Neurostructural Correlates of Body Mass Index & Waist Circumference in Adolescents with Bipolar Disorder

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

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University of Toronto

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Department of Pharmacology and Toxicology

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Abstract – 149/150 words

Obesity among youth with bipolar disorder (BD) is associated with greater illness severity. In adult BD, obesity is associated with neurostructural differences. We examined this topic for the first time in youth. T1-weighted images of 40 BD and 48 psychiatrically healthy controls (HC) were processed using FreeSurfer to derive cortical region of interest (ROI) volumes/cortical thickness for frontal lobe (FL), prefrontal cortex (PFC), and orbitofrontal cortex (OFC), as well as subcortical ROI volumes for amygdala and hippocampus. Our results show that there was a significant BMI-by-group interaction effect on FL and OFC volumes. In the BD group only, BMI was significantly negatively associated with OFC volume, as well as FL, OFC, and PFC cortical thickness. Our results suggest that elevated BMI is associated with neurostructural changes in youth with BD but not in HC. Treatment studies examining the effect of optimizing weight on brain structure in BD are warranted.
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List of Abbreviations

1. Introduction

1.1 Statement of Problem

The prevalence of obesity is increased among youth and adults with bipolar disorder (BD) (Wang et al. 2006; McElroy et al. 2002; Fiedorowicz et al. 2008). Up to 68% percent of adult (Fagiolini et al. 2002) and 42% of adolescent patients with BD are overweight (OW) or obese (OB) (Goldstein et al. 2008), prevalence rates that are much higher than general population (McIntyre et al. 2007).

While negative health outcomes associated with elevated body mass index (BMI) are well documented for the general population, obese individuals with BD show increased morbidity and mortality from conditions such as hypertension, diabetes, cardiovascular diseases (CVD), and certain types of cancers (Tsuang, Woolson, and Fleming 1980; Kilbourne et al. 2004; Osby et al. 2001). Obesity poses as a particularly concerning condition in BD because CVD is the leading cause of mortality in BD (Goldstein, Liu, et al. 2011). Related to this, a recent scientific statement from American Heart Association established that BD predisposes youth to premature CVD and atherosclerosis (Goldstein et al. 2015).

Additionally, obesity in adult BD has been associated with a more pernicious course of psychiatric illness, including increased frequency of mood episodes, poor cognitive function, and increased suicidal ideation and attempts (Fagiolini et al. 2003; Gomes et al. 2010). Similar to adult BD findings, obesity in adolescents with BD has been associated with greater prevalence of substance use disorder, self-injurious behaviour, suicidality, and greater number of psychiatric hospitalizations (Goldstein et al. 2008; Shapiro et al. 2016). One study showed that OW/OB adolescents had a significantly earlier age of BD-onset (Goldstein et al. 2008). The brain phenotypes and
the putative neurobiological mechanisms underlying the link between obesity and increased illness burden are relatively unknown.

The neurostructural correlates of BMI, and other proxies of obesity have been studied and reviewed extensively in the general population (Willette and Kapogiannis 2015). In youth, obesity is most consistently associated with reduced GM volume in frontal lobe (FL), specifically the prefrontal cortex (PFC) and orbitofrontal cortex (OFC) (Willette and Kapogiannis 2015; Alosco et al. 2014; Yokum, Ng, and Stice 2012; Yau et al. 2014; Ross, Yau, and Convit 2015; Ou et al. 2015). There is also evidence of reduced volume of medial temporal lobe (MTL) structures such as the hippocampus and parahippocampal gyrus in OB/OW adolescents (Bauer et al. 2015). The FL regions are of particular interest because they are associated with higher-level cognitive processes, such as executive functioning, decision-making, and inhibitory control, and are also known to modulate emotion processing (Willette and Kapogiannis 2015; Taki et al. 2008).

BD diagnosis is also associated with morphometric changes in the brain. A recent-meta analysis established that adolescents with BD have smaller amygdala compared to non-BD control participants. Smaller hippocampal volumes and reduced GM in PFC have also been reported in youth with BD (Pfeifer et al. 2008; Frazier et al. 2005) (Dickstein et al. 2005; Selvaraj et al. 2012).

Despite the large number of brain structure studies examining obesity and BD independently, investigation of the relationship between obesity and BD from a neuroimaging perspective is limited. Although one recent voxel-based morphometry (VBM) study has shown that BMI is associated with unique neurostructural changes among adults with BD (Bond et al. 2014; Bond et al. 2011), this topic has not yet been
addressed in the adolescent population. The adult study found that higher BMI in BD patients was associated with reduced grey matter (GM) and white matter (WM) volume in clusters within the frontal, temporal, and subcortical limbic structures in contrast to normal-weight controls that showed reduced occipital lobe GM only (Bond et al. 2014). Focusing on adolescent population may serve to optimize signal to noise ratio in the data by virtue of shorter duration of illness and less confounding health complications (e.g. age-related neurodegeneration and chronic diseases of aging). Importantly, if there are discernible and unique neurostructural changes associated with obesity among adolescents with BD, these findings could help improve our understanding on the etiology and progression of BD. Indeed, research in this realm may help to identify novel neuroimaging biomarkers that can advance diagnostic accuracy, treatment, and prognosis of individuals with BD. Moreover, obesity could potentially serve as a pharmacological and behavioural treatment target.

1.2 Purpose of Study and Objectives
The purpose of this study was to ascertain the relationship between proxies of obesity (i.e. BMI and WC) and brain structure (volume and cortical thickness) of select a priori regions of interest (ROIs) in the brain among adolescents with and without BD. The ROIs that were chosen have been implicated in both BD and obesity and include the following cortical ROIs: (1) FL, (2) PFC, and (3) OFC and subcortical ROIs: (4) amygdala, and (5) hippocampus. Note: for cortical ROIs we examined both volume and cortical thickness as separate ROI measures. The primary analyses examined BMI as our main measure of obesity in keeping the majority of the prior literature and because a
substantial number of participants did not have WC measures, which were added to the study mid-way. WC was examined in secondary analyses.

The objectives of this study were to:

1. Determine if there is a main effect of BMI/WC on brain structure among BD adolescents.
2. Examine for unique neurostructural correlates of BMI/WC among BD adolescents as compared to HC adolescents. That is, to determine if there is a Diagnosis group by BMI/WC interaction effect.
3. Identify additional neurostructural correlates of BMI/WC using an atheoretical whole-brain analysis.

1.3 Statement of Research Hypotheses and Rationale for Hypotheses

We hypothesize that there will be a negative main effect of BMI/WC in predicting ROI measures. We also hypothesize that there will be a group-by-BMI/WC interaction effect in predicting ROI measures. Specifically, the negative correlation between BMI/WC and ROI measures will be greater in BD than in HC participants.

