & Haynes, 2013). The current study found that BDNF is associated with cIMT, only among symptomatic BD adolescents. We speculate that levels of BDNF and inflammatory markers among symptomatic BD adolescents may confer risk for the development and progression of atherosclerosis and CVD in adulthood.

This is the first study to assess putative biomarkers and non-invasive atherosclerosis proxies, in a BD-control sample in adolescents. The lack of a significant difference in atherosclerosis proxies (despite our prior demonstration of the validity of cIMT and FMD in this population) (Hatch et al., 2014) highlights that there is key opportunity for preventative treatment, to potentially alter the course of illness and delay or reduce CVD outcomes. Adolescence comprises a crucial window of opportunity to intervene, prevent or delay CVD in BD. If our preliminary findings are replicated in future studies, this may suggest that reduced BDNF and elevated inflammation among symptomatic adolescents with BD may serve as surrogate targets for intervention to modify both symptomatic course and CVD outcomes. For example, investigation of lifestyle modifications including those related to exercise, diet, and sleep, in addition to pharmacological approaches, may increase BDNF and reduce inflammation (Berk et al., 2013; Neeper, Gomez-Pinilla, Choi, & Cotman, 1995; Pashkow, 2011). Finally, present findings provide preliminary support for the concept of BD as a multi-system disease in which vascular pathology may play an important role. This concept can potentially be leveraged to yield much needed progress in terms of novel therapeutics,
biomarker discovery, and stigma reduction in BD.

**Clinical Points**

1. Adolescents with BD are at an elevated risk for premature atherosclerosis.

2. Inflammatory and neurotrophic factors may underlie the BD-CVD link.

3. Despite elevated inflammation in BD adolescents, especially when symptomatic, ultrasound CVD proxies did not differ from healthy controls. This indicates a potential window of opportunity for prevention of accelerated atherosclerosis.
Table I. Demographic, biological, and clinical characteristics across groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adolescents with BD; mean (SD)</th>
<th>Healthy Adolescents; mean (SD)</th>
<th>Statistic (FDR corrected p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
<td><strong>Patients (n=40)</strong></td>
<td><strong>Patients (n=20)</strong></td>
<td></td>
</tr>
<tr>
<td>Demographic and Clinical Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>32.5</td>
<td>50</td>
<td>$\chi^2 = 1.727, p=0.189$</td>
</tr>
<tr>
<td>Age (years)</td>
<td>17.41 (1.64)</td>
<td>16.06 (1.67)</td>
<td>U=580; p=0.005 (.016)</td>
</tr>
<tr>
<td>Socio-economic Status</td>
<td>4.03 (1.21)</td>
<td>4.35 (0.81)</td>
<td>U=386.5, p=0.832</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>90</td>
<td>75</td>
<td>$\chi^2 = 4.478, p=0.345$</td>
</tr>
<tr>
<td>Age at onset of illness (years)</td>
<td>13.59 (3.38)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>3.37 (3.03)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mania score</td>
<td>9.67 (12.00)</td>
<td>0.63 (1.38)</td>
<td>U=528.5, p=0.004 (.015)</td>
</tr>
<tr>
<td>Depression score</td>
<td>12.63 (12.38)</td>
<td>0.32 (0.95)</td>
<td>U=659.5, p&lt;0.0001 (.0006)</td>
</tr>
<tr>
<td>Functioning score</td>
<td>62.28 (14.32)</td>
<td>89.85 (6.16)</td>
<td>U=46.0, p &lt;0.0001 (.0006)</td>
</tr>
<tr>
<td>Medication (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>11.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>47.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Antidepressant (SSRI)</td>
<td>30.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Stimulant</td>
<td>17.5</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>20.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Biological Markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.01 (0.58)</td>
<td>0.783 (0.36)</td>
<td>U=480.5, p=0.100</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.31 (0.65)</td>
<td>1.98 (0.55)</td>
<td>U=462, p=0.081</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.40 (0.36)</td>
<td>1.44 (0.32)</td>
<td>U=338.0, p=0.592</td>
</tr>
<tr>
<td>Fasting Glucose (mmol/L)</td>
<td>4.58 (0.44)</td>
<td>4.69 (0.49)</td>
<td>U=321.0, p=0.411</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>81.72 (11.67)</td>
<td>75.64 (8.63)</td>
<td>U=547.5, p=0.021</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.50 (5.29)</td>
<td>21.76 (3.43)</td>
<td>U=608.5, p&lt;0.0001 (.0006)</td>
</tr>
<tr>
<td>Systolic Blood pressure (mmHg)</td>
<td>114.23 (11.07)</td>
<td>110.26 (7.16)</td>
<td>U=458.5, p=0.192</td>
</tr>
<tr>
<td>Diastolic Blood pressure (mmHg)</td>
<td>71.72 (10.17)</td>
<td>76.42 (4.93)</td>
<td>U=242.5, p=0.027</td>
</tr>
<tr>
<td>Pulse Pressure (mmHg)</td>
<td>42.31 (10.57)</td>
<td>33.84 (6.69)</td>
<td>U=561.5, p&lt;0.0001 (.0046)</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>8.93 (7.71)</td>
<td>4.96 (6.38)</td>
<td>U=516.0, p&lt;0.0001 (.0006)</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>16.55 (22.48)</td>
<td>26.08 (86.89)</td>
<td>U=432.0, p=0.026</td>
</tr>
<tr>
<td>BDNF (pg/mL)</td>
<td>90.10 (98.07)</td>
<td>114.50 (121.44)</td>
<td>U=283.0, p=0.591</td>
</tr>
<tr>
<td>Imaging Measures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum cIMT (mm)</td>
<td>0.52 (0.07)</td>
<td>0.54 (0.07)</td>
<td>U=330.0, p=0.335</td>
</tr>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.44 (0.04)</td>
<td>0.45 (0.04)</td>
<td>U=334.0, p=0.370</td>
</tr>
<tr>
<td>Maximum FMD (mm)</td>
<td>6.18 (3.83)</td>
<td>6.63 (4.94)</td>
<td>U=386.0, p=0.949</td>
</tr>
<tr>
<td>Mean FMD (mm)</td>
<td>1.91 (2.69)</td>
<td>2.18 (3.10)</td>
<td>U=314.0, p=0.349</td>
</tr>
</tbody>
</table>
Table I. Abbreviations and Caption

*Socio-economic status score (1-5) based on Hollingshead scale, with higher scores reflecting higher socio-economic status. Mania score is the Mania Rating Scale for the past month. The depression score is the Depression Rating Scale score for the past month and the functioning score is the current Children’s Global Assessment Scale score. False Discovery Rate-corrected p-value (q) is presented for values remaining significant after correcting for multiple comparisons.

Abbreviations: BD = bipolar disorder, BDNF = brain derived neurotrophic factor, BMI = body mass index, cIMT = carotid intima media thickness, FDR = False discovery rate, FMD = flow mediated dilation, HDL = high density lipoprotein, IL-6 = Interleukin-6, LDL = low density lipoprotein, SSRI = selective serotonin reuptake inhibitor, TNF-α = tumor necrosis factor-alpha
Figure I. Peripheral IL-6 Levels Across Groups

A

B

Figure I. Caption
Mean values are reported with error-bars denoting 95% confidence intervals.
Figure II. Peripheral TNF-α Levels Across Groups

**Figure II. Caption**
Mean values are reported with error-bars denoting 95% confidence intervals.
**Statement of Significance and Impact**

BD is associated with increased rates of CVD. BDNF and inflammatory markers are leading biomarkers in BD. We examined whether these biomarkers underlie the link between BD and CVD proxies among adolescents with BD. By investigating PIMs and BDNF in adolescents with BD in a case-controlled study we were able to assess the between group differences in HC compared to affected adolescents. This provided an indication of early limits of detection, as well as the degree to which adolescents with BD are at risk for CVD compared to their peers at this point in their disease progression, addressing Aims 2 and 3. PIMs and BDNF were selected as relevant factors for assessment with CVD risk as they have been consistently reported to be associated with BD in adults, and have are increasingly reported to be associated with CVD risk and progression (Berk et al., 2011; Groner et al., 2006; Hatch et al., 2014). Non-invasive measures of vascular structure (cIMT) and function (FMD) were chosen as methods due to their robust association with traditional CVRFs, predictive value and our experience with using them as assessment tools (Flammer et al., 2012; Urbina et al., 2009).

Non-parametric analyses (Mann-Whitney U) and bivariate correlation analyses (Spearman’s correlation coefficient), general linear models and linear regressions (model inclusion p<.20) were completed as appropriate. Log transformed IL-6 was used in linear models. FDR was used to correct for multiple comparisons, with maximum acceptable FDR α set to 0.05 (Benjamini, 2010; Benjamini and Hochberg, 1995). General linear models were used to assess between group differences (e.g. HC v BD, BD-I v BD-II v BD-NOS v HC, Symptomatic BD v Asymptomatic BD, v HC), for INF, BDNF and CVRFs. Linear Regressions were used to assess these group differences while holding pertinent covariates constant (i.e. Age and BMI). Previous literature has shown that BMI and age are associated with CVD as well as INF and BDNF (Berk et al., 2011; Fiedorowicz et al., 2008; Ridker, 1999;
Therefore, in order to assess the association of these markers with CVD, independent of these factors they needed to be included in the model, as done in similar studies (Berk et al., 2011; Fiedorowicz et al., 2009; Jarvisalo et al., 2004; Jourdan et al., 2005; Munkholm et al., 2015; Woo et al., 2004b).

First we wanted to examine between group differences for CVRFs, INF and BDNF. We found that BD adolescents had significantly greater waist circumference, BMI, pulse pressure TNF-α and IL-6 levels, compared to HC. This indicates that adolescents with BD have elevated levels of INF and CVRFs compared to HC adolescents. IL-6 and TNF-α remained significantly greater in BD adolescents after controlling for age and BMI in linear regressions. This further supports that INF is elevated in BD adolescents and that it is not due to age or BMI, which are pertinent covariates.

However, traditional CVRFs (i.e. waist circumference, blood pressure, BMI, cholesterol, HDL, LDL, fasting glucose and triglycerides) were not significantly associated with INF or BDNF in BD adolescents and there were no significant differences between groups in cIMT or FMD measures. This study therefore found that while there is elevated INF in BD adolescents, these factors are not related to traditional CVRFs or to non-invasive vascular measures. Importantly, we did not find poor vascular structure (cIMT) or function (FMD) in adolescents with BD compared to HC. Thus, while adolescents are at risk for premature CVD, we did not find evidence of CVD development in adolescents. This highlights a potential opportunity for CVD prevention in adolescents with BD, perhaps via anti-inflammatory and BNDF elevating therapies and interventions (e.g. exercise) (Neeper et al., 1995; Woo et al., 2004b).
Summary and General Discussion

Mortality due to CVD is excessive and premature in BD, and research has highlighted a robust link between BD and CVRFs, and CVD (Goldstein et al., 2015a). Recently, AHA released a scientific statement positioning adolescent BD as an exceptionally prevalent tier-II moderate risk conditions for accelerated atherosclerosis (Goldstein et al., 2015a). Studying the BD-CVD link in adolescence provides an opportunity to assess early limits of detection and gathering evidence for modifiable risk factors, via pharmacological treatment, lifestyle modifications, and appropriate assessment. Therefore the aim of my PhD was to examine the underlying biology of the BD-CVD, in adolescents with BD.

My first study aimed to examine the association of cIMT and FMD with CVD risk factors among adolescents with BD, by assessing adolescents with BD using high-resolution ultrasonography and traditional CVRFs. Our findings revealed that cIMT and FMD are associated with traditional CVD risk factors among adolescents with BD, despite the presence of multiple potential confounds. Therefore, non-invasive vascular ultrasound approaches may be used as CVD risk proxies among adolescents with BD as they are for other adolescents and further research is warranted to further assess CVD risk and early detection limits in this population.

Due to the prevalent and premature CVD mortality and greater occurrence and dysregulation of CVRFs in adults with BD, it is important to assess CVR risk proxies in adolescents with BD to determine early detection limits (Goldstein et al., 2015a). Importantly, due to the fluctuating symptomatic polarities, comorbid psychiatric conditions and polypharmacy, there are several confounding variables that may preclude cIMT and FMD from being valid measures in adolescent BD (Fiedorowicz et al., 2012; Goldstein, Carnethon, et al.,...
2015; Urbina et al., 2009). I therefore set out to examine CVRFs and non-invasive vascular proxies to assess whether CVD proxies are valid measures in a clinical heterogeneous adolescent BD sample. Results indicate that in adolescents with BD traditional CVRFs, such as triglycerides, elevated blood pressure, greater waist circumference and low HDL, are significantly associated with poor vascular structure (i.e. cIMT) and function (i.e. FMD) (Hatch et al., 2014). To further examine CVD risk in adolescents with BD, I compared the observed cIMT and FMD values to aggregated data of psychiatrically healthy adolescents of the same age group (Hatch et al., 2014; Jourdan et al., 2005; Urbina et al., 2009). cIMT and FMD measures indicated that adolescents with BD had significantly poorer vascular structure and function compared to their psychiatrically healthy counterparts (Hatch et al., 2014). Additionally, previous literature has reported that age is not associated with cIMT and FMD in adolescents until approximately 18 years of age (Jourdan et al., 2005; Stein et al., 2004a). We found that in adolescents with BD, age was significantly associated with non-invasive CVD proxies, which may indicate that adolescents with BD may exhibit premature vascular aging. Therefore, cIMT and FMD can be used as valid CVD risk proxies in adolescents with BD, however longitudinal studies examining cIMT and FMD over-time are needed for the assessment of a vascular risk and vascular aging (Hatch et al., 2014).