The rationale for these hypotheses come from the fact that BD and obesity have been independently associated with volumetric reductions in the ROIs we will be examining. In BD, cortical thinning and reduced GM volumes have been observed in several areas of the FL, specifically the PFC (Selvaraj et al. 2012). A recent meta-analysis also showed that amygdala sizes were smaller in adolescents with BD when compared to HC (Pfeifer et al. 2008). There is also extant evidence which shows that
obesity/overweight in general youth population is associated with reduced GM volume in the ROIs, which include the FL and specific regions within it (i.e. the PFC and OFC) as well as subcortical MTL structures (including the hippocampus) (Alosco et al. 2014; Yokum, Ng, and Stice 2012; Yau et al. 2014; Ross, Yau, and Convit 2015; Ou et al. 2015).

In addition to this, the study in adult BD observed reduced volumes in frontal, temporal, and limbic structures with increasing BMI in BD but not in HC (Bond et al. 2014; Bond et al. 2011). We therefore expect that the effect of BMI/WC on brain structure will differ depending on whether or not the participant has BD.

1.4 Review of Literature

1.4.1 Bipolar Disorder in Adolescents

BD is a mood disorder, characterized by recurrent episodes of mania or hypomania, usually alternative with periods of depression, and separated by intervening asymptomatic periods described as euthymia (Jann 2014). BD is recognized as one of the most debilitating psychiatric illnesses, affecting 2-5% of adolescents and adults (Jann 2014; Kessler et al. 2009). Onset of BD typically occurs in late adolescence, or early adulthood (Jann 2014). Recent evidence suggests that child- or adolescent-onset of BD is associated with a more severe and progressive course of illness (Goldstein and Levitt 2006; Perlis et al. 2009). For example, youth with BD are reported to have greater proportion of time suffering from syndromal mood symptoms, particularly depression and mania (Birmaher et al. 2006).
In addition to the direct burden of psychiatric symptoms, BD is associated with a wide-range of medical problems, but particularly cardiovascular disease (CVD) and related conditions such as diabetes, hyperlipidemia, hypertension, and obesity, which contribute to the increased and premature mortality in BD (Tsuang, Woolson, and Fleming 1980; Kilbourne et al. 2004; Osby et al. 2001). Furthermore, compared to psychiatrically healthy controls, adolescents with BD have greater functional impairment, poorer neurocognitive functioning, increased suicidality, and greater rates of comorbid psychiatric disorders (Mann-Wrobel, Carreno, and Dickinson 2011). Indeed BD significantly lowers the quality of life in individuals who suffer from it (Saarni et al. 2010).

1.4.2 Neuroanatomical Correlates of BD

Recent advances in neuroimaging techniques have paved the way for an abundance of research examining the neuroanatomical correlates of BD. Although discordant findings exist, a recent synthesis of studies determined that the psychopathology of BD is associated with anomalous structural and functional differences in the brain, namely in regions that govern emotional behaviour (Strakowski et al. 2012).

*Functional correlates of BD*

There are two well-recognized networks originating from the prefrontal cortex (PFC) that modulate emotional control and are implicated in BD. Both networks form iterative feedback loops that work to curb and nuance the activity of limbic structures such as the amygdala (Strakowski et al. 2012; Townsend and Altshuler 2012). One
network, originating from the ventrolateral PFC (vlPFC), is thought to regulate external emotional stimuli; the other, originating from the ventromedial PFC (also known as the orbitofrontal cortex [OFC]) is thought to modulate internal emotional stimuli (Blond, Fredericks, and Blumberg 2012). Both of these networks innervate the amygdala, which along with other limbic structures, are putative key players of complex emotion regulation and perception (Townsend and Altshuler 2012).

Indeed, PFC-amygdala networks have been strongly implicated in BD (Blond, Fredericks, and Blumberg 2012). In particular, a number of studies have reported abnormal and excessive activation of the amygdala during face emotion processing tasks, across a variety of mood states (Kalmar et al. 2009; Rich et al. 2006). Consistent with the evidence of dysfunctional amygdala, studies have also found atypical PFC activity as well (Blond, Fredericks, and Blumberg 2012). Specifically, decreased functional activity as measured by functional magnetic resonance imaging (fMRI) as well as positron emission tomography (PET) was seen in these ventral PFC regions during a variety of cognitive and emotional tasks (Chen et al. 2011; Blumberg et al. 1999). Interestingly, some studies have also shown that PFC activity recovers when BD individuals are asymptomatic during their euthymic periods (Strakowski et al. 2004; Adler et al. 2004). Collectively, these studies suggest that abnormal amygdala activation paired with reduced PFC modulation leads to emotional extremes of mania and depression that are observed in BD (Strakowski et al. 2012). To reinforce this notion, multiple studies have also reported deficits in functional connectivity between the PFC and the amygdala in BD (Foland et al. 2008). In fact, studies done in youth at familial-risk for BD (i.e. offspring of individuals with BD) observed similar anomalies, suggesting that these
functional connectivity differences may exist before the onset of BD and likely precedes the functional impairment observed in the PFC and amygdala (Kafantaris et al. 2009).

**Neurostructural Correlates of BD**

Several studies report structural brain differences in BD when compared to psychiatrically healthy control counterparts (Hanford et al. 2016). In particular, regions that have shown abnormal functional MRI findings also show structural differences. For example, a recent-meta analysis established that adolescents with BD have smaller amygdala compared to non-BD control participants (Pfeifer et al. 2008). Smaller hippocampal volumes and reduced GM in PFC have also been reported in youth with BD (Frazier et al. 2005) (Dickstein et al. 2005; Selvaraj et al. 2012). Several studies also report cortical thinning in the FL, specifically in the dorsolateral PFC, vIPFC, and the OFC (Lan et al. 2014; Elvsashagen et al. 2013; Lyoo et al. 2006). Studies have also shown that cortical thinning in FL and limbic regions are correlated with greater affective symptom severity and cognitive impairments in BD (Oertel-Knochel et al. 2015). In addition to these regions, cortical thinning in the anterior cingulate cortex (ACC), an integrative structure that is responsible for impulse control and emotion processing, has been reported in a number of different studies (Hanford et al. 2016; Fornito et al. 2008).

Taken together, structural and functional differences in the PFC, amygdala, and surrounding limbic structures, supports a hypothetical model for abnormal emotional regulation and cognitive dysfunction in BD.
1.4.3 The Relationship between BD and Obesity

A number of epidemiological studies have found that comorbid obesity is highly prevalent in individuals living with Bipolar Disorder (BD) (Wang et al. 2006; McElroy et al. 2002; Fiedorowicz et al. 2008). Sixty-eight percent of adult (Fagiolini et al. 2002) and 42% of adolescent patients with BD are overweight (OW) or obese (OB) (Goldstein et al. 2008), prevalence rates that are much higher than general population (McIntyre et al. 2007). There is also evidence to suggest that the prevalence rates of OW/OB are markedly higher in Americans vs. Europeans with BD (McElroy et al. 2002). Recent meta-analyses have confirmed that BD patients have an increased prevalence of metabolic syndrome and obesity (Vancampfort et al. 2013) (Zhao et al. 2016). Related to this, individuals with BD are also known to have greater waist-to-hip ratios and are more likely to be centrally obese compared to non-BD individuals (Taylor and MacQueen 2006). Individuals with mood disorders have increased difficulty in balancing food intake and exercise and hence are more likely to binge-eat (particularly in relation to emotional dysregulation) and be physically inactive (McElroy and Keck 2012). Similar findings have also been observed in youth with BD (Martin et al. 2016; Jewell et al. 2015). In addition to this, specific pharmacotherapies commonly used in BD also confer risk for meaningful weight gain, including lithium and second-generation antipsychotics (SGAs) (Nashed, Restivo, and Taylor 2011).