After validating cIMT and FMD as CVD risk proxies in adolescents with BD, my second project aimed to examine whether psychiatric and cardiovascular characteristics (traditional CVRFs, as well as cIMT and FMD) were associated with peripheral oxidative stress markers among adolescents with BD. I focused on assessing whether serum markers of OS, specifically LPH and PC, were associated with traditional CVRFs as well as noninvasive CVD proxies (cIMT and FMD). Our findings revealed that thicker mean and maximum cIMT was significantly associated with greater levels of LPH (r=.455, p=.015; r=.620, p<0.0001,
respectively) and greater levels of LPH were also significantly associated with CVRFs. Therefore, OS is significantly associated with a proxy measure of atherosclerosis and may explain in part the CVD-BD link.

Moreover, OS and CVRFs were investigated to assess whether OS was detectable in adolescents with BD, and whether OS is associated with a validated proxy measure of atherosclerosis and CVD risk. Our results indicate that greater cIMT measurements were associated with greater OS, as measured via serum LPH levels. There has been recent research to highlight the role of OS in vascular impairment, for example a recent atherosclerosis study investigating OS in haemodialysis patients reported that elevated peripheral OS was significantly associated with worse cIMT (Dursun et al., 2008; Hatch et al., 2015; Madamanchi, Vendrov, & Runge, 2005). Despite evidence that vascular function is associated with OS we did not observe a significant association of LPH or PC OS factors with FMD in our adolescent BD sample (Groner et al., 2006; Hatch et al., 2015; Schnabel & Blankenberg, 2007). Since we did observe lower levels of OS compared to adults with BD, this may be due to the early stage in illness course when OS has not yet overwhelmed antioxidant defense systems (Andreazza et al., 2009; Berk et al., 2011; Hatch et al., 2015; Versace et al., 2013). Currently, there have not been studies examining the link between OS and vascular structure or function among adults with BD.

Lastly, my third study aimed to examine whether lower levels of BDNF and elevated levels of PIMs were associated with CVD proxies and CVRFs among adolescents with BD. We hypothesized that (a) elevated PIMS, OS and lower levels of BDNF would be associated with poor vascular structure (thick cIMT) and function (low FMD), (b) symptomatic and asymptomatic adolescents with BD will have elevated PIM levels and lower levels BDNF compared to HC adolescents and (c) cIMT will be thicker and FMD will be worse in
adolescents with BD compared to HC and that (d) poor FMD and cIMT will be associated with: older age, male sex, BD-I subtype, more severe mood symptom burden, use of second-generation antipsychotics and family psychiatric history.

As evidenced by prior literature, BD is associated with elevated PIMs and decreased neurotrophic factors (Goldstein and Young, 2013; Kauer-Sant’Anna et al., 2009; Mitchell and Goldstein, 2014; O’Brien et al., 2006; Ray et al., 2014). Similarly, elevated PIMs and decreased neurotrophic factors have also been noted in CVD (Bozzini et al., 2009; Kaess et al., 2015; Kermani & Hempstead, 2007; Meyer et al., 2006; Ridker, 1999). We therefore set out to examine the association of these markers with CVD proxies in adolescents with BD who are at increased risk for early CVD. Our results revealed that adolescents with BD had higher levels of PIM IL-6 compared to HC, and that this difference was observed in BD-I and BD-II but not BD-NOS. Moreover, IL-6 was highest in symptomatic BD, followed by asymptomatic BD, followed by HC. Likewise, PIM TNF-α was elevated in BD compared to HC, specifically elevated in BD-I compared to HC, and was significantly higher in symptomatic BD compared to HC. Finally, lower BDNF levels were associated with significantly thicker mean cIMT in symptomatic adolescents with BD, but there was no significant association with FMD, cIMT and PIMs or BDNF in the other groups.

Therefore, we found evidence of elevated inflammation in adolescents with BD, and importantly PIM levels were significantly higher in those with BD-I, and in currently symptomatic subjects. This is in agreement with adult literature that symptomatic status, and severity are associated with greater levels of peripheral inflammation (Berk et al., 2011; Dickerson et al., 2007; Goldstein, Kemp, et al., 2009; O’Brien et al., 2006). Furthermore, in agreement with prior literature investigating symptom burden in adult BD, we have found that CVD risk is related to symptomatic status (Fiedorowicz, 2014; Fiedorowicz et al., 2009;
Stillman et al., 2013). Adult literature investigating symptomatic burden and CVD risk did not include neurotrophins (Fiedorowicz, 2014; Fiedorowicz et al., 2009; Stillman et al., 2013). The current study extends the adult literature and revealed that lower BDNF is associated with poor vascular structure, only among symptomatic BD adolescents.

In summary, I first assessed whether cIMT and FMD were valid measures of CVD in adolescent BD, despite the presence of multiple confounding factors inherent to the pathology. I determined that cIMT and FMD are associated with traditional CVRFs in adolescent BD, even in a heterogeneous clinical sample, and that compared to aggregated data of HC in the same age-group, adolescents with BD had significantly worse vascular structure and function. Second, I confirmed that peripheral OS is detectable in adolescence with BD, and that elevated OS as measured by LPH, is significantly associated with poor vascular structure. Lastly, I confirmed that while adolescents with BD do have elevated levels of PIMs compared to HC, they do not have detectable levels of structural or functional vascular impairment.

Therefore, this work provides support for the concept of BD as a multi-system disease, in which vascular pathology plays a significant role. Currently, there are no clinically used biomarkers used to assess BD, and thus subjective clinical measures are used for diagnosis, monitoring and staging (Goldstein and Young, 2013). Establishing biomarkers for BD could provide clinically relevant treatment indications that could improve quality of life, reduce CVD risk and improve prognoses (Fiedorowicz et al., 2008; Goldstein et al., 2012; Young & Grunze, 2013). Conceptualizing BD as a multi-system disease with measurable biological markers, can aid in the discovery of novel and re-applied therapeutics, prevention strategies, biomarker discovery, and stigma reduction in BD.
Conclusions

Our method validation of standard measures revealed that despite fluctuating disease state, medication(s) and comorbid conditions in adolescent BD, cIMT and FMD are associated with traditional CVRFs, and that they may be used in further research as a proxy for atherosclerosis. We then established that peripheral OS markers are detectable in adolescents with BD, and that OS is associated with non-invasive proxy measures of atherosclerosis. This supports the hypothesis that there are underlying biological mechanisms that link BD and CVD pathology and progression. Finally, while we found that adolescents with BD had elevated INF compared to HC adolescents, there was no evidence of structural or functional vascular impairment in adolescent BD. In this case-controlled study we also found that lower levels of BDNF were associated with poor vascular structure in symptomatic BD adolescents, suggesting that there is possible interplay between biomarkers and vascular risk in BD.

Together, these three studies extend the current literature on CVD risk and progression in BD. While INF, neurotrophins and OS may play a role in BD symptomology and CVD progression, adolescents with BD have not yet developed distinguishable differences in CVD progression, compared to HC adolescents. Importantly, this indicates that there is an opportunity for prevention of CVD progression in adolescent BD. Understanding and targeting the shared biology in BD and CVD can assist in early detection, risk assessment, medication and disease monitoring, as well as prevention planning.

It is possible that while cIMT and FMD are already linked with CVRFs, the duration of illness among our sample of adolescents with BD has not been sufficient to confer vascular impairment (Berk et al., 2011; Kablak-Ziembicka et al., 2011; Kapczinski, Vieta, Andreazza, et al., 2008; Kauer-Sant’Anna et al., 2009). This indicates that there is potential for prevention of accelerated atherosclerotic development in BD during adolescence. For instance, there is
evidence that exercise regimens can improve BDNF levels and decrease INF (Berk et al., 2013; Lucassen et al., 2010; Matthews et al., 2009; Rothman, Griffioen, Wan, & Mattson, 2012; Swardfager et al., 2011; Ziccardi et al., 2002). Similarly, diet modifications can decrease INF, improve anti-oxidant capacity and reduce OS levels (Anthony, George, & Eaton, 2014; Berk, 2009; Chrysohoou, Panagiotakos, Pitsavos, Das, & Stefanadis, 2004; Grattagliano et al., 2008; Jacka, Kremer, et al., 2011; Jacka, Mykletun, Berk, Bjelland, & Tell, 2011; Pistell et al., 2012; Sodhi et al., 2012). Literature has shown that exercise and diet can improve measurable levels in these biomarkers, but also improve symptomatic burden, for example, improving cognition (Andreaazza et al., 2009; Berk et al., 2011; Goldstein, Carnethon, et al., 2015; Goldstein, Kemp, et al., 2009; Maes, Ruckoanich, Chang, Mahanonda, & Berk, 2011; McIntyre et al., 2007; Neepet al., 1995; Pistell et al., 2012). Investigating medications that have anti-inflammatory and/or anti-oxidant capacity may also prove beneficial for psychiatric symptoms as well as CVD risk. For instance, curcumin is a natural health product that has been shown to have anti-inflammatory properties and may be beneficial in both the depressive symptoms of BD as well as reducing CVD risk (Kapakos, Youreva, & Srivastava, 2012; Lopresti, Maes, Maker, Hood, & Drummond, 2014; Lopresti, Hood, & Drummond, 2012; Shehzad, Rehman, & Lee, 2013; Wongcharoen & Phrommintikul, 2009; Zhang et al., 2012). This could prove to be beneficial, as lithium is a standard mood stabilizing treatment for BD, and is also known to be a potent anti-oxidant (Bakare et al., 2009; Cui et al., 2007). It is possible that new avenues for pharmacological targets may be revealed, improved and/or better understood and monitored, by further understanding the link between BD and CVD.

Moreover, previous literature notes that many mood-stabilizing and antidepressant medications are found to increase neurotrophin levels and have anti-inflammatory properties (Berk et al., 2011; Fiedorowicz et al., 2014; Goldstein, Kemp, et al., 2009). In these three
studies adolescents with BD sample were medicated and/or on more than one medication/mood stabilizer (Hatch et al., 2014; Hatch et al., 2015). Future studies with larger sample sizes would enable the assessment of atherosclerosis proxies and the role of INF, with the inclusion of important covariates such as symptomatic status and medication class, as well as the ratification of potential interactions. Additionally, investigating atherosclerotic risk proxies and biomarkers among adolescents at genetically at higher risk for developing BD (i.e. offspring of one or more parent with BD, or a full-biological sibling with BD) over-time may provide greater insights of disease course of BD, CVD and CVRF progression, as well as the potential impact of medication and treatment on these associations (Goldstein, 2012; Scola et al., 2016).

Taken together, my thesis contributes to the current literature examining biomarkers and CVD risk in BD. This work addressed the literature gap in adolescent BD vascular risk and biomarker assessment. We conclude that INF, neurotrophins and OS may play a role in BD symptomology and CVD progression. Importantly, this work provides evidence that despite the detectable levels of OS and elevated PIMs found in adolescents with BD, there is a window of opportunity to potentially prevent CVD. Biomarkers provide an avenue to assess psychiatric characteristics, disease progression, and monitoring of treatment response. Understanding and identifying biomarkers in adolescent BD are clinically relevant for both psychiatric care (e.g. monitoring of symptoms, treatment response and diagnostics), as well as CVD prevention and risk assessment (e.g. metabolic and vascular health, treatment response).
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Appendix I

Copyright Release: Chapter 1

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Copyright Release: Chapter 2

Title: Cardiovascular and Psychiatric Characteristics associated with Oxidative Stress Markers among Adolescents with Bipolar Disorder

Authors: Jessica Hatch, Ana Andreazza, Omodele Olowoyeye, Gislane Tezza Rezin, Alan Moody, Benjamin I. Goldstein

Journal: Journal of Psychosomatic Research

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Letter of Acceptance: Chapter 3

From: jclinpsych@psychiatrist.com [jclinpsych@psychiatrist.com]
Sent: July-28-16 10:37 AM
To: Goldstein, Dr. Benjamin
Cc: goldsteinbi@upmc.edu
Subject: Manuscript J16-M10762R Decision Letter

Dear Dr. Goldstein:

RE: J16-M10762R
Inflammatory Markers and Brain-Derived Neurotrophic Factor as Potential Bridges Linking Bipolar Disorder and Cardiovascular Risk among Adolescents

I am pleased to inform you that your manuscript has been accepted for publication in The Journal of Clinical Psychiatry. I am forwarding it to our publisher, Physicians Postgraduate Press, for final editing, and their office will contact you about a month before your article is published. http://www.psychiatrist.com

Congratulations to you and your co-authors!

Thank you.