While negative health outcomes associated with elevated BMI are well documented for the general population, obese individuals with BD show increased morbidity from conditions such as hypertension, diabetes, cardiovascular diseases
(CVD), and certain types of cancers (Tsuang, Woolson, and Fleming 1980; Kilbourne et al. 2004; Osby et al. 2001).

Additionally, obesity in adult BD has been associated with a more pernicious course of psychiatric illness, including increased frequency of mood episodes, poor cognitive function, and increased suicidal ideation and attempts (Fagiolini et al. 2003; Gomes et al. 2010). Convergent with adult BD findings, overweight/obesity in adolescents with BD has been associated with greater substance use disorder, self-injurious behaviour, suicidality, and greater number of psychiatric hospitalizations (Goldstein et al. 2008; Shapiro et al. 2016).

The mechanisms underlying the mood disorders and obesity may share common pathophysiological pathways (Zhao et al. 2016). For example, there is compelling evidence supporting the fact that obesity and BD, particularly during symptomatic intervals, are both associated with low-grade inflammation (Kivimaki et al. 2009; Avila et al. 2015). Elevated levels of inflammatory cytokines (e.g. interleukin family of cytokines) and C-reactive protein have been found in BD and obesity (Avila et al. 2015). Furthermore, abnormalities in the hypothalamic-pituitary adrenal (HPA) axis have been observed in bipolar depression similar to those seen in obese individuals (Taylor, McIntyre, et al. 2012). There is also evidence of similar phenotypic expression of brain reward pathways in overeating/food-seeking behaviour and hypomania (Galvez et al. 2015). Other neuroimaging studies have also suggested that reward pathways in the brain, mediated by dopamine and serotonin, may be involved in regulation of mood as well as energy balance (Vannucchi et al. 2014). Lastly, obesity and BD are both
independently associated with cognitive dysfunction in overlapping cognitive domains such as executive functioning and impulsivity (Niciu et al. 2014).

The question arises as to the direction of the association between BD and OW/OB in youth. Although there are no studies to date regarding the direction of the association between OW/OB and BD in youth, a systematic review of literature on the relationship between early-onset depression and adult body weight showed that obese adolescent females are more likely to develop depressive illness later in life (Korczak et al. 2013). The review also reported that depressed adolescents are more likely to become overweight adults. Another recent study has shown that onset of obesity and depression occurred concurrently in early adolescence but not later in development. Prospective evidence from the same study showed that depression in early adolescence predicted onset of obesity during late adolescence in females (Marmorstein, Iacono, and Legrand 2014).

1.4.4 Neuroanatomical Correlates of Obesity in General Youth Population

In the general adolescent population, the most consistent associations between obesity measures and brain changes were found in FL. Most studies used GM volume or cortical thickness of either entire frontal lobe or an ROI within the frontal cortex (e.g. PFC, OFC, or frontal gyrus) and found a negative association with obesity (Alosco et al. 2014; Yokum, Ng, and Stice 2012; Yau et al. 2014; Ross, Yau, and Convit 2015; Ou et al. 2015). Alosco et al. (2014) found a negative correlation between BMI and GM volume of the entire FL. Maayan et al. (2011) found reduced OFC volume in obese adolescents compared to normal-weight counterparts. Ross, Yau, and Convit (2015) and Yau et al. (2014) both found that obese adolescents had thinner OFC compared to
normal-weight individuals. Yokum, Ng, and Stice (2012) conducted a longitudinal study and reported a trend (p=0.06) toward reduced GM volumes in superior and middle frontal gyri in relation to increasing BMI over one year.

Obesity has also been negatively associated with the volumes of MTL and related limbic structures. Bauer et al. (2015) reported that overweight/obese children showed reduced left hippocampal volumes. Similarly, Alosco et al. (2014) reported a negative association between BMI and limbic regions, which was defined as the combination of the cingulum, hippocampus, parahippocampus, and amygdala. A longitudinal study determined that hippocampal and parahippocampal gyri volumes increased more in adolescents that had less increase in BMI over a 3-year period (Hashimoto et al. 2015).

1.4.5 Neurostructural Implications of Obesity in Adults with BD

Bond and colleagues investigated the relationship between increased BMI and brain volumes in euthymic adults with BD (Bond et al. 2014; Bond et al. 2011). Using voxel-based morphometry they found reduced GM and WM in frontal, temporal, and subcortical limbic structures (areas which are implicated in the pathophysiology of BD) but not in the HC participants (Bond et al. 2014). Of note, the authors discuss that the reduced WM volume associated with the elevated BMI mirrors neurostructural changes observed later in the course of BD illness. This could imply that higher BMI can potentially exacerbate BD progression in adults.
1.4.6 Summary of Literature and Rationale

In summary, it is evident from prior literature that neurostructural changes are implicated in both obesity and BD in youth. A single study has examined the neurostructural correlates of BMI among middle-aged adults with BD, but no prior study has examined this topic among youth early in their course of BD. Studying obesity as it relates to adolescents with BD may serve to optimize signal detection by virtue of shorter duration of illness and less confounding health complications. Importantly, if there are unique neurostructural correlates of BMI/WC among adolescents with BD, these findings could enhance our understanding on the neuropathology and etiology of BD, and suggest a novel treatment target.

2. Materials & Methods

2.1 Study Design

This investigation is a cross-sectional and observational study examining the neurostructural correlates of BMI and WC in adolescents with Bipolar Disorder (BD) and psychiatrically healthy control (HC) adolescents. All participants completed gold standard semi-structured diagnostic interviews, psychiatric assessments, anthropometric measurements, and underwent neurostructural MRI scans.

2.2 Participant Selection

2.2.1 Participant Recruitment

This study includes 40 adolescents with BD (type I, II or not otherwise specified [NOS]) and 48 HC. Participants with BD were recruited primarily from within the Centre
for Youth Bipolar Disorder (CYBD), a subspecialty clinical-research program at Sunnybrook Health Sciences Centre in Toronto, Ontario. Control subjects were recruited from the community via advertisements (newspaper ads, local print media) on public transit and in institutions in the GTA. Psychiatric diagnoses were assessed, for both the BD and HC groups, using the Kiddie- Schedule for Affective Disorders and Schizophrenia- Present and Lifetime version (K-SADS-PL), semi-structured interview tool intended to obtain current episode and lifetime history of psychiatric disorders (Kaufman et al. 1997).

The research ethics board at Sunnybrook Health Science Centre approved this study (Appendix 1). Written informed consent was obtained from participants and their parent/guardian prior to study commencement (Appendix 2).

2.2.2 Inclusion Criteria

English-speaking adolescents between the ages of 13-20 years were recruited for this study. BD participants met DSM-V diagnostic criteria for BD (I, II, or NOS). HC participants had no history of major psychiatric disorders (i.e. no lifetime mood or psychotic disorders, no alcohol or drug dependence in the past 3 months, and no anxiety disorders in the past 3 months) and had no first or second-degree relatives with BD or psychosis family.

2.2.3 Exclusion Criteria

Participants were excluded if they were:
1) unable to give informed consent (e.g. severe mania or psychosis or unable to speak English)

2) had a pre-existing cardiac condition, auto-immune illness, or inflammatory illness

3) taking any anti-inflammatories, anti-platelet, anti-lipidemic, anti-hypertensive or hypoglycemic agents (including insulin and metformin)

4) had an infectious disease within the past 14 days

5) had contradictions with MRI (e.g. cardiac pacemaker, intrauterine device, any metal implant in the body)

6) had a health condition or physiological impairment that prohibits intense exercise

7) had a neurological or severe cognitive impairment (e.g. autism)

2.3 Study Chronology

Study intake usually proceeded in two separate visits. During the first visit, the participant and accompanying parent/guardian completed a semi-structured diagnostic interview (takes 1-4 hours to complete). During the second visit, eligible participants provided a clinical research interview, to evaluate current mood symptoms and collect family medical/psychiatric history, and underwent a T1-weighted MRI scan.

Figure 1 outlines an overview of study.
2.4 Participant Demographics, Psychiatric, and Medical History

Patient characteristics such as age, sex, diagnosis, and medication status were ascertained during the first visit (i.e. the clinical intake interview) at the CYBD. Other demographic and clinical variables (e.g. family psychiatric history, medical history, and current and past medication use) were collected during the parent and child research interview and post-interview questionnaires.

2.5 Primary Interview Instruments

Demographics and current mood states were assessed during a clinical interview using the Affective Disorder and Schizophrenia for School Aged Children, Present and Life Version – mania and depression scales (K-SADS-PL/ MRS and DEP-P respectively), Medication listing, Children’s Global Assessment Scale (C-GAS), family
medical history screen, CARDIA Medical and Family Medical history, and tobacco use form.

The CARDIA Family Medical History is a questionnaire that obtains cardiovascular disease, stroke, and metabolic syndrome history about 1st and 2nd degree relatives (Burke et al. 1991). The Family History Screen is a tool used to detect psychiatric disorders in 1st and 2nd degree relatives (Weissman et al. 2000).

The Medication Listing asks the participant about medication recently taken the day of the study and the day before. The psychotropic medication status is queried during the initial clinical interview (average dose per week and type of psychotropic is recorded).

The Wechsler Abbreviated Scale of Intelligence (WASI) is an assessment of intellectual ability, consisting of four main subtests that measure different aspects of intelligence: vocabulary, block design, similarities, and matrix reasoning (Kehoe et al. 2015).

2.6 Anthropometric Data

<table>
<thead>
<tr>
<th>Measure</th>
<th>Method of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>Stadiometer (Seca Inc; Chino, CA, USA)</td>
</tr>
<tr>
<td>Weight</td>
<td>Body Mass Analysis Scale (Conair Consumer Products; Woodbridge, ON, Canada)</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>Measuring tape</td>
</tr>
</tbody>
</table>

BMI was calculated as weight in kilograms divided by square of height in meters. BMI percentiles were calculated for participants under the age of 20 based on growth charts from the Centre for Disease Control (CDC). An adjusted BMI score was also
obtained using an adjusted weight score to account for the approximate weight of clothes worn by the participant.

2.7 Structural Imaging & Analysis

2.7.1 Image Acquisition

MRI data were collected with a 3 Tesla Philips Achieva system using body coil transmission and an 8-channel head receiver coil. Structural images were acquired via T1-weighted high resolution fast-field echo imaging (TR/TE/TI = 9.5/2.3/1400 ms, spatial resolution 0.94×1.17×1.2 mm, 256×164×140 matrix, flip angle 8°, scan duration 8:56 min). 3D T1-weighted Fast Field Echo (FFE) scans quantified GM and WM using a single slab Fast Field Echo sequence with 140 slices, an echo time of 2.3 milliseconds (ms), repetition time of 9.5ms, and flip angle of 8 degrees. Field view was 240mm x 191mm. Time of acquisition was 8 minutes and 56 seconds.

2.7.2 Pre-processing Quality Control

Two independent raters visually inspected T1-weighted images of all subjects. A score between 0-3 was given for each T1 image with respect to quality (See Figure 2). Raters were trained to score based on overall image quality (i.e. graininess, contrast between WM and GM, and number of artifacts due to excessive movement while in the scanner). In cases where there were incongruent scores between raters, images were inspected a second time and consensus was reached following discussion. T1-weighted images that were scored a “3” (i.e. poor quality images) were discarded from the dataset.
before being processed by FreeSurfer. In total, 6 T1 images were excluded from dataset during this QC step: 2 BD and 4 HC participants.

![Grading scheme for T1 image quality](image)

**Figure 2. Grading scheme for T1 image quality**

2.7.3 Pre-processing and Surface Morphometry

T1-weighted images for individual participants were processed into surface based morphometric data using the fully automated reconstruction function in FreeSurfer v5.3.0. The automated pre-processing included re-sampling of 3D coronal images (1mm isotropic voxels), intensity normalization correction, registration (12 degrees of freedom) to MNI space and additional intensity normalization with automated skull stripping. Individual .mgz files were visually inspected to ensure there were no errors in skull stripping, registration, and alignment. Automated parcellation proceeded (Fischl, et al., 2002, Fischl, et al., 2004) with cortical surface reconstruction which included generation of binary white matter masks in two hemispheres which were used to produce a triangle-based mesh of the white matter surface and smoothed to remove voxel-based effects
(Dale, et al., 1999) and corrected for topological defects (Fischl, et al., 2001, Segonne, et al., 2005). White matter and pial surfaces were then estimated with a deformation algorithm and surfaces were spherically inflated to be registered to a canonical template. Finally, a parcellation algorithm used the Desikan cortical atlas, spatial landmarks, curvatures, sulcal depth to assign 33 gyral regions of interest. Individual participants’ mean average thickness, cortical volume, and surface areas were extracted from native space.

2.7.4 Post-processing Quality Control: Correcting Parcellation Errors

After the T1 images were processed on the automated FreeSurfer pipeline, they were individually reviewed to check accuracy of parcellation algorithm. Errors in the parcellation stream were manually corrected using the FreeView tool in the FreeSurfer package and re-run on the FreeSurfer pipeline. The individual who reviewed the parcellation was blinded to the participants’ diagnosis. In total, 5 participants were excluded from dataset during this QC step: 3 BD and 2 HC participants. The following steps were employed:

1) **Reviewed parcellation contours:** Used “Freeview” tool on the FreeSurfer package to examine the contour lines that were generated automatically i.e. outlining the gray matter-white matter interface (white surface) and the gray matter-pial boundary (pial surface). See example of erroneous parcellation in Figure 3.
2) **Corrected GM classification:** Added missed voxels (usually mistakenly labeled as GM) to the WM mask. Erased voxels where bright dura matter at the edge of the brain was erroneously labeled as white matter.