Sincerely,

Karen Dineen Wagner, M.D., Ph.D.
Deputy Editor
Special Editor for Focus on Childhood and Adolescent Mental Health
Appendix II

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

For Adolescents 13-19 years of age

TITLE OF PROJECT:
Inflammation and Brain-Derived Neurotrophic Factor: At the Heart of Cardiovascular Risk among Adolescents with Bipolar Disorder

PRINCIPAL INVESTIGATOR:
Benjamin I. Goldstein, MD, PhD, FRCPC
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Toronto, Ontario M4N 3M5

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

SPONSOR: Heart and Stroke Foundation of Ontario
GENERAL INFORMATION
You are being asked to consider taking part in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood.

This form explains the purpose of this research study, provides information about the study, the tests and procedures involved, possible risks and benefits, and the rights of participants.

Please read this form carefully and ask any questions you may have. You may have this form and all information concerning the study explained to you. If you wish, someone may be available to verbally translate this form into your preferred language. You may take as much time as you wish to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. Please ask the study staff or the study doctor to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

Participating in this study is your choice (voluntary). You have the right to choose not to participate, or to stop participating in this study at any time.

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, in some cases, waist circumference is collected.

WHY IS THIS STUDY BEING DONE?
Bipolar disorder (manic-depressive illness) has been connected to early heart disease among adults. Standard heart disease risk factors such as high blood pressure, obesity, and abnormalities in blood sugar and cholesterol are common among adults with bipolar disorder. Previous research with sophisticated blood vessel imaging techniques has also shown that adolescents and adults with mood disorders may have reduced responsiveness of blood vessel walls and increased thickness of blood vessel walls. Overall, it appears that adolescents with bipolar disorder may be at increased risk of early heart disease.

This study aims to measure specific biological and genetic markers in the blood among adolescents with bipolar disorder and to find out whether these markers are associated with changes in their blood vessels. The levels of the biological markers and their association with changes in blood vessel response will be compared with those of adolescents without bipolar disorder.
The purpose of this research study is to help the study doctors better understand the underlying biology connecting bipolar disorder with heart disease, in order to help guide future research on prevention and treatment of heart disease among people suffering from 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 drugs which will take about 3 hours.

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**

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 8 hours prior to the blood draw). The blood draw will take 15 – 35 minutes. In addition, your height, weight, abdominal circumference and blood pressure will be measured, and you will complete two blood vessel imaging tests. One of the tests involves placing an ultrasound device on the outside of your neck while you are lying flat. This will take about 15 minutes. The other test involves placing an ultrasound over a blood vessel on your arm. The ultrasound will gather information while you are resting. Then a blood pressure cuff will be tightly inflated on your arm for 4 minutes so that it prevents blood flow. The ultrasound will again gather information one minute after the blood pressure cuff is released. This will take about 30 minutes.

Your parent can accompany you to the blood draw, and can wait just outside the ultrasound room. However, because the ultrasound procedures must be the same for everyone, parents may not be inside the ultrasound room.

The psychiatric interviews will take approximately 1-4 hours and the bloodwork and blood vessel imaging will take approximately 60-90 minutes. The total duration of study procedures may therefore take up to 5.5 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>
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<tr>
<td>TOTAL TIME:</td>
<td>1 – 4 hours</td>
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<tr>
<td>Informed Consent = 45 minutes</td>
<td>Ultrasound of neck = 15 minutes</td>
</tr>
<tr>
<td>Screening = 10 – 15 minutes</td>
<td>Ultrasound of arm = 30 minutes</td>
</tr>
<tr>
<td>Psychiatric Interview / complete self – report forms</td>
<td>Blood draw = 15 – 35 minutes</td>
</tr>
<tr>
<td></td>
<td>Measurements = 10 minutes</td>
</tr>
</tbody>
</table>
High resolution 2D - Doppler ultrasound machine that will be used:

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

It is expected that about 75 adolescents and their parents will take part in this study.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?

Although participation in this study is entirely voluntary, you are responsible for completing the full procedure for each visit, as outlined above. If you choose not to complete any of the requirements, you will not be able to participate in the study.

Please note the following information regarding the use and storage of the blood sample you will provide at visit 2:

Duration of Storage of Information

All blood 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 and heart disease). 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 RISKS OR HARMs OF PARTICIPATING IN THIS STUDY?

You may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be other side effects that are not expected. If you decide to take part in this study, you should contact the study doctor (Dr. Benjamin Goldstein) or study staff during business
hours with questions or concerns regarding any side effects or study-related injuries that you experience. The telephone number for this purpose is: 416-480-5328.

<table>
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<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
</thead>
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<tr>
<td>Mild discomfort from blood pressure cuff</td>
<td>Very Likely (30-100%)</td>
<td>Mild</td>
<td>Temporary</td>
</tr>
<tr>
<td></td>
<td>Likely (10-30%)</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Less Likely (1-10%)</td>
<td>Severe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rare (0-1%)</td>
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<td></td>
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<tr>
<td>Bruising from blood draw</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Infection from blood draw</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Emotional Discomfort</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
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</table>

It is possible that you will experience mild discomfort, bruising during or, rarely, infection as a result of the blood draw. You are also asked to start fasting 8 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 12 hours. You may also experience discomfort during the 4 minutes that the blood pressure cuff is tightly inflated during the ultrasound 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 ultrasound or 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.

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

You may or may not benefit directly from participating 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.
WHAT OTHER CHOICES ARE THERE?
For those who do not wish to participate in this study, they will not be affected in any way.

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?
You will not be paid to participate in this study.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?
You have the right to have any information about you and your health that is collected, used or disclosed for this study to be handled in a confidential manner.

If you decide to participate in this study, the investigator and study staff will look at your personal health information and collect only the information they need for this study. Personal health information refers to health information about you that could identify you because it includes information such as your:

• Name,
• Address,
• Telephone number,
• Date of birth,
• New and existing medical records, or
• The types, dates and results of various tests and procedures.

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

The following people may come to the hospital to look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines:

• Representatives of the Sunnybrook Research Ethics Board, a group of people who oversee the ethical conduct of research studies at Sunnybrook

Access to your personal information will take place under the supervision of the Principal Investigator.

“Study data" is information about you that is collected for the study, but that does not directly identify you. Any study data that is sent outside of the hospital will have a study code and will not contain your name or address or any information that directly identifies you. Study data that is sent outside of the hospital will be used for the research purposes explained in this consent form.

The investigator(s), study staff and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying you from the study data is very small, it can never be completely eliminated.

All study data will be stored in a secure and confidential location 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.

When the results of this study are published, your identity will not be disclosed.

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 the study doctor: Dr. Benjamin Goldstein, 416-480-5328.

DO THE INVESTIGATORS 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?
You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout this study.

If you have any questions about this study, you are encouraged to contact the study doctor: 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. Philip C. Hébert, Chair of the Sunnybrook Research Ethics Board at (416) 480-4276.

______________________________

Documentation of Informed Consent

You will be given a copy of this informed consent form after it has been signed and dated by you and the study staff.

Inflammation and Brain-Derived Neurotrophic Factor: At the Heart of Cardiovascular Risk among Adolescents with 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 authorize access to my personal health information (medical record) and research study data as explained in this form
• I have agreed to participate in this study
• I understand that my parent/s and I, and my family doctor, will be notified of abnormal findings in my blood sugar or cholesterol.

Name of Participant (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

Version December 16, 2011
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

For Parents of Adolescents 13-19 years of age

TITLE OF PROJECT:
Inflammation and Brain-Derived Neurotrophic Factor: At the Heart of Cardiovascular Risk among Adolescents with Bipolar Disorder

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

CO-INVESTIGATORS:

Krista Lanctot, PhD
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Anthony Levitt, MD, FRCPC
Sunnybrook Health Sciences Centre
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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

SPONSOR: Heart and Stroke Foundation of Ontario
GENERAL INFORMATION

You are being asked to consider taking part in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood.

This form explains the purpose of this research study, provides information about the study, the tests and procedures involved, possible risks and benefits, and the rights of participants.

Please read this form carefully and ask any questions you may have. You may have this form and all information concerning the study explained to you. If you wish, someone may be available to verbally translate this form into your preferred language. You may take as much time as you wish to decide whether or not to participate. Feel free to discuss it with your friends and family, or your family doctor. Please ask the study staff or the study doctor to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

Participating in this study is your choice (voluntary). You have the right to choose not to participate, or to stop participating in this study at any time.

INTRODUCTION

You are being asked to participate in this research study because your adolescent is either being treated for bipolar disorder through the Youth Psychiatry Division of Sunnybrook or because your adolescent 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, in some cases, waist circumference is collected. Blood work can also be routine practice for some patients.

WHY IS THIS STUDY BEING DONE?

Bipolar disorder (manic-depressive illness) has been connected to early heart disease among adults. Standard heart disease risk factors such as high blood pressure, obesity, and abnormalities in blood sugar and cholesterol are common among adults with bipolar disorder. Previous research with sophisticated blood vessel imaging techniques has also shown that adolescents and adults with mood disorders may have reduced responsiveness of blood vessel walls and increased thickness of blood vessel walls. Overall, it appears that adolescents with bipolar disorder may be at increased risk of early heart disease.

This study aims to measure specific biological and genetic markers in the blood among adolescents with bipolar disorder and to find out whether these markers are associated with changes in their blood vessels. The levels of the biological markers and their association with changes in blood vessel response will be compared with those of adolescents without bipolar disorder.
The purpose of this research study is to help the study doctors better understand the underlying biology connecting bipolar disorder with heart disease, in order to help guide future research on prevention and treatment of heart disease among people suffering from 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 and to answer questions regarding his/her medical history as well as his/her eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs all of which will take about 3 hours.

If he/she meets 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**

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 8 hours prior to the blood draw). The blood draw will take 15 – 35 minutes. In addition, his/her height, weight, abdominal circumference and blood pressure will be measured, and he/she will complete two blood vessel imaging tests. One of the tests involves placing an ultrasound device on the outside of your adolescent’s neck while he/she is lying flat. This will take about 15 minutes. The other test involves placing an ultrasound over a blood vessel on your adolescent’s arm. The ultrasound will gather information while he/she is resting. Then a blood pressure cuff will be tightly inflated on his/her arm for 4 minutes so that it prevents blood flow. The ultrasound will again gather information one minute after the blood pressure cuff is released. This will take about 30 minutes.

You may accompany your adolescent to the blood draw, and can wait for him/her just outside the ultrasound room. However, because the ultrasound procedures must be the same for everyone, parents may not be inside the ultrasound room.

The psychiatric interviews will take approximately 1-4 hours and the blood work and blood vessel imaging will take approximately 60-90 minutes. The total duration of study procedures may therefore take up to 5.5 hours.

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

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<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
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<tr>
<td><strong>TOTAL TIME:</strong></td>
<td>1 – 4 hours</td>
<td>60 – 90 minutes</td>
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<tr>
<td><strong>Visit 1</strong></td>
<td>Informed Consent = 45 minutes</td>
<td>Ultrasound of neck = 15 minutes</td>
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<td></td>
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<td>Ultrasound of arm = 30 minutes</td>
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<td>Screening = 10 – 15 minutes</td>
<td>Blood draw = 15 – 35 minutes</td>
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<tr>
<td>Psychiatric Interview / complete self – report forms = 3 hours</td>
<td>Measurements = 10 minutes</td>
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High resolution 2D – Doppler ultrasound machine that will be used:

**HOW MANY PEOPLE WILL TAKE PART IN THE STUDY**

It is expected that about 75 adolescents and their parents will take part in this study.

**WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?** Although participation in this study is entirely voluntary, you and your adolescent are responsible for completing the full procedure for each visit, as outlined above. If you or your adolescent chooses not to complete any of the requirements, you will both not be able to participate in the study.

Please note the following information regarding the use and storage of the blood sample your adolescent will provide at visit 2:

**Duration of Storage of Information**

All blood 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 your adolescent cannot be reached, your adolescent’s 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 and heart disease). 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 RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?**
Your adolescent may experience side effects from participating in this study. Some side effects are known and are listed below, but there may be other side effects that are not expected. If your adolescent decides to take part in this study, he/she should contact the study doctor (Dr. Benjamin Goldstein) or study staff during business hours with questions or concerns regarding any side effects or study-related injuries that he/she experiences. The telephone number for this purpose is: 416-480-5328.

<table>
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<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
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<td></td>
<td>Very Likely</td>
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<td></td>
<td>(30-100%)</td>
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<tr>
<td>Mild discomfort from blood pressure cuff</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Bruising from blood draw</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Infection from blood draw</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Emotional Discomfort</td>
<td>X</td>
<td>X</td>
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</table>

It is possible that your adolescent will experience mild discomfort, bruising during or, rarely, infection as a result of the blood draw. Your adolescent is also asked to start fasting 8 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 12 hours. Your adolescent may also experience discomfort during the 4 minutes that the blood pressure cuff is tightly inflated during the ultrasound 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 ultrasound or 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.

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

You may or may not benefit directly from participating 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.

**WHAT OTHER CHOICES ARE THERE?**

For those who do not wish to participate in this study, their adolescent’s care will not be affected in any way.