3) **Corrected outline of pial surface and removed non-brain tissue:** Erased brain mask when pial surface extended beyond the boundary of brain. Typically the cerebral spinal fluid would force the parcellation to effectively end. In other words, this step corrected the “over-extension” of GM.

4) **Corrected WM classification:** Reinforced steps 2 and 3 by exploiting the use of “control points” to enhance white matter detection. These cue the algorithm to assign that specific vertex to take the value of white matter. For every two dimensional slice, control points were added to sagittal, coronal, and axial slices.

5) **Re-processed the corrected masks on FreeSurfer:** The brain measures derived from the re-processed images were then used for analysis.

![Image](image_url)

**Figure 3. Example of parcellation error in FS pipeline indicated by white arrows**
2.8 Defining Regions of Interest

Lobar ROI volumes were created by summing individual labels from the Desikan-Killany atlas i.e. default atlas used for the cortical parcellation algorithm on FreeSurfer. Mean cortical thickness measurements for each ROI were averaged using the same labels. Note: Although PFC often includes the OFC within it, for our purposes we chose to keep the ROIs as distinct entities. The definition used for PFC was taken from this study.
The ROIs were created as a sum of individual labels from the DKT atlas

1) Frontal Lobe (FL)
Superior frontal gyrus, rostral and caudal middle frontal gyri, pars opercularis, pars triangularis, pars orbitalis, lateral and medial orbitofrontal, precentral gyrus, paracentral gyrus, and frontal pole.

2) Prefrontal Cortex (PFC)
Rostral middle frontal gyrus, caudal middle frontal gyrus, caudal anterior cingulate, and superior frontal

3) Orbitofrontal Cortex (OFC)
Medial orbitofrontal cortex, lateral orbitofrontal cortex

4) Amygdalar and 5) Hippocampal volumes
These ROI volumes were derived from the subcortical segmentation algorithm built into FreeSurfer version 5.3.

2.9 Intracranial Volume (ICV) Correction

There are a few used methods for ICV correction in neuroimaging research. One practice known as the “proportion method” is to divide a raw ROI by ICV and use the ROI-to-ICV ratio in statistical analysis (Liu et al. 2014). Another commonly used method is the analysis of covariance (ANCOVA) method in which ICV is included as a covariate in a regression model whose dependent variable is a ROI. It has been shown to
perform better than the proportion method (Sanfilipo et al. 2004). The ANCOVA method relaxes the proportionality assumption on the relationship between a ROI and ICV, but assumes that ROI is linearly related to ICV. For our analyses, we decided to use ICV as an explanatory variable in our GLM analysis.

2.10 Statistical Analyses

Normality of all continuous variables was assessed using the Shapiro-Wilks test. Non-parametric tests were used for non-normally distributed variables as appropriate. Continuous variables were compared between groups using independent-samples t-tests and Mann-Whitney U-tests. One-way ANOVA test was used to compare continuous variables when three groups were analyzed. Categorical variables were compared between groups using Chi-squared $\chi^2$ tests. Effect sizes were reported as Cohen’s d for continuous variable comparisons, and Cramer’s V for categorical comparisons. Spearman’s rho coefficients were reported in the bivariate correlations. Fisher’s Z tests were used to compare correlation coefficients between BD and HC. Results from the bivariate correlation analysis were further analyzed using General Linear Model (GLM) analysis. In the GLM, BMI or WC was the continuous variable of interest; and age, sex and intracranial volume (ICV) were used as covariates. Medication, psychiatric comorbidities, and BD subtype were controlled for in sensitivity analyses. Benjamini-Hochberg False Discovery Rate (FDR) method was used to correct for multiple comparisons, with FDR-adjusted p-values expressed as q-values (Benjamini and Hochberg 1995). Statistical significance was defined as p<0.05. All statistical tests were performed using SPSS version 22 (IBM Inc, Armonk, New York, USA)
2.11 Whole Brain Exploratory Analyses

To conduct the whole-brain exploratory analyses, we used the QDEC (Query, Design, Estimate, Contrast) tool, included in the FreeSurfer package (Fischl 2012).

Vertex to vertex contrasts of cortical thickness was performed for HCs versus BD patients. For this, an average normal control surface was generated, and thickness data from each subject were registered to this surface and smoothed. Then contrast was entered into an analysis of covariance GLM design matrix, including diagnosis, intracranial volume, sex, and age as covariates. We then added BMI as our continuous variable of interest. Results were thresholded at a surface-wide i.e. primary threshold $P < 0.05$. We corrected for multiple comparisons with Monte Carlo simulation and cluster analysis, as implemented in FreeSurfer 5.3 (Liem et al. 2015).

In the main analysis for cortical volume, we used age, sex, and ICV as covariates, and BMI as the continuous variable of interest. For cortical thickness measurements, we removed ICV as prior studies have shown that ICV does not explain thickness and surface area (Liem et al. 2015).

The primary threshold was set at 0.05. Correction for multiple comparisons was done using a Monte Carlo cluster-wise simulation approach (threshold = 1.3 corresponding to $p = 0.05$). Cluster-wise thresholding accounts for the fact that individual vertex-wise differences are not independent of the differences of their neighbouring vertices (Wang and Li 2013).
3. Results

3.1 Demographic and Clinical Characteristics between Groups

The BD group was significantly older than the HC group (p=0.001), had significantly greater BMI (p=0.001), waist circumference (p=0.002), ADHD (p=0.005), ODD (p<0.001), anxiety (p<0.001), substance use disorder (p<0.001), and tobacco use (p=0.004). The BD group also presented with a higher prevalence of family history with depression (p<0.001), anxiety (p<0.001), and ADHD (p=0.002). All of these significant differences survived FDR correction for multiple comparisons. Refer to Table 1.

3.2 Correlation Analyses of BMI/WC vs. ROI measures

In the whole sample (including BD together with HC), BMI negatively correlated with frontal (p=0.004), PFC (p=0.002), and OFC (p=0.001) cortical thickness. WC also negatively correlated with frontal (p=0.013), PFC (p=0.008), and OFC (p=0.023) cortical thickness. Refer to Table 3A.

Within the BD group, BMI negatively correlated with frontal lobe (p=0.022), PFC (p=0.016), and OFC (p=0.001) volume, as well as PFC (p=0.007) and OFC (p=0.013) cortical thickness. WC was negatively correlated only with OFC cortical thickness. Within the HC group, BMI positively correlated with the OFC volume (p=0.030). Refer to Table 3B.

Following up with Fisher’s Z test, the following correlations between BMI and ROIs differed significantly between BD and HC groups: FL volume (z=-3.23, p=0.001), PFC volume (z=-2.99, p=0.001), PFC cortical thickness (z = -2.01, p=0.022), and OFC volume (z=-3.68, p<0.001). The following correlations between WC and ROIs differed
significantly between BD and HC groups: FL volume \((z=-2.711, p=0.003)\), PFC volume \((z=-2.308, p=0.01)\), PFC cortical thickness \((z=-1.669, p=0.048)\), OFC volume \((z=-3.351, p<0.001)\), OFC cortical thickness \((z=-1.665, p=0.048)\), and hippocampal volume \((z=-2.30, p=0.011)\). Refer to Table 3C.
## Table 2. Comparison of demographic and clinical variables between BD & HC groups

<table>
<thead>
<tr>
<th>Sociodemographic</th>
<th>BD (n=40)</th>
<th>HC (n=48)</th>
<th>t, U, or X²</th>
<th>Effect-Size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>17.03 ± 1.44</td>
<td>15.85 ± 1.74</td>
<td>572.5</td>
<td>0.739</td>
<td>0.001**</td>
</tr>
<tr>
<td>Sex (female), n (%)</td>
<td>24 (60%)</td>
<td>25 (52%)</td>
<td>0.554</td>
<td>0.079</td>
<td>0.457</td>
</tr>
<tr>
<td>Socio-economic Status</td>
<td>52.35 ± 12.38</td>
<td>52.98 ± 10.31</td>
<td>-0.26</td>
<td>0.051</td>
<td>0.797</td>
</tr>
<tr>
<td>Race (Caucasian), n (%)</td>
<td>32 (80%)</td>
<td>31 (65%)</td>
<td>2.55</td>
<td>0.170</td>
<td>0.110</td>
</tr>
</tbody>
</table>

### Bipolar Subtype

| BD-I, n (%) | 14 (35%) | 14 (35%) | 2.55 | 0.170 | 0.110 |
| BD-II, n (%) | 14 (35%) | 14 (35%) | - | - | - |
| BD-NOS, n (%) | 12 (30%) | 12 (30%) | - | - | - |

### Clinical characteristics

| Waist Circumference | BD (82.15 ± 9.48) | HC (74.62 ± 6.97) | 317 | 0.905 | 0.001** |
| Adjusted BMI | 24.44 ± 4.32 | 21.22 ± 2.98 | 470 | 0.868 | <0.001** |
| Resting Systolic BP | 112.68 ± 12.84 | 109.85 ± 17.13 | 0.86 | 0.187 | 0.395 |
| Resting Diastolic BP | 68.58 ± 7.81 | 68.07 ± 10.00 | 0.26 | 0.057 | 0.795 |
| Lifetime Tobacco Use, n (%) | 6 (25%) | 0 (0%) | 4.70 | 0.389 | 0.030** |
| Lifetime Abuse | 3 (8%) | 0 (0%) | 1.49 | 0.132 | 0.222 |

### Lifetime comorbid diagnoses

| ADHD, n (%) | BD (17 (44%)) | HC (5 (10%)) | 11.78 | 0.372 | 0.001** |
| Anxiety, n (%) | 29 (73%) | 2 (4%) | 43.88 | 0.710 | <0.001** |
| SUD, n(%) | 9 (23%) | 0 (0%) | 12.03 | 0.370 | <0.001** |
| ODD, n (%) | 12 (30%) | 0 (0%) | 16.67 | 0.435 | <0.001** |
| CD, n (%) | 3 (8%) | 0 (0%) | 3.73 | 0.206 | 0.054 |

### Family History (1st or 2nd degree)

| Mania/Hypomania | BD (22 (55%)) | HC (27 (68%)) | 21.45 | 0.494 | <0.001** |
| Depression, n(%) | 9 (19%) | 9 (19%) | 12.57 | 0.378 | <0.001** |
| Anxiety | 22 (55%) | 9 (19%) | 9 (23%) | 1 (2%) | 9.03 | 0.320 | 0.003** |

### Concomitant Medications

| Second generation antipsychotics | BD (21 (53%)) | HC (8 (20%)) | - | - | - |
| Lithium | 11 (28%) | 0 (0%) | - | - | - |
| Non-SSRI Antidepressants | 4 (10%) | 0 (0%) | - | - | - |
Table 3. Correlation analyses between BMI/WC & ROI measures

### Table 3A. Whole Sample

<table>
<thead>
<tr>
<th></th>
<th>Adjusted BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>P-value</td>
</tr>
<tr>
<td>Frontal Lobe Vol.</td>
<td>-0.112</td>
<td>0.298</td>
</tr>
<tr>
<td>Mean Frontal Thickness</td>
<td>-0.271</td>
<td>0.011*</td>
</tr>
<tr>
<td>PFC Vol.</td>
<td>-0.159</td>
<td>0.140</td>
</tr>
<tr>
<td>Mean PFC Thickness</td>
<td>-0.297</td>
<td>0.005*</td>
</tr>
<tr>
<td>OFC Vol.</td>
<td>-0.158</td>
<td>0.141</td>
</tr>
<tr>
<td>Mean OFC Thickness</td>
<td>-0.275</td>
<td>0.010*</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.120</td>
<td>0.265</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>-0.081</td>
<td>0.453</td>
</tr>
</tbody>
</table>

### Table 3B. BD Sample

<table>
<thead>
<tr>
<th></th>
<th>Adjusted BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>P-value</td>
</tr>
<tr>
<td>Frontal Lobe Vol.</td>
<td>-0.363</td>
<td>0.022*</td>
</tr>
<tr>
<td>Mean Frontal Thickness</td>
<td>-0.296</td>
<td>0.063</td>
</tr>
<tr>
<td>PFC Vol.</td>
<td>-0.377</td>
<td>0.016*</td>
</tr>
<tr>
<td>Mean PFC Thickness</td>
<td>-0.421</td>
<td>0.007*</td>
</tr>
<tr>
<td>OFC Vol.</td>
<td>-0.496</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean OFC Thickness</td>
<td>-0.388</td>
<td>0.013*</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.075</td>
<td>0.644</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>-0.121</td>
<td>0.458</td>
</tr>
</tbody>
</table>

### Table 3C. HC Sample

<table>
<thead>
<tr>
<th></th>
<th>Adjusted BMI</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rho</td>
<td>P-value</td>
</tr>
<tr>
<td>Frontal Lobe Vol.</td>
<td>0.327</td>
<td>0.024*</td>
</tr>
<tr>
<td>Mean Frontal Thickness</td>
<td>-0.039</td>
<td>0.792</td>
</tr>
<tr>
<td>PFC Vol.</td>
<td>0.262</td>
<td>0.073*</td>
</tr>
<tr>
<td>Mean PFC Thickness</td>
<td>-0.002</td>
<td>0.987</td>
</tr>
<tr>
<td>OFC Vol.</td>
<td>0.267</td>
<td>0.066</td>
</tr>
<tr>
<td>Mean OFC Thickness</td>
<td>-0.068</td>
<td>0.645</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.224</td>
<td>0.125</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.048</td>
<td>0.745</td>
</tr>
</tbody>
</table>
3.3 General Linear Models

3.3.1 Main Effect of BMI on ROIs in Whole Sample

The first linear regression model included BMI as the continuous predictor variable of interest and age, sex, and ICV as covariates. In the whole sample, BMI was negatively correlated with mean FL (β=-0.009, η²_p=0.078, p=0.009), PFC (β=-0.009, η²_p=0.078, p=0.009) and OFC (β=-0.009, η²_p=0.078, p=0.009) cortical thickness, when accounting for covariates. The linear model of BMI in predicting ROIs in the whole group is presented in Table 4A. The full model output including all predictors and corresponding statistics from SPSS, can be found in Appendix 3.