**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 and your adolescent can also choose to end participation at any time without having to provide a reason. If your adolescent chooses to withdraw, his/her choice will not have any effect on his/her 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 OF PARTICIPATING IN THIS STUDY?**

There is no cost for participation.

**WHAT HAPPENS IF I HAVE A RESEARCH RELATED INJURY?**

If your adolescent becomes sick or injured as a direct result of his/her participation in this study, his/her 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 or your adolescent do not give up any of your legal rights.

**ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?**

You and your adolescent will not be paid to participate in this study.

**HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?**

Your adolescent has the right to have any information about him/her and his/her health that is collected, used or disclosed for this study to be handled in a confidential manner.
If your adolescent decides to participate in this study, the investigator and study staff will look at his/her personal health information and collect only the information they need for this study. Personal health information refers to health information about your adolescent that could identify him/her because it includes information such as your adolescent’s:

• Name,
• Address,
• Telephone number,
• Date of birth,
• New and existing medical records, or
• The types, dates and results of various tests and procedures.

Your adolescent has the right to access, review and request changes to his/her personal health information.

The following people may come to the hospital to look at your adolescent’s personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines:

• Representatives of the Sunnybrook Research Ethics Board, a group of people who oversee the ethical conduct of research studies at Sunnybrook

Access to your adolescent’s personal information will take place under the supervision of the Principal Investigator.

“Study data” is information about your adolescent that is collected for the study, but that does not directly identify your adolescent. Any study data that is sent outside of the hospital will have a study code and will not contain your adolescent’s name or address or any information that directly identifies him/her. Study data that is sent outside of the hospital will be used for the research purposes explained in this consent form.

The investigator(s), study staff and the other people listed above will keep the information they see or receive about you confidential, to the extent permitted by applicable laws. Even though the risk of identifying your adolescent from the study data is very small, it can never be completely eliminated.

All study data will be stored in a secure and confidential location 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.

When the results of this study are published, your adolescent’s identity will not be disclosed.
You and your adolescent have the right to be informed of the results of this study once the entire study is complete. If either of you would like to be informed of the results of this study, please contact the study doctor: Dr. Benjamin Goldstein, 416-480-5328.

**DO THE INVESTIGATORS 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?**

You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout this study.

If you have any questions about this study, you are encouraged to contact the study doctor: 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. Philip C. Hébert, Chair of the Sunnybrook Research Ethics Board at (416) 480-4276.

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**Documentation of Informed Consent**

You will be given a copy of this informed consent form after it has been signed and dated by you and the study staff.

Inflammation and Brain-Derived Neurotrophic Factor: At the Heart of Cardiovascular Risk among Adolescents with 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 not be accessed for the purpose of this study
• I understand that my adolescent and I, and his/her family doctor, will be notified of abnormal findings in his/her blood sugar or cholesterol.
• I have agreed to participate in this study

Name of Parent/Caregiver (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

Version December 16, 2011
Source Document-Consenting Process

Name: __________________________________

Date: _____________ Time: _____________

Consenting for: ____________________________________________________

Points of Study Reviewed:
☐ Purpose of study
☐ Procedures
☐ Risk/Benefits
☐ Cost of participation
☐ Alternatives
☐ Confidentiality
☐ Voluntary participation
☐ Contact info

Questions Asked: _________________________________________________________
________________________________________________________________________
________________________________________________________________________

☐ Comprehension Assessed (please see attached self report form)

Additional comments: _____________________________________________________
________________________________________________________________________
________________________________________________________________________

Person Obtaining Consent Date
(signature)

Primary Investigator Date
(signature)
Informed Consent Evaluation Feedback Tool

Title of Study:

REB#:

1. The purpose of the research is:  or I’m not sure

2. Benefits of the research include:  or I’m not sure

3. Possible risks of the research include:  or I’m not sure

4. I have no other treatment options other than this study:
   True  False

5. The research study cost me money.
   True  False

6. Participation is voluntary.
   True  False

7. I must continue in the study until completion.
   True  False

8. I may not benefit by taking part in this study.
   True  False
Appendix III
**K-SADS MANIA RATING SCALE**

**BIPOLAR DISORDERS**

This rating scale is based on the items from the WASH-U-KSADS (Barbara Geller, M.D.) and the 4th Revision of the KSADS-P (Joaquim Puig-Antich, M.D. and Neal Ryan, M.D.). The following items are to determine the presence of mania or hypomania during a period of time prescribed by the rater/study. At the end of the scale, the rater should note the onset and offset of the time period being rated. If any of the items are judged present, inquire in a general way to determine how s/he was behaving at the time with such questions as, "When you were this way, what kind of things were you doing? How did you spend your time?" If there have been manic periods it is exceedingly important that they are clearly delineated. Whenever two or more items are scored positively, it is important to determine if they occurred at the same time.

If the subject has only described dysphoric mood, the following questions regarding the manic syndrome should be introduced with a statement such as, "I know you have been feeling (____), however, many people have other feelings mixed in or at different times too." The most difficult patients to assess are those in whom manic and depressed symptoms simultaneously coexist, superimposed on each other during the same times (Mixed States). The rater should keep this possibility in mind as s/he goes through this section.

### 1. Elation, Expansive Mood

Elevated mood and/or optimistic attitude toward the future which lasted at least 4 hours and was out of proportion to the circumstances. Differentiate from normal mood in chronically depressed subjects. Do not rate positive if mild elation is reported in situations like Christmas gifts, birthdays, amusement parks, which normally overstimulate and make children very excited.

- Have [ ] [ ] [ ] been times when you felt very good or too cheerful or high or terrific or great, or just not your normal self? If unclear: When you felt on top of the world or as if there was nothing you couldn’t do?... That this is the best of all possible worlds?
- Have you felt that everything would work out just the way you wanted? If people saw you, would they think you were just in a good mood or something more than that?
- Did you get as if you were drunk? Did you laugh a lot, get silly? Did you feel super happy? When did this happen?

**Most Severe Past Episode**

- P [ ]
- C [ ]
- S [ ]

### Worst week in past month

- No information [ ] [ ] [ ]
- Not at all, normal, or depressed [ ] [ ] [ ]
- Slight: Good spirits, more cheerful than most people in his/her circumstances, but of only possible clinical significance. [ ] [ ] [ ]
- Mild: Definitely elevated mood and optimistic outlook that is somewhat out of proportion to his/her circumstances. [ ] [ ] [ ]
- Moderate: Mood and outlook are clearly out of proportion to circumstances. Noticeable to others. [ ] [ ] [ ]
- Severe: Quality of euphoric mood way out of proportion to circumstances. [ ] [ ] [ ]
- Extreme: Clearlly elated, almost constantly elated, expression, or expansive. [ ] [ ] [ ]

### 2. IRRITABILITY AND ANGER

Subjective feeling of irritability, anger, crankiness, bad temper, short tempered, resentment, or annoyance, externally directed, whether expressed overtly or not. Rate the intensity and duration of such feelings. Do not rate here if irritability is due to depression or disruptive disorders.

- Do you get annoyed and irritated or cranky at little things?
- What kinds of things?
- Have you been feeling mad or angry also (even if you don’t show it)? How angry?
- More than before?
- What kinds of things make you feel angry?
- Do you sometimes feel angry and/or irritable, and/or cranky and don’t know why? Does this happen often?
- Do you lose your temper?
- With your family? Your friends? Who else? At school? What do you do?
- Has anybody said anything about it?
- How much of the time do you feel angry, irritable, and/or cranky: All of the time? Lots of the time? Just now and then? None of the time?
- When you get mad, what do you think about?
- Do you think about killing others? Or about hurting them or torturing them?
- Whom: Do you have a plan? How?

**Most Severe Past Episode**

- P [ ]
- C [ ]
- S [ ]

### Worst week in past month

- No information [ ] [ ] [ ]
- Not at all, clearly of no clinical significance [ ] [ ] [ ]
- Slight and doubtful clinical significance [ ] [ ] [ ]
- Mild: Often (at least 3X/1 hrs. or less) feels definitely more angry, irritable than called for by the situation, relatively frequent but not very intense. Or often argumentative, quick to express annoyance. No homicidal thoughts; [ ] [ ] [ ]
- Moderate: Most days irritable/angry or over 50% of the time. Often shouts, loses temper. Occasional homicidal thoughts. [ ] [ ] [ ]
- Severe: At least most of the time child is aware of feeling very irritable or quite angry or has frequent homicidal thoughts (no plan) or thoughts of hurting others. Or throws and breaks things around the house. [ ] [ ] [ ]
- Extreme: Most of the time feels extremely angry or irritable, to the point s/he “can’t stand it.” Or frequent uncontrollable tantrums. [ ] [ ] [ ]

---

**ID:**

**Date:** MM / DD / YYYY

**11503**
3. DECREASED NEED FOR SLEEP

Less need for sleep than usual in order to feel rested (average for several days when needed less sleep). (Refer to norms on insomnia)

Have you needed less sleep than usual to feel rested? How much sleep do you ordinarily need?
How much do you sleep when you are feeling so good?
When you wake up do you feel good and rested?

When you cannot fall asleep or when you get up through the night, what types of things do you do?
Watch TV? Read? or do you do active things? (e.g., rearrange furniture? clean house? exercise?)
Do you have a lot of thoughts go through your mind when awake? What kinds of thoughts?
Do you worry? About what types of things?
How long are you awake? How often during the night? During the week?

Most Severe Past Episode □ □ □

Worst week in past month

P C S
[ ] [ ] [10] No information
[ ] [ ] [11] No change or more sleep needed
[ ] [ ] [12] Up to 1 hour less than usual
[ ] [ ] [13] Up to 2 hours less than usual
[ ] [ ] [14] Up to 3 hours less than usual
[ ] [ ] [15] Up to 4 hours less than usual
[ ] [ ] [16] 4 or more hours less than usual

4. UNUSUALLY ENERGETIC

More active than his/her usual level without expected fatigue.

Have you had more energy than usual to do things?
Did people tell you that you were (are) non-stop?
Did you agree with them? Did it seem like too much energy? Do you know why? Were you doing too many things? Did you feel tired?
When did this happen? (example)

Most Severe Past Episode □ □ □

Worst week in past month

P C S
[ ] [ ] [10] No information
[ ] [ ] [11] No difference than usual or less energetic
[ ] [ ] [12] Slightly more energetic but of questionable significance
[ ] [ ] [13] Little change in activity level but less fatigued than usual
[ ] [ ] [14] Somewhat more active than usual with little or no fatigue
[ ] [ ] [15] Much more active than usual with little or no fatigue
[ ] [ ] [16] Unusually active all day long with little or no fatigue

5a. INCREASE IN GOAL-DIRECTED ACTIVITY

As compared with usual level. Consider changes in scholastic, social, sexual, or leisure involvement or activity level associated with work, family, friends, new projects, interests, or activities (e.g., telephone calls, letter writing)

Is there any time when you were more active or involved in things compared to the way you usually are? What about in school, at your club, scouts, church, at home, friends, hobbies, new projects or interests?
Were you doing a lot of things?
How much of your day has been spent in this?
Were you trying to do so many different things that you couldn’t keep up?
When did this happen? (example)

Most Severe Past Episode □ □ □

Worst week in past month

P C S
[ ] [ ] [10] No information
[ ] [ ] [11] No change or decrease
[ ] [ ] [12] Slightly more interest or activity but of questionable significance
[ ] [ ] [13] Mild but definite increase in general activity level involving several areas
[ ] [ ] [14] Moderate generalized increase in activity level involving several areas
[ ] [ ] [15] Marked increase and almost constantly involved in numerous activities in many areas
[ ] [ ] [16] Extreme, e.g., constantly active in a variety of activities from awakening until going to sleep
5b. MOTOR HYPERACTIVITY

Visible manifestations of generalized motor hyperactivity which occurred during a period of abnormally elevated, expansive, or irritable mood. Make certain that the hyperactivity actually occurred and was not merely a subjective feeling of restlessness. Make sure it is not chronic but episodic hyperactivity.

When you were ( ), were there times when you were (high, feeling so good, so angry) that you were always moving, could not stay put, were unable to sit still or you always had to be moving, pacing up and down? Or are you always like that?

Most Severe Past Episode

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6. GRANDIOSITY

Increased self-esteem and appraisal of his/her worth, power, or knowledge (up to grandiose delusions) as compared with usual level. Persecutory delusions should not be considered evidence of grandiosity unless that subject feels the persecution is due to some special attributes of his/her e.g., power, knowledge).

Have you felt more self-confident than usual? Have you felt much better than others? ...smarter? ...stronger? Why? Have you felt that you are a particularly important person or that you had special talents or abilities? What about special plans? When did this happen? (example)

Most Severe Past Episode

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7. ACCELERATED, PRESSURED OR INCREASED AMOUNT OF SPEECH

When you were ( ), were there times that you talked very rapidly or talked on and on and couldn’t be stopped? Did people say you were talking too much? Could people understand you?

Most Severe Past Episode

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8a. RACING THOUGHTS
Subjective experience that thinking was markedly accelerated.
When you were (___), were there times when your thoughts raced through your mind?
Did you have more ideas than usual or more than you could handle?