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>β</th>
<th>η²_p</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Lobe Vol.</td>
<td>-195.56</td>
<td>0.004</td>
<td>-0.60</td>
<td>0.552</td>
</tr>
<tr>
<td>Mean Frontal Thickness</td>
<td>-0.009</td>
<td>0.078</td>
<td>-2.67</td>
<td>0.009</td>
</tr>
<tr>
<td>PFC Vol.</td>
<td>-202.27</td>
<td>0.012</td>
<td>-0.988</td>
<td>0.326</td>
</tr>
<tr>
<td>PFC Thickness</td>
<td>-0.009</td>
<td>0.086</td>
<td>-2.81</td>
<td>0.006</td>
</tr>
<tr>
<td>OFC Vol.</td>
<td>-47.22</td>
<td>0.007</td>
<td>-0.74</td>
<td>0.462</td>
</tr>
<tr>
<td>OFC Thickness</td>
<td>-0.012</td>
<td>0.107</td>
<td>-3.17</td>
<td>0.002</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>6.96</td>
<td>0.002</td>
<td>0.39</td>
<td>0.697</td>
</tr>
<tr>
<td>Amygdala</td>
<td>11.968</td>
<td>0.020</td>
<td>1.31</td>
<td>0.193</td>
</tr>
</tbody>
</table>

a Multiple linear regression models were also run for WC (substituting BMI). Similar GLM results were observed.
In the second GLM, we introduced Diagnosis as a fixed factor in the model and included a “Diagnosis x BMI” interaction term in the model (refer to Table 4B). The interaction term was significant for FL volume ($\beta=-1701.75$, $\eta^2_p=0.062$, $p=0.023$) and OFC volume ($\beta=-356.78$, $\eta^2_p=0.072$, $p=0.014$), when controlling for age, sex, BMI, Diagnosis, and ICV. In other words, the association between BMI and FL volume and the association between BMI and OFC volume is significantly different between BD and HC. When using WC as obesity proxy, we observed significant interaction effect in the OFC ($p=0.048$). To appreciate this interaction effect, Figure 5 and Figure 6 present scatterplots showing BMI vs. Uncorrected FL and OFC volumes, respectively.
### Table 4C. BD sample: Main Effect of BMI on ROI measures

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>β</th>
<th>η²_p</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Lobe Vol.</td>
<td>-791.75</td>
<td>0.081</td>
<td>-1.76</td>
<td>0.087</td>
</tr>
<tr>
<td>Mean Frontal Thickness</td>
<td>-0.010</td>
<td>0.137</td>
<td>-2.39</td>
<td>0.022*</td>
</tr>
<tr>
<td>Prefrontal Cortex (PFC) Vol.</td>
<td>-371.86</td>
<td>0.044</td>
<td>-1.27</td>
<td>0.213</td>
</tr>
<tr>
<td>Mean PFC Thickness</td>
<td>-0.011</td>
<td>0.154</td>
<td>-2.56</td>
<td>0.01*</td>
</tr>
<tr>
<td>Orbitofrontal Cortex (OFC) Vol.</td>
<td>-187.12</td>
<td>0.149</td>
<td>-2.49</td>
<td>0.018*</td>
</tr>
<tr>
<td>Mean OFC Thickness</td>
<td>-0.016</td>
<td>0.239</td>
<td>-3.36</td>
<td>0.002*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>17.83</td>
<td>0.015</td>
<td>0.73</td>
<td>0.468</td>
</tr>
<tr>
<td>Amygdala</td>
<td>9.75</td>
<td>0.016</td>
<td>0.75</td>
<td>0.456</td>
</tr>
</tbody>
</table>

### Table 4D. HC sample: Main Effect of BMI on ROI measures

<table>
<thead>
<tr>
<th>Region of Interest</th>
<th>β</th>
<th>η²_p</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Lobe Vol.</td>
<td>861.169</td>
<td>0.049</td>
<td>1.49</td>
<td>0.144</td>
</tr>
<tr>
<td>Mean Frontal Thickness</td>
<td>0.001</td>
<td>0.001</td>
<td>0.25</td>
<td>0.804</td>
</tr>
<tr>
<td>Prefrontal Cortex (PFC) Vol.</td>
<td>286.51</td>
<td>0.014</td>
<td>0.78</td>
<td>0.439</td>
</tr>
<tr>
<td>Mean PFC Thickness</td>
<td>0.009</td>
<td>0.000</td>
<td>0.02</td>
<td>0.987</td>
</tr>
<tr>
<td>Orbitofrontal Cortex (OFC) Vol.</td>
<td>161.54</td>
<td>0.041</td>
<td>1.36</td>
<td>0.182</td>
</tr>
<tr>
<td>Mean OFC Thickness</td>
<td>-0.002</td>
<td>0.002</td>
<td>-0.26</td>
<td>0.796</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>-12.27</td>
<td>0.003</td>
<td>-0.36</td>
<td>0.722</td>
</tr>
<tr>
<td>Amygdala</td>
<td>14.97</td>
<td>0.017</td>
<td>0.87</td>
<td>0.392</td>
</tr>
</tbody>
</table>

### 3.3.3 Main Effect of BMI on ROI Measures within BD and HC groups

The linear regression model was analyzed independently within each group to examine the main effect of BMI on ROI measures. In the BD group, BMI was negatively correlated with OFC volume ($\beta=-181.12$, $\eta^2_p=0.149$, $p=0.018$); and FL ($\beta=-0.010$, $\eta^2_p=0.137$, $p=0.022$), PFC ($\beta=-0.011$, $\eta^2_p=0.154$, $p=0.01$), and OFC ($\beta=-0.016$, $\eta^2_p=0.239$, $p=0.018$) volumes. In the HC group, BMI was positively correlated with FL ($\beta=0.001$, $\eta^2_p=0.000$, $p=0.804$), PFC ($\beta=0.009$, $\eta^2_p=0.000$, $p=0.987$), and OFC ($\beta=-0.002$, $\eta^2_p=0.002$, $p=0.796$) volumes.
p=0.002) cortical thickness. There were no significant associations between BMI or WC with any ROI measures in the HC group (p>0.182).

Figure 5. Scatterplot of BMI vs. uncorrected FL volume (mm$^3$)

Figure 6. Scatterplot of BMI vs. uncorrected OFC volume (mm$^3$)
3.5 Sensitivity Analyses within BD subgroup: Controlling for Medication and Psychiatric Co-morbidities

Multiple regression models within the BD subgroup controlling for additional nuisance variables were conducted on the ROIs that had significant diagnosis x BMI interaction effect (FL and OFC volume). Note: The control analyses is exploratory and therefore does not require correction for multiple comparisons.

GLMs with a medication x BMI interaction term, while controlling for age, sex, and ICV were conducted. The medications controlled for included: (1) lithium, (2) second-generation antipsychotics, and (3) antidepressants (both SSRI and non-SSRIs). No significant interaction terms were found.