Most Severe Past Episode  P  C  S

Worst week in past month
[ ] [ ] [ ] No information
[ ] [ ] [1] Not at all
[ ] [ ] [2] Doubtful
[ ] [ ] [3] Mild: Occasional racing thoughts at least 3 times per week
[ ] [ ] [4] Moderate: Racing thoughts at least 50% of awake time
[ ] [ ] [5] Severe: Racing thoughts most of the time
[ ] [ ] [6] Extreme: Almost constant racing thoughts

8b. FLIGHT OF IDEAS (Observed or reported by informant)
Accelerated speech with abrupt changes from topic to topic, usually based on understandable associations, distracting stimuli or play on words. In rating severity consider speed of associations, inability to complete ideas and sustain attention in a goal-directed manner. When severe, complete or partial sentences may be galloping on each other so fast that apparent sentence to sentence derailment and/or sentence incoherence may also be present. An extreme example of this symptom is "You have to be quick to be sad. Everything having to do with it is quiet on the q.t., sit, sob, sigh, sin, sorrow, suicide, sought, sand, sweet mother’s love and salvation."
Have there been times when people could not understand you? When they said you did not make sense? Could you give me an example?

Most Severe Past Episode  P  C  S

Worst week in past month
[ ] [ ] [ ] No information
[ ] [ ] [1] Not at all or some other form of
[ ] [ ] [2] Slight: Occasional instances, which are of doubtful clinical significance
[ ] [ ] [3] Mild: Occasional instances of abrupt change in topic with some impairment in understandability, >5% of sentence to sentence transitions are abrupt
[ ] [ ] [4] Moderate: Frequent instances with moderate impairment in understandability. >10%
[ ] [ ] [5] Severe: Very frequent instances with definite impairment in understandability. >25%
[ ] [ ] [6] Extreme: Most of speech consists of such rapid changes of topic that is impossible to follow. >50%

9. POOR JUDGEMENT
Excessive involvement in dangerous activities without recognizing the high potential for painful consequences.
When you were (___), did you do anything that caused trouble for you or your family...or friends?
What about anything that could have?
Did you do things you normally wouldn’t do (like giving away a whole lot of things or taking a whole lot of chances)?
Did you think of what would happen before you did it?
Was there anything that you did that you now think you should not have done?

Most Severe Past Episode  P  C  S

Worst week in past month
[ ] [ ] [ ] No information
[ ] [ ] [1] Not at all
[ ] [ ] [2] Slight: Of doubtful clinical significance
[ ] [ ] [3] Mild: e.g., Calls friends at odd hours
[ ] [ ] [4] Moderate: e.g., Purchases many things she/he doesn’t need and can’t afford or gives money away
[ ] [ ] [5] Severe: e.g., On impulse, goes to places without plans or money and takes too many chances
[ ] [ ] [6] Very Severe: Attempts activities with potentially very dangerous consequences
10. DISTRACTIBILITY (Observed or reported by informant)

Child presents evidence of difficulty focusing his/her attention on the questions of the interviewer, jumps from one thing to another, cannot keep track of his/her answers, and is drawn by irrelevant stimuli he cannot shut off. Not to be confused with avoidance of uncomfortable themes.

Have you ever been told that you have trouble sticking to what you are supposed to do? Did you?
Can you give me an example?
Has a teacher told you that you "always" get distracted?

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11. HALLUCINATIONS

Sometimes children, when they are alone, hear voices or see things, or smell things and they don't quite know where they come from.

Has this happened to you?
Do you ever hear voices when you are alone?
Have you ever seen things that were not there?
When did you?
What did you see?
What did you hear?
Has there been anything unusual about the way things sounded?

How often have you heard these voices (noises)? (smell, feeling, visions) is it some of the time, only now and then, most of the time, or all of the time?

What do you think it is?
Do you think it is your imagination or real?
Did you think it was real when you (heard, saw, etc.) it?
Do you think it's real or your imagination?
What did you do when you (heard, saw, etc.) it?

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12. DELUSIONS

Do you know what imaginations is? Tell me.
Sometimes does your imagination play tricks on you? What kind of tricks?

Tell me more about them.
Do you have any ideas about things that you don't tell anyone because they might not understand? What are they?
Do you have any secret thoughts? Tell me about them.
Do you believe in other things that other people don't believe in? Like what?
Is anybody out to hurt you?
Does anybody control your mind or body (like a robot)?
Is anything happening to your body?
Do you ever think you are an important or great person? Who?

Are you sure that this (...?) is this way?
Could there be any other reason for it?
Who do you know that it happens as you say?
Any other possible explanation?
Do you enjoy making up stories like this?
Or is it different from making up stories?
(you might suggest other possible explanations and see how the subject reacts to them)

Did you ever think that this was your imagination?
Do you think it could be your imagination?
What did you do about it?

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**13. MOOD LABILITY**

Changability of mood; rapid mood variation with several mood states (angry, elated, depressed, anxious, relaxed) within a brief period of time; appears internally driven without regard to circumstances or not related to anything external to the patient. Could be an exaggerated mood change in regard to minor slights, frustrations or positive events.

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Current time period rated:

Onset / / Offset

Most severe past time period rated:

Onset / / Offset

To score this interview:

Add the summary scores for items 1-13. Note: Use the higher score for items 5a and 5b and the higher score for items 8a and 8b.

If the number answered >10, calculate the MRS score by:

\[
\text{(total} \times \left( \frac{13}{\text{number answered}} \right) \right) - 13
\]

Check if Reliability □ Yes

Child's Initials □□ Interviewer's Initials: □□

ID: □□□ 11503
K-SADS-P DEPRESSION SECTION

1. DEPRESSED MOOD

Worst week in past month

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<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>1 Not at all or less than once a week</td>
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<td>2 Slight: Occasionally has dysphoric mood at least once a week for more than 1 hour</td>
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<td>3 Mild: Often experiences dysphoric mood at least 3 times a week for more than 3 hours each</td>
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<td>4 Moderate: Most days feels &quot;depressed&quot; (including weekends) or over 50% of awake time</td>
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<td>5 Severe: Most of the time feels depressed and it is almost painful. Feels wracked</td>
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<td>6 Extreme: Most of the time feels extreme depression which i can't stand</td>
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<td>7 Very Extreme: Constant unrelieved extremely painful feelings of depression</td>
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2. IRRITABILITY AND ANGER

Worst week in past month

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<td>0 No Information</td>
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<td>[ ]</td>
<td>[ ]</td>
<td>1 Not at all clearly of no clinical significance</td>
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<tr>
<td>[ ]</td>
<td>[ ]</td>
<td>2 Slight and doubtful clinical significance</td>
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<td>3 Mild: Often (at least 3 times/3 hours each week) feels definitely more angry, irritable than called for by the situation, relatively often but never very intense. Or often argumentative, quick to express annoyance. No homicidal thoughts</td>
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<td>4 Moderate: Most days feels irritable/angry or over 50% of awake time. Or often shouts, loses temper. Occasional homicidal thoughts</td>
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<td>5 Severe: At least most of the time feels extremely irritable or angry or has frequent homicidal thoughts (no plan) or thoughts of hurting others. Or throws and breaks things around the house</td>
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<td>[ ]</td>
<td>6 Extreme: Most of the time feels extremely irritable or angry, to the point he can't stand it. Or frequent uncontrollable</td>
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<td>[ ]</td>
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<td>7 Number 6 plus homicidal plan</td>
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Most Severe Past Episode

ID:  
Date: MM/DD/YYYY

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3. EXCESSIVE OR INAPPROPRIATE GUILT

...self reproach, for things done or not done, including delusions of guilt. Rate according to proportion between intensity of guilt feelings or severity of punishment child thinks he deserves and the actual misdeeds.

When people say or do things that are good, they usually feel good, and when they say or do something bad they feel bad about it. Do you feel bad about anything you have done? What is it? How often do you think about it? When did you do that? What does it mean if I said I feel guilty about something? How much of the time do you feel like this? Most of the time? A lot of the time? A little of the time? Not at all?

What kind of things do you feel guilty about? Do you feel guilty about things you have not done? or are actually not your fault? Do you feel guilty about things your parents or others do? Do you feel you cause bad things to happen? Do you think you should be punished for this? What kind of punishment do you feel you deserve? Do you want to be punished? How do your parents usually punish you? Do you think it's enough?

For many young children it is preferable to give a concrete example such as: I am going to tell you about three children and you tell me which one is most like you. The first is a child who does something wrong, then feels bad about it, goes and apologizes to the person, the apologies are accepted, and he just forgets about it from then on. The second child is like the first but after his apologies are accepted, he just cannot forget about what he had done and continues to feel bad about it for one to two weeks. The third is a child who has not done much wrong, but who feels guilty for all kinds of things which are really not his fault like... Which one of these three children is like you? It is also useful to double check the child's understanding of the questions by asking him to give an example, like the last time he felt guilty "like the child in the story."

4. NEGATIVE SELF-IMAGE

Includes feelings of inadequacy, inferiority, failure and worthlessness, self-deprecation, self-betitling. Rate with disregard of how "realistic" the negative self evaluation is.

How do you feel about yourself? Are you down on yourself? Do you like yourself as a person? Why? or Why not? Describe yourself. Do you ever think of yourself as ugly? When? How often? Do you think you are bright or stupid? Why? Do you often think like that? Do you think you are better or worse than your friends? Is any one of your friends worse than you are? What things are you good at? Any others? What things are you bad at? How often do you feel this way about yourself? What would you like to change about you?

Most Severe Past Episode

Worst week in past month

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<tr>
<td>1</td>
<td>Not at all</td>
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<tr>
<td>2</td>
<td>Slight: Occasional feelings of inadequacy</td>
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<td>3</td>
<td>Mild: Often feels somewhat inadequate, or would like to change his looks or brains or his personality</td>
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<td>Moderate: Often feels like a failure, or would like to change 2 of the above</td>
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<td>Severe: Frequent feelings of worthlessness or would like to change all 3. Occasionally says he hates himself</td>
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<td>6</td>
<td>Extreme: Pervasive feelings of being worthless or a failure. Says he hates himself</td>
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5. HOPELESSNESS, HELPLESSNESS, DISCOURAGEMENT, PESSIMISM

Negative outlook toward the future, regarding his life and his current problems. This item refers to ideational content and not to feelings.

**Worst week in past month**

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<td>Not at all discouraged about the future</td>
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<td>Slight: Occasional feelings of mild discouragement about future</td>
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<td>Mild: Often discouraged. Doubts he will get better</td>
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<td>Moderate: Often feels quite pessimistic about the future. Doubts he will make it to being a grown up</td>
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<td>Severe: Pervasive feelings of intense pessimism. Has given up. Helpless</td>
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<td>Extreme: Delusions or hallucinations that he is doomed, or that the world is coming to an end</td>
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ANHEDONIA, LACK OF INTEREST, APATHY, LOW MOTIVATION, OR BOREDOM

This is a summary rating synthesizing anhedonia, boredom and loss of interest.

**Boredom** is a term all children understand and which frequently refers to loss of ability to enjoy (anhedonia) or to loss of interest or both. Loss of pleasure and loss of interest are not mutually exclusive and may coexist.

What are the things you do for fun? Enjoy?
(Examples: Nintendo, sports, friends, favorite games, school subjects, outings, family activities, favorite TV programs, computer or video games, music, dancing, playing alone, reading, going out, etc.)

Do you feel bored a lot of the time?
Are you bored because you don't enjoy things or because you are not interested in even starting them?
Do you feel bored when you think about doing these things you used to do before you began feeling (sad, etc.)? (Give examples mentioned above.)
Does this stop you from doing these things?
Do you (also) feel bored while you are doing things you used to enjoy?

**Anhedonia** refers to partial or complete (pervasive) loss of ability to get pleasure, enjoy, have fun during participation in activities which have been attractive to the child like the ones listed above. It also refers to basic pleasures like those resulting from eating favorite foods and, in adolescents, sexual activities.

Do you still do the things you used to do for fun before you began to feel (_____)?
Do you do less than you used to? How much less?
Do you have as much fun doing them as you used to before you began feeling (sad, etc.)?
If less fun, do you enjoy them a little less? Much less? Not at all?
Do you have as much fun as your friends?
How many things are less fun now than they used to be?
How many are as much fun? More fun?
What are your favorite foods?
Do you enjoy them as much as you used to?
Are there any foods you really enjoy eating? Do they taste as good?

In adolescents: (If sexually active)
Do you enjoy sex as much as you used to?
Are you less sexually active than you used to be?
Do you find that you start to do things that interest you, but then find you are not enjoying them as much?

**Loss of interest, apathy and low motivation** refer to partial or complete (pervasive) loss of ability to anticipate enjoyment and to be interested and/or to have the motivation to pursue activities which have been attractive to the child. The child does not desire to engage in activities and does not initiate them. There is a lack of enthusiasm and anticipatory excitement, not caring about, apathy, lack of motivation in the contemplation of doing things he/she would normally look forward to.

Do you look forward to doing the things you used to enjoy? (Give examples)
Do you try to get into them?
Do you have to push yourself to do your favorite activities? Do they interest you?
Do you get excited or enthusiastic about doing them? Why not?

Have you stopped even trying to do things that you used to do because they just don't excite you anymore?
How many things are less interesting now than they were before you started feeling (sad, etc.)?
How many things are as interesting? More interesting?

**WHAT ABOUT DURING THE LAST WEEK?**

This item does not refer to inability to engage in activities (loss of ability to concentrate on reading, games, TV, or school subjects).

Two comparisons should be made in each assessment: Enjoyment as compared to that of peers and/or enjoyment as compared to that of child when not depressed. The second is not possible in episodes of long duration because normally children's preferences change with age. Severity is determined by the number of activities which are less enjoyable to the child, and by the degree of loss of ability to enjoy.

Do not confound with lack of opportunity to do things which may be due to excessive parental restrictions.
### Fatigue, Lack of Energy, Tiredness

This is a subjective feeling. (Do not confuse with lack of interest) (Rate presence even if subject feels it is secondary to insomnia). Differentiate from drowsiness, sleepiness, etc. which should not be rated here.

- **Have you been feeling tired? How often?**
- **Do you feel tired?**
- **All of the time?**
- **Most of the time?**
- **Some of the time?**
- **Now and then?**
- **When did you start feeling so tired?**
- **Was it after you started feeling (______)?**
- **Tell me more about this feeling; is it sleepiness or that you just do not have the energy?**
- **Do you spend much time resting? How much?**
- **Do you have to rest?**
- **Do your limbs feel heavy?**
- **Is it very hard to get going? ... to move your legs?**

#### Worst week in past month

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<td>2 Slight: Possible less energy than usual</td>
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<td>3 Mild: At times definitely more tired or less energy than usual</td>
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<td>4 Moderate: Often feels tired without energy. Has to rest (not sleep) during the day</td>
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<td>5 Severe: Almost all the time feels very tired or without energy or spends a great deal of time resting, (not sleeping). Limbs may feel heavy and hard to move</td>
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<td>6 Extreme: Constant feeling of extreme fatigue or lack of energy or spends most of the time resting. Limbs feel heavy and hard to move</td>
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#### Most Severe Past Episode

### Difficulty Concentrating, Inattention, Slowed Thinking

(School information may be crucial to proper assessment of this item).

Complaints (or evidence from teacher) of diminished ability to think or concentrate which was not present to the same degree before onset of present episode. Distinguish from lack of interest or motivation. (Do not include if associated with formal thought disorder). Distinguish from ADHD.

- **Do you know what it means to concentrate?**
- **Sometimes children have a lot of trouble concentrating. For instance, they have to read a page from a book, and can’t keep their mind on it so it takes much longer to do it or they just can’t do it, can you pay attention?**
- **Have you been having this kind of trouble? When did it begin?**
- **Is your thinking slowed down?**
- **If you push yourself very hard can you concentrate?**
- **Does it take longer to do your homework?**
- **When you try to concentrate on something, does your mind drift off to other thoughts?**
- **Can you pay attention in school?**
- **Can you pay attention when you want to do something you like?**
- **Do you forget about things a lot more?**
- **What things can you pay attention to?**
- **Is it that you can’t concentrate? or is it that you are not interested, or don’t care?**
- **Did you have this kind of trouble before?**
- **When did it start?**

#### Worst week in past month

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<td>1 Not at all</td>
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<td>2 Slight: Slight and of doubtful clinical significance</td>
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<td>3 Mild: Definitely aware of limited attention span but causes no difficulties other than substantially increased effort in schoolwork</td>
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<td>4 Moderate: Interferes with school marks. Forgetful</td>
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<td>5 Severe: Interferes with school work and most other activities. Can’t concentrate even when he wants to. Very forgetful</td>
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<td>6 Extreme: Unable to do the simplest tasks, e.g., watch TV, or engage in a conversation</td>
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#### Most Severe Past Episode

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**NOTE: IF CHILD HAS ATTENTION DEFICIT DISORDER, DO NOT RATE POSITIVELY, UNLESS THERE WAS A WORSENING OF THE CONCENTRATION PROBLEMS ASSOCIATED WITH THE ONSET OF DEPRESSED MOOD.**
**9. PSYCHOMOTOR AGITATION**

Includes inability to sit still, pacing, fidgeting, repetitive lip or finger movement, wringing of hands, pulling at clothes, and non-stop talking. To be rated positive, such activities should occur **while the subject feels depressed, not associated with the manic syndrome, and not limited to isolated periods when discussing something upsetting. Do not include subjective feelings of tension or restlessness, which are often incorrectly called agitation.** To arrive at your rating, take into account your observations during the interview, the child’s report and the parent’s report about the child’s behavior during the episode. **Distinguish from ADHD.**

When you feel so (sad), are there times when you can’t sit still, or you have to keep moving and can’t stop?
Do you walk up and down?
Do you wring your hands? (demonstrate)
Do you pull or rub on your clothes, hair, skin or other things?
Do people tell you not to talk so much?
Did you do this before you began to feel (sad)?
When you do these things, is it that you are feeling (sad) or do you feel high or great?

If someone was taking movies of you while you were eating breakfast and talking to your (mother), and they took these movies before you got (depressed) and again while you were (depressed) would I be able to see a difference?
What would it be?
What would I see?
What would I hear?
Probe: Would it take longer before or while you were (depressed)?
A little longer?
Much longer?
If I saw a videotape or heard an audiotape of your child at home while he/she was depressed and another when he/she wasn’t depressed, could I tell the difference? If yes, what would I see (hear) different?

Make sure it does not refer to content of speech or acts or to facial expression. Refer only to speed and tempo.

**NOTE:** IF CHILD HAS ATTENTION DEFICIT DISORDER, DO NOT RATE THE PSYCHOMOTOR AGITATION ITEM POSITIVELY UNLESS THERE WAS A WORSENING OF AGITATION THAT CORRESPONDED WITH THE ONSET OF THE DEPRESSED MOOD.

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<td>Not at all, retarded, or associated with manic</td>
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<td>Slight: Increase which is of doubtful significance</td>
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<td>Mild: Unable to sit quietly in a chair without fidgeting or pulling and/or rubbing</td>
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<td>Moderate: Frequent temper tantrums, or marked inability to sit in class, almost always disruptive to some degree</td>
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<td>Marked: Pacing, hand-wringing, or very frequent temper tantrums. Increased activity both at home and school</td>
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<td>Extreme: Almost constantly moving or pacing about or nonstop talking. Agitated in all settings</td>
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**10. PSYCHOMOTOR RETARDATION**
Visible, generalized slowing down of physical movement, reactions and speech. It includes long speech latencies. Make certain that slowing down actually occurred and is not merely a subjective feeling. To arrive at your rating take into account your observations during the interview, the child's report and the parent's report about the child's behavior during the episode.

Since you started feeling (sad) have you noticed that you can't move as fast as before?
Have you found it hard to start talking?
Has your speech slowed down?
Do you talk a lot less than before?
Since you started feeling sad, have you felt like you are moving in slow motion?
Have other people noticed it?

If someone was taking movies of you while you were eating breakfast and talking to your (mother), and they took these movies before you got (depressed) and again while you were (depressed) would I be able to see a difference?
What would it be?
What would I see?
What would I hear?

*Probe:* Would it take longer before or while you were (depressed)? A little longer? Much longer?
If I saw videotape or heard an audiotape of your child at home while he/she was depressed and another when he/she wasn't depressed, could I tell the difference? If yes, what would I see (hear) different?

Make sure it does not refer to content of speech or acts or to facial expression. Refer only to speech and tempo.

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**14. INSOMNIA**
Sleep disorder, including initial, middle and terminal difficulty in getting to sleep or staying asleep.
Do not rate if he feels no need for sleep.

Take into account the estimated number of hours slept and the subjective sense of lost sleep.
Normally a 6-8 year old child should sleep about 10 hours ± 1 hour;
For 9-12 year olds = 9 hours ± 1 hour;
For 12-16 year olds = 8 hours ± 1 hour.

Distinguish from other possible causes of insomnia.

Have you had trouble sleeping? What kind of trouble?
How long does it take you to fall asleep?
Do you wake up in the middle of the night? How many times? Any reason for it (urinating, nightmares)?

At what time do you wake up in the morning?
Is that later or earlier than usual?
Do you wake up before you want, or have to get up? Or before your mother calls you?
Do you feel you would sleep more if you could?

For how long have you been having trouble sleeping?
Are you having this trouble every night? Almost every night?
Sometimes? Only now and then?
Do you feel rested when you wake up?
Do you feel not rested through 3 hours after being up?
Have you slept, at some point during the day and been awake during the right, and just could not sleep?

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**Worst week in past month**

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<td>2 Slight, and of doubtful clinical significance</td>
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<td>3 Mild: Convesation is noticeably retarded but not strained, and/or slowed body movements</td>
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<td>4 Moderate: Conversation is difficult to maintain, and/or hardy moves at all</td>
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<td>5 Marked: Conversation is difficult to maintain, and/or moves very slowly</td>
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<td>6 Extreme: Conversation is almost impossible, mute and immobile most of the time (depressive stupor)</td>
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</table>

**Most Severe Past Episode**

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**Worst week in past month**

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<tbody>
<tr>
<td></td>
<td></td>
<td>0 No information</td>
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<td>1 Not at all, or feels no need for any sleep</td>
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<td></td>
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<td>2 Slight: Occasional difficulty</td>
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<td>3 Mild: Often (at least 2 times a week) has some significant difficulty. (At least 1 hour to fall asleep, or bedtime delayed for one hour. No middle or terminal insomnia.)</td>
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<td>4 Moderate: Usually has considerable difficulty. (Either at least 2 hours initial insomnia, or any middle or terminal insomnia unrelated to urination, lasting up to half an hour). Feeling of unrestorative sleep</td>
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<td>5 Severe: Almost always has great difficulty. Either at least 3 hours initial insomnia or any middle or terminal insomnia lasting over one hour total. Considerable circadian reversal</td>
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<td>6 Extreme: Claims he almost never sleeps and feels exhausted the next day or complete circadian inversion</td>
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</table>

**Most Severe Past Episode**

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ID: 13932
**12. HYPERSOMNIA**
Do not rate positive if daytime sleep time plus nighttime true
sleep equals normal sleep time (compensatory naps).
Increased need for sleep, sleeping more than usual. Inquire about
hypersomnia even if insomnia was rated 3–6. Sleeping more than
norms in 24-hour period.

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Are you sleeping longer than usual?
Do you go back to sleep after you wake up in the morning?
When did you start sleeping longer than usual?
What about taking long naps during the day?
Did you use to take naps before?
When did you start to take naps?
How many hours did you use to sleep before you started to feel so
(sad)?

Parents may say that if child was not awakened he/she would
regularly sleep >11-12 hours and he/she actually does so, every
time he is left on his own. This should be rated 3.

**13. ANOREXIA**
Appetite compared to usual or to peers if episode is of long duration.
Make sure to differentiate between decrease of food intake because
of dieting and because of loss of appetite.
Rate here loss of appetite only.

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How is your appetite? Do you feel hungry often?
Are you eating more or less than before?
Do you leave food on your plate?
When did you begin to lose your appetite?
Do you sometimes have to force yourself to eat?
When was the last time you felt hungry?
Are you on a diet? What kind of diet?

**14. WEIGHT LOSS**
Total weight loss from usual weight since onset of the present episode
(or maximum of 12 months). Make sure he has not been dieting. In
the assessment of weight loss it is preferable to obtain recorded weights
from old hospital charts or the child’s pediatrician. Failure to gain 1.5
kg over a 6-month period for children between 5 and 11 years old
qualifies as weight loss, as does loss of percentile grouping over a
6-month period (low tables). Groupings are: Under 3rd %ile:
between 3-10; 10-25; 25-50; 50-75; 75-95; 95-97; and over 97th %ile.
Rate this item even if later he regained weight or became overweight.
If possible, rater should have verified weights available at time of
interview.

Have you lost any weight since you started feeling sad?
How do you know?
Do you find your clothes are looser now?
When was the last time you were weighed?
How much did you weigh then?
What about now? (measure it).

NOTE: DO NOT RATE POSITIVELY IF CHILD HAS ANOREXIA.
15. INCREASED APPETITE
As compared to usual. Inquire about this item even if anorexia and/or weight loss were rated 3-6.

Have you been eating more than before? Since when?
Is it like you feel hungry all the time? Do you feel this way every day?
Do you eat less than you would like to eat? Why?
Do you have cravings for sweets?
What do you eat too much of?

Most Severe Past Episode

16. WEIGHT GAIN
Total weight gain from usual weight during present episode (or a maximum of the last 12 months) not including gaining back weight previously lost or not gained according to the child's usual percentile for weight.

Have you gained any weight since you started feeling sad?
How do you know?
Have you had to buy new clothes because the old ones did not fit any longer?
What was your last weight?
When were you weighed last?

Most Severe Past Episode

17. SUICIDAL IDEATION
This includes preoccupation with thoughts of death or suicide and auditory command hallucinations where the child hears a voice telling him to kill himself or even suggesting the method. Do not include mere fears of dying.

Sometimes children who get upset or feel bad think about dying or even killing themselves.
Have you ever had such thoughts?
How would you do it?
Do you have a plan?
Have you told anybody (about suicidal thoughts)?
When did you start to think about suicide?
Have you actually tried to kill yourself? When? What did you do?
Any other thing? Did you really want to die? How close did you come to actually doing it?

Most Severe Past Episode

ID:
18. Number of discrete suicidal acts (gestures or attempts) since onset of present episode (or up to the last 12 months)*
   Note: "0" indicates none or no information

Worst week in past month
P: [ ] [ ] C: [ ] [ ] S: [ ] [ ]

Most Severe Past Episode
P: [ ] [ ] C: [ ] [ ] S: [ ] [ ]

19. SUICIDAL ACTS—SERIOUSNESS
Judge the seriousness of suicidal intent as expressed in his suicidal act like: Likelihood of being rescued; precautions against discovery; actions to gain help during or after attempt; degree of planning; apparent purpose of the attempt (manipulative or truly suicidal intent).

How did you try to kill yourself?
Was anybody in the room? In the apartment?
Did you tell them in advance?
How were you found?
Did you really want to die?
Did you ask for any help after you did it?

Worst week in past month
P: [ ] [ ] [ ] C: [ ] [ ] [ ] S: [ ] [ ] [ ]
   0 No information or no attempt
   1 Obviously no intent, purely manipulative gestures
   2 Not sure or only minimal intent
   3 Definite but very ambivalent
   4 Serious
   5 Very serious
   6 Extreme (every expectation of death)

Most Severe Past Episode
P: [ ] [ ] C: [ ] [ ] S: [ ] [ ]

20. SUICIDAL ACTS—MEDICAL LETHALITY
Actual medical threat to life or physical condition following the most serious suicidal act.

Take into account the method, impaired consciousness at time of being rescued, seriousness of physical injury, toxicity of ingested material, reversibility, amount of time needed for complete recovery and how much medical treatment needed.

How close were you to dying after your (most serious suicidal act)?

Worst week in past month
P: [ ] [ ] C: [ ] [ ] S: [ ] [ ] [ ]
   0 No information or no attempt
   1 No danger, e.g., no effects, held pills in hand
   2 Minimal, e.g., scratch on wrist
   3 Mild, e.g., took 10 aspirin, mild gastritis
   4 Moderate, e.g., took 10 seconds, had brief unconsciousness
   5 Severe, e.g., cut throat, hanging
   6 Extreme, e.g., respiratory arrest, prolonged coma

Most Severe Past Episode
P: [ ] [ ] C: [ ] [ ] S: [ ] [ ]
21. RECURRENT THOUGHTS OF DEATH

(Not just fear of dying). The patient has not made suicidal gestures or statements but has verbalized, and/or has had thoughts of death, or being better off dead.

Sometimes children who get upset or feel bad, wish they were dead or feel they'd be better off dead. Have you ever had these type of thoughts? When? Do you feel that way now? Was there ever another time you felt that way?

Worst week in past month

P C S

[ ] [ ] [ ] 0 No information

[ ] [ ] [ ] 1 Not present

[ ] [ ] [ ] 2 Slight: Transient, infrequent, thoughts of wishing to be dead. One time per week or less, for a very brief period of time

[ ] [ ] [ ] 3 Mild: Occasional thoughts of death, 2-3 times a week. Occasional statements like "I wish I was dead" in the context of anger or frustration

[ ] [ ] [ ] 4 Moderate: Often has thoughts of death, i.e., almost every day and often verbalizes thoughts of being better off dead

[ ] [ ] [ ] 5 Severe: Frequent statements re: desire to be dead, daily or several times per day

[ ] [ ] [ ] 6 Extreme: Constant preoccupation with dying, wishing to be dead

Most Severe Past Episode

P C S

Unset / / 

Utset / / 

Most severe past time period rated:

Unset / / 

Utset / / 

To score this interview:

Add the summary scores for the following ****items.

1. Depressed Mood
2. Irritable Mood
3. Guilt
4. Anhedonia
5. Fatigue
6. Difficulty Concentrating
7. Psychomotor Agitation
8. Psychomotor Retardation
9. Insomnia
10. Hypersomnia
11. Anorexia
12. Increased Appetite
13. Suicidal Idation

If the number answered > 10, then:

(total x (13/ number answered)) - 13

Check if Reliability __ Yes
ID: [ ] [ ] [ ]
Interviewer's Initials: [ ] [ ] [ ]
ALIFE BASE
(Psychosocial Functioning at Initial Visit)

Please rate the following categories of the subject's psychosocial functioning with regard to the past 3 months.

<table>
<thead>
<tr>
<th>Interviewers Initials</th>
<th>Child's Initials</th>
</tr>
</thead>
</table>

1. **Student Work**
   - If subject has **not** been enrolled in a student program at all during the past month, was this due to psychopathology?  ○ Yes  ○ No
   - and/or for some other reason?  ○ Yes  ○ No
   --------IF YES, SKIP TO INTERPERSONAL RELATIONS--------
   Degree of impairment in student work: 

2. **Interpersonal Relations with Family**
   A. Biological parents
   B. Adoptive parents
   C. Foster parents
   D. Step-parents
   E. Other guardian
   F. Siblings
   G. Girlfriend/boyfriend
   H. Other important relatives

2a. **Interpersonal Relationships with Friends**

3. **Work**
   Rate up to three categories if appropriate:

4. **Employment or Self-Employment**
   - If subject has **not** been employed at all during the past month, was this due to psychopathology?  ○ Yes  ○ No
   - and/or for some other reason?  ○ Yes  ○ No
   --------IF YES, SKIP TO HOUSEHOLD DUTIES--------
   How many hours a week during the past week were spent in employment activities?
   Degree of impairment in work activities:

_This form is completed at the initial assessment ONLY. The date below should be the date of the Initial Assessment._

| ID | DATE MM/ DD/ YYYY | 60919 |
5. Household Duties
If subject has not performed any household duties at all during the past month, was this due to psychopathology? ○ Yes ○ No
and/or for some other reason? ○ Yes ○ No

------------IF YES, SKIP TO RECREATION-----------

Degree of impairment in household activities: 

6. Recreation

7. Sexual Functioning
   A. Marital status
   B. Sexual orientation
   C. Sexual activities
   D. Frequency of sexual activities
   E. Number of partners
   F. Level of Sexual Risk

8. Satisfaction

9. Global Social Adjustment
CHILDREN'S GLOBAL ASSESSMENT SCALE (C-GAS)

INTAKE ASSESSMENT

Directions: Rate the subject's most impaired level of general functioning for the specified time period by selecting the lowest level which describes his/her functioning on a hypothetical continuum of health-illness. Use intermediary levels (e.g., 35, 58, 82). Rate actual functioning regardless of treatment or prognosis. The examples of behavior provided are only illustrative and are not required for a particular rating.

100-91 Superior functioning in all areas (at home, at school and with peers), involved in a range of activities and has many interests (e.g., has hobbies or participates in extracurricular activities or belongs to an organized group such as Scouts, etc.) Likeable, confident, "everyday" worries never get out of hand. Doing well in school. No symptoms.

90-81 Good functioning in all areas. Secure in family, school and with peers. There may be transient difficulties and "everyday" worries that occasionally get out of hand (e.g., mild anxiety associated with an important exam, occasionally "blow-up" with siblings, parents or peers).

80-71 No more than slight impairment in functioning at home, at school, or with peers. Some disturbance of behavior or emotional distress may be present in response to life stresses (e.g., parental separations, are only minimally disturbing to others and are not considered deviant by those who know them.

70-61 Some difficulty in a single area, but generally functioning pretty well (e.g., sporadic or isolated antisocial acts, such as occasionally playing hookey or petty theft; consistent minor difficulties with school work, mood changes of brief duration; fears and anxieties which do not lead to gross avoidance behavior, self-doubts). Has some meaningful interpersonal relationships. Most people who do not know the child would not consider him/her deviant but those who do know him/her might well express concern.

60-51 Variable functioning with sporadic difficulties or symptoms in several but not all social areas. Disturbance would be apparent to those who encounter the child in a dysfunctional setting or time but not to those who see the child in other settings.

50-41 Moderate degree of interference in functioning in most social areas or severe impairment of functioning in one area, such as might result from, for example, suicidal preoccupations and ruminations, school refusal, and other forms of anxiety, obsessive rituals, major conversion symptoms, frequent anxiety attacks, frequent episodes of aggressive or other anti-social behavior with some preservation of meaningful social relationships.

40-31 Major impairment in functioning in several areas and unable to function in one of those areas, i.e., disturbed at home, at school, with peers, or in the society at large, e.g., persistent aggression without clear instigation; markedly withdrawn and isolated behavior due to either mood or thought disturbance; suicidal attempts with clear lethal intent. Such children are likely to require a special schooling and/or hospitalization or withdrawal from school (but this is not a sufficient criterion for inclusion in this category).

30-21 Unable to function in almost all areas, e.g., stays at home, in bed or in bed all day without taking part in social activities OR severe impairment in communication (e.g., sometimes incoherent or inappropriate).

20-11 Needs considerable supervision to prevent hurting others or self, e.g., frequently violent repeated suicide attempts OR gross impairment in all forms of communication, e.g., severe abnormalities in verbal and gestural communication, marked social aloofness, stupor, etc.

10-1 Needs constant supervision (24-hour care) due to severely aggressive or self-destructive behavior or gross impairment in reality testing, communication, cognition, affect, or personal hygiene.

Most severe past episode Past year highest level of functioning Current episode (Past month)

Rater Initials

ID INTAKE DATE MM DD YYYY

14029
From the first text box, please indicate the week span that is being used.

186

<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</table>

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**Do Not Rate Both Mania and Hypomania Positively**

1. Does not meet DSM-IV criteria for mania (GO TO HYPOMANIA LINE).
2. Definite criteria for mania, almost always in an acute, current episode, but no prominent psychosocial or extraneous impairment in functioning.
3. Definite criteria (Severe). Meets DSM-IV criteria for definite episode and no other prominent psychosocial or extraneous impairment in functioning.
4. Does not meet definite DSM-IV criteria, but more than minimal impairment in functioning.
5. Meets definite DSM-IV criteria for MDE, but no extraneous impairment in functioning.
6. Meets definite DSM-IV criteria, but other prominent psychosocial or extraneous impairment in functioning.

---

**Psychiatric Status Rating**

Week: 0 1 2 3 4 5 6 7 8 9 10

---

**Date:**

---

**Number:**

---
**Family Medical History**

*Interview Date: [ ] / [ ] / 20[ ]*

*Please answer the following questions in terms of your biological mother and father:*

1. Is your biological mother living?  ○ Yes  ○ No  ○ Don't know
   
   *IF YES, GO TO QUESTION 4. IF DON'T KNOW, GO TO QUESTION 6.*

2. Approximately how old was your biological mother when she died? [ ] years of age  ○ Don't know

3. What was the cause of your mother's death?
   ○ Accident  ○ Cancer  ○ Heart Attack  ○ Stroke  ○ Don't Know
   ○ Other: [ ]

4. How old is she now? [ ] years old  ○ Don't know

5. Did your biological mother ever have any of the following?
   
   A. Diabetes  ○ Yes  ○ No  ○ Don't know
   B. High Blood Pressure  ○ Yes  ○ No  ○ Don't know
   C. Obesity  ○ Yes  ○ No  ○ Don't know
   D. Cholesterol Problems  ○ Yes  ○ No  ○ Don't know
   E. Stroke  ○ Yes  ○ No  ○ Don't know
   F. Angina (chest pain related to heart disease)  ○ Yes  ○ No  ○ Don't know
   G. Gout  ○ Yes  ○ No  ○ Don't know
   H. Toxemia, preclampsia, or high blood pressure during pregnancy  ○ Yes  ○ No  ○ Don't know
   I. Heart attack
      
      *About how old was she when she had it? [ ] years of age*
6. Is your biological father living?  ○ Yes  ○ No  ○ Don't know

7. Approximately how old was your biological father when he died?  □ years of age  ○ Don't know

8. What was the cause of your father's death?
   ○ Accident  ○ Cancer  ○ Heart Attack  ○ Stroke  ○ Don't Know
   ○ Other: □ □ □ □ □

9. How old is he now?  □ □ years old  ○ Don't know

10. Did your biological father ever have any of the following?
    A. Diabetes  ○ Yes  ○ No  ○ Don't know
    B. High Blood Pressure  ○ Yes  ○ No  ○ Don't know
    C. Obesity  ○ Yes  ○ No  ○ Don't know
    D. Cholesterol Problems  ○ Yes  ○ No  ○ Don't know
    E. Stroke  ○ Yes  ○ No  ○ Don't know
    F. Angina (chest pain related to heart disease)  ○ Yes  ○ No  ○ Don't know
    G. Gout  ○ Yes  ○ No  ○ Don't know
    H. Heart attack  ○ Yes  ○ No  ○ Don't know

   About how old was he when he had it?  □ □ years of age

ID: □ □ □
11. How many full brothers and sisters do your biological parents have?
Please include any aunts and uncles who may have died, but do not include half or step-brothers and sisters. Check the **don't know** box if you don't know.

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<thead>
<tr>
<th>Uncles</th>
<th>Don't know</th>
<th>Aunts</th>
<th>Don't know</th>
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</table>

12. Please complete the following regarding the second degree relatives who may have one or more of the following medical issues. Fill in the circle ONLY if the relative has the medical issue listed at the top of the column.

<table>
<thead>
<tr>
<th>2nd Degree Relative #</th>
<th>Diabetes</th>
<th>High BP</th>
<th>Obesity</th>
<th>Choles. Issues</th>
<th>Stroke</th>
<th>Angina</th>
<th>Gout</th>
<th>Toxemia, preeclampsia, high BP during pregnancy</th>
<th>Heart Attack</th>
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</tbody>
</table>

ID: 58633
## Medical History

### 1. High blood pressure or hypertension?
- **No**
- **Yes**
- **Not sure**

<table>
<thead>
<tr>
<th>Age first told?</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>At what age were you first told this?</td>
<td></td>
</tr>
</tbody>
</table>

FOR FEMALE: Was this during pregnancy only?
- **No**
- **Yes**

### 2. High blood cholesterol?
- **No**
- **Yes**
- **Not sure**

<table>
<thead>
<tr>
<th>Age first told?</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>At what age were you first told this?</td>
<td></td>
</tr>
</tbody>
</table>

### 3. Heart problems?
- **No**
- **Yes**
- **Not sure**

<table>
<thead>
<tr>
<th>Age first told?</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>What type? (CHECK ALL THAT APPLY)</td>
<td></td>
</tr>
<tr>
<td>Heart attack</td>
<td></td>
</tr>
<tr>
<td>Angina</td>
<td></td>
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<tr>
<td>Rheumatic heart disease</td>
<td></td>
</tr>
<tr>
<td>Mitral valve prolapse</td>
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<tr>
<td>Other, specify:</td>
<td></td>
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</tbody>
</table>

### 4. Diabetes (high sugar in blood or urine)?
- **No**
- **Yes**
- **Not sure**

FOR FEMALE: Was this during pregnancy only?
- **No**
- **Yes**

Have you ever been treated for your diabetes without insulin?
For example, with diet and pills only?
- **No**
- **Yes**

<table>
<thead>
<tr>
<th>Age first told?</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>At what age were you first told this?</td>
<td></td>
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</tbody>
</table>

### 5. Kidney problems?
- **No**
- **Yes**
- **Not sure**

<table>
<thead>
<tr>
<th>Age first told?</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>What type? (CHECK ALL THAT APPLY)</td>
<td></td>
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<tr>
<td>Urine infection from your kidney (pyelonephritis)</td>
<td></td>
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<tr>
<td>Kidney stones</td>
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</tr>
<tr>
<td>Kidney problems such as nephritis or glomerulonephritis</td>
<td></td>
</tr>
<tr>
<td>Kidney failure, dialysis or kidney transplant</td>
<td></td>
</tr>
<tr>
<td>Other, specify:</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Had in past year?</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>No</strong></td>
<td><strong>Yes</strong></td>
<td><strong>Yes</strong></td>
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</tbody>
</table>
6. Liver
   • No
   • Yes
   • Not sure
   What type? (CHECK ALL THAT APPLY)
   • Hepatitis A
   • Hepatitis B
   • Hepatitis C
   • Hepatitis, Unknown type
   • Cirrhosis
   • Other, specify
   Age first told? years
   Had in past year? No Yes
   • No
   • Yes

7. Gallstones or gallbladder disease?
   • No
   • Yes
   • Not sure
   At what age were you first told this? years
   Have you had this in the past year? No Yes

8. Migraine headaches?
   • No
   • Yes
   • Not sure
   At what age were you first told this? years
   Have you had this in the past year? No Yes

9. Peripheral vascular disease (problems with circulation, blocked arteries to leg)?
   • No
   • Yes
   • Not sure
   At what age were you first told this? years

10. Cancer or malignant tumor?
    • No
    • Yes
    • Not sure
    What type? (CHECK ALL THAT APPLY)
    • Lung
    • Breast
    • Cervical
    • Blood/lymph glands
    • Testes/ scrotum
    • Bone
    • Melanoma
    • Other, specify
    Age first told? years
    What type? (CHECK ALL THAT APPLY)
    • Skin
    • Brain
    • Stomach
    • Colon
    • Uterine
    • Prostate
    Age first told? years
    ID: 13329
<table>
<thead>
<tr>
<th>Question</th>
<th>What type?</th>
<th>Age first told?</th>
<th>Had in past year?</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Thyroid problem?</td>
<td>○ No ○ Yes ○ Not sure ○ Hypothyroidism or underactive thyroid (low thyroid)</td>
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<tr>
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<td>○ Hypothyroidism or underactive thyroid (low thyroid)</td>
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<td></td>
<td>○ Hyperthyroidism or overactive thyroid (Grave’s disease)</td>
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<td></td>
<td>○ Other, specify</td>
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<td>12. Digestive disease?</td>
<td>○ No ○ Yes ○ Not sure ○ Ulcer</td>
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<td>○ No ○ Yes</td>
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<td>○ Ulcer</td>
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<td>○ Other (such as Crohn’s or ulcerative colitis; specify)</td>
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<td>13. Gout?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<td>14. Asthma?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<td>15. Epilepsy (seizures)?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<td>16. Pneumonia?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<td>17. Tuberculosis or a positive skin test for tuberculosis?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<td>18. Emphysema?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<td>19. Chronic bronchitis?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<tr>
<td>20. Chronic Obstructive Pulmonary Disease (COPD)?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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<tr>
<td>21. Stroke or TIA (Transient Ischemic Attack)?</td>
<td>○ No ○ Yes ○ Not sure</td>
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<td>○ No ○ Yes</td>
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</tbody>
</table>
22. Multiple sclerosis?  
- No  
- Yes  
- Not sure  
- Age first told?  
- Had in past year?  

23. Blood clot in your leg vein or lung requiring blood thinning med?  
- No  
- Yes  
- Not sure  
- Age first told?  
- Had in past year?  

24. HIV?  
- No  
- Yes  
- Not sure  
- Age first told?  
- Had in past year?  

25. Any other major diseases or health problems?  
- No  
- Yes  
- Not sure  
- Please specify:  
- Age first told?  

26. Are you taking medications for high blood pressure?  
- No  
- Yes  
- Not sure  

27. Are you taking medications to lower your blood cholesterol?  
- No  
- Yes  
- Not sure  

28. Are you taking medications for any heart conditions?  
- No  
- Yes  
- Not sure  

29. Are you taking medications for asthma or any breathing problem?  
- No  
- Yes  
- Not sure  

30. Are you taking medications for diabetes?  
- No  
- Yes  
- Not sure  

31. Are you currently taking any other prescription medications (FOR FEMALE: excluding birth control pills)?  
- No  
- Yes  
- Not sure  

32. Are you currently taking aspirin at least three times a week (do not include Tylenol, Aleve, Advil or other non-aspirin pain relievers)?  
- No  
- Yes  
- Not sure
199

33. During the past five years, have you been on a weight reducing diet?
   ○ No
   ○ Yes
   Are you on or were you on such a diet because of a health problem or disease? ○ No ○ Yes
   Are you on such a diet now? ○ No ○ Yes
   If yes, what type of diet? [ ]

34. Do you have any medical problem(s) that interfered with your ability to exercise over the past twelve months?
   ○ No ○ Yes Specify:
   ○ Not sure
   How much did the medical problem(s) interfere with your ability to exercise?
   A little ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 Very much

MALE: END OF QUESTIONNAIRE, FEMALE: PLEASE CONTINUE

FOR FEMALES:

35. Did a doctor or nurse ever tell you that you had a polycystic ovarian syndrome or polycystic ovarian disease?
   ○ No ○ Yes Age first told? [ ] years
   ○ Not sure
   What medical treatments have you received for this condition?
   (CHECK ALL THAT APPLY)
   ○ None
   ○ Surgery on the ovary(ies)
   ○ Medications for hair growth or acne
   ○ Medications for irregular menstrual
   ○ Medications for infertility
   ○ Other, please specify [ ]
   ○ Don’t know

36. Have you ever taken birth control pills or other birth control medication?
   ○ No ○ Yes
   ○ Not sure

37. Are you currently taking hormones other than birth control pills?
   ○ No ○ Yes
   ○ Not sure

38. Have you ever been pregnant?
   ○ No ○ Yes Are you currently pregnant? ○ No ○ Yes ○ Not sure
   ○ Not sure Are you currently breast feeding? ○ No ○ Yes ○ Not sure

ID: [ ]
Menstrual History Interview

Date: __ __ / __ __ / __ __

On average, over the past 3 months, how many days were between your periods? __ __

On average, over the past 3 months, how many days did your period last? __ __

When did your last period start? (Interviewer shows participant a calendar)

( __ __ / __ __ / __ __ ) MM/DD/YYYY

Number of days since last period: __ __

Initials: __ __

ID: __ __
PETERSEN PUBERTAL DEVELOPMENT SCALE

Please darken the circle in front of the answer that best describes what is happening to you. Please choose only one answer for each question.

I am:  O Male  O Female

1. Would you say that your growth in height:
   O has not yet begun to spurt ("spurt" means more growth than usual)
   O has barely started
   O is definitely underway
   O seems completed

2. And how about the growth of body hair ("body hair" means underarm and pubic hair)? Would you say that your body hair has:
   O not yet started growing
   O barely started growing
   O is definitely underway
   O seems completed

BOYS ONLY

4a. Have you noticed a deepening of your voice?
   O not yet started changing
   O has barely started changing
   O voice change is definitely underway
   O voice change seems completed

4b. Have you begun to grow hair on your face?
   O not yet started growing hair
   O has barely started growing hair
   O facial hair growth is definitely underway
   O facial hair growth seems completed

GIRLS ONLY

4. Have your breasts begun to grow?
   O not yet started growing
   O has barely started changing
   O breast growth is definitely underway
   O breast growth seems completed

6. Have you begun to menstruate?
   O NO  O YES

If you answered YES, how old were you when you first menstruated?

   Years  [ ]  Months  [ ]

Height: I am [ ] feet and [ ] inches tall.

Weight: I weigh [ ] pounds.

ID [ ]

Date [ ] / [ ] / [ ]

32144
### TOBACCO USE - LIFETIME

THIS FORM IS TO BE COLLECTED ONCE TO CAPTURE LIFETIME TOBACCO USE.

<table>
<thead>
<tr>
<th>Code: 0 = No Information; 1 = No; 2 = Yes</th>
<th>Parent</th>
<th>Child</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Have you ever smoked cigarettes?</td>
<td>0 1 2</td>
<td>0 1 2</td>
<td>0 1 2</td>
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<tr>
<td>If yes, have you ever smoked cigarettes regularly?</td>
<td>(1 cigarette a day or more for at least 30 days)</td>
<td>( ) ( ) ( )</td>
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<tr>
<td>2. Have you ever chewed tobacco?</td>
<td>0 1 2</td>
<td>0 1 2</td>
<td>0 1 2</td>
</tr>
<tr>
<td>If yes, have you ever chewed tobacco regularly?</td>
<td>(1 dip/chew a day or more for at least 30 days)</td>
<td>( ) ( ) ( )</td>
<td>( ) ( ) ( )</td>
</tr>
</tbody>
</table>

If no evidence of REGULAR cigarette/chewing tobacco use, STOP HERE.

<table>
<thead>
<tr>
<th>Cigarette Use: (1 pack = 20 cigarettes)</th>
<th>Parent</th>
<th>Child</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Age, in years, of first regular use (1 cigarette a day or more for at least 30 days):</td>
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<tr>
<td>4. Greatest amount of use - lifetime:  Rate in cigarettes per day.</td>
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<tr>
<td>5. Age, in years, at greatest amount of use - lifetime:</td>
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<tr>
<td>6. Current use: Rate in cigarettes per day.</td>
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<tr>
<td>7. Ever attempted to quit?</td>
<td>0 1 2</td>
<td>0 1 2</td>
<td>0 1 2</td>
</tr>
<tr>
<td>If yes, code longest # of months:</td>
<td>( ) ( ) ( )</td>
<td>( ) ( ) ( )</td>
<td>( ) ( ) ( )</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Chewing Tobacco Use: (1 can = 20 dips/chews)</th>
<th>Parent</th>
<th>Child</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>9. Age, in years, of first regular use (1 dip/chew a day or more for at least 30 days):</td>
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<tr>
<td>10. Greatest amount of use - lifetime: Rate in dips/chews per day.</td>
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<td></td>
<td></td>
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<tr>
<td>11. Age, in years, at greatest amount of use - lifetime:</td>
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<tr>
<td>12. Current use: Rate in dips/chews per day.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13. Ever attempted to quit?</td>
<td>0 1 2</td>
<td>0 1 2</td>
<td>0 1 2</td>
</tr>
<tr>
<td>If yes, code longest # of months:</td>
<td>( ) ( ) ( )</td>
<td>( ) ( ) ( )</td>
<td>( ) ( ) ( )</td>
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</tbody>
</table>

If subject meets DSM-IV criteria for nicotine dependence, code on PSR.

<table>
<thead>
<tr>
<th>ID:</th>
<th>46919</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MM / DD / YYYY</td>
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</tbody>
</table>