GLMs with a psychiatric comorbidity x BMI interaction term, while controlling for age, sex, and ICV were also conducted. The comorbidities we controlled for included: (1) ADHD, (2) anxiety, (3) SUD, and (4) ODD. The ADHD x BMI interaction effect was significant in predicting FL (p=0.04) and OFC (p=0.01) volumes (Table 5 and Table 6). To appreciate this interaction effect, Figure 7 presents a scatterplot showing BMI vs. Uncorrected FL volume in both ADHD and Non-ADHD comorbid groups in BD sample.
Table 5. Linear model of predictors for OFC volume in BD group

<table>
<thead>
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<tr>
<td>ADHD * BMI</td>
<td>458.904</td>
<td>2.740</td>
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Table 6. Linear model of predictors for FL volume in BD group

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Figure 7. Scatterplot of BMI vs. uncorrected FL volume (mm$^3$)

Figure 8. Scatterplot of BMI vs. uncorrected FL volume (mm$^3$)
Although the effect of BMI in predicting FL/OFC volumes was not significantly different between non-ADHD BD and HC group, the overall pattern of group differences remained and the main effect of BMI was still significantly negative in the non-ADHD BD group.
Figure 9. Whole brain vertex-wise analysis (BD>HC) of BMI-Cortical Volume correlations. The colour scale indicates Log-P.

**LH volume**
- Cluster Talairach Coordinates: -7.8, 40.3, -15.8
- CW P-value=0.02730
- Size (mm$^2$) = 1544.41
- Number of vertices=2765
- **Cluster peak: Medial Orbitofrontal**
  - Encompasses: Caudal ACC, Rostral ACC, Superior Frontal

**RH Volume**
- Cluster Talairach Coordinates: 13.3, 31.6, 17.7
- CW P-value=0.00010
- Size (mm$^2$) = 3197.35
- Number of vertices = 6505
- **Cluster peak: Caudal Anterior Cingulate**
  - Encompasses: Posterior cingulate, superior frontal, rostral ACC, medial & lateral OFC

### 3.6 Whole-brain Analysis

BMI-to-Volume correlation was significantly lower in BD compared to HC in two clusters. In the left-hemisphere, there was a single cluster with its peak vertex in the medial OFC, and the cluster circumscribed regions in the anterior cingulate cortex as well as the superior frontal gyrus. The second cluster, in the right hemisphere, had its peak vertex in the caudal ACC and extended into the posterior cingulate, superior frontal, rostral ACC, and both lateral and medial OFC. Similar clusters were seen when WC was used in the GLM instead of BMI; however, the clusters did not survive FDR-correction.
Figure 10. Whole brain vertex-wise analysis (BD>HC) of BMI-Cortical Thickness correlations. The colour scale indicates Log-P.

In the uncorrected maps, one can see that the BMI-Thickness correlation is lower in BD than in HC in clusters located around ACC and dispersed through temporal and parietal lobes. However, there were no significant clusters to be seen in the BMI-to-Cortical Thickness correlation maps after cluster-wise correction.
4. Discussion

4.1 Summary of Findings

This study examined the relationship between BMI/WC and neurostructural MRI measures in adolescents with BD compared to HC participants. The ROIs we examined were ones that were previously associated with the pathophysiology of both obesity and BD independently. Findings from this study suggest that there are unique neurostructural correlates of elevated BMI and WC in adolescents with BD, which are not observed in HC. In particular, we observed significant negative correlations between BMI/WC and FL, PFC, and OFC volume/cortical thickness in the BD group. This finding remained significant in the GLM analysis when controlling for age, sex, and ICV. Similar results were found when WC was used as the obesity proxy in the GLMs.

When controlling for additional nuisance variables as a part of sensitivity analysis, we found that there was significant interaction effect between ADHD diagnosis and BMI in the BD group. Subsequent within-BD analyses showed that there was stronger negative correlation between BMI and FL and OFC volumes in BD participants with, vs. without, comorbid ADHD. In contrast, medication-related analyses indicated that there were no statistically significant effects of lithium, SGA, or antidepressants in predicting ROI measures.

Exploratory whole-brain volumetric analyses showed that there were significant group-by-BMI interaction effects in clusters that predominantly encompassed the ACC and the OFC. Specifically, the negative correlation between BMI and the volumes within those clusters were greater in BD than in HC. These clusters overlapped with many of the sub-regions defining the ROIs in our primary analyses. Although clusters in similar areas
were seen in primary thresholded map of cortical thickness, no clusters survived multiple corrections.

In summary, this is the first study to demonstrate a relationship between BMI/WC and reduced brain volumes in adolescents with BD. The results from our study could indicate that brain changes in relation to elevated BMI/WC are localized to the FL in adolescents. This could imply that the FL may be an area of early-susceptibility in the onset of BD. Furthermore, our findings can promote the idea of a potential neurobiological mechanism subserving the link between obesity and poor health outcomes observed in BD. Importantly, these findings suggest the need for treatment studies that seek to evaluate the effect of optimizing BMI and WC on brain structure among adolescents with BD.

4.2 Interpretation of Findings

The results from our study partially support the hypothesis of an interaction effect between diagnosis and BMI in predicting the ROI volumes/cortical thickness. In particular, we observed the diagnosis x BMI interaction effect only in the FL and OFC volume. It is worth noting that the interaction effect was consistently in the negative direction for the other cortical ROIs and nearly significant (p-value range between 0.067 – 0.106). Within the BD group only, GLM results showed that the main effect of BMI was negative in the OFC volume, as well as FL, PFC, and OFC cortical thickness measures. No significant correlations between either BMI or WC and ROIs were found in the HC group.
Consistent with the study in adult BD, elevated BMI in our adolescent sample was associated with FL GM volume reductions (Bond et al. 2014). However, in contrast to that study, we did not observe any negative correlations between BMI and any of our limbic ROIs (i.e. amygdala or hippocampus) – or any limbic structures in the whole-brain analysis.

There is extant literature supporting the fact that the BMI-related volume reductions observed in our BD sample were specifically in brain regions relevant to the pathophysiology of BD. Firstly, volumetric reductions in these FL regions have been observed in youth with BD (Lopez-Larson et al. 2002) (Najt et al. 2007). Additionally, a number of studies have implicated the role of OFC in modulating the function and activity of limbic structures in perceiving and processing emotion (Townsend and Altshuler 2012). There is also strong evidence linking decreased functional activity in these FL areas during a variety of cognitive and emotional tasks in BD (Chen et al. 2011). Moreover, there are studies that show that the functional connectivity between the OFC and limbic structures is disrupted early in the course of BD (Adler et al. 2006; Frazier et al. 2007).

The etiopathological factors that underlie the significant interaction based on diagnosis are as yet uncertain. As suggested earlier in the review of literature (see Introduction), FL and MTL have been negatively associated with obesity among youth in the general population. Therefore, our finding of no negative associations in the HC sample is inconsistent with prior literature. One explanation to justify this inconsistent finding is the fact that our HC sample is healthier than those in prior population studies, both in terms of BMI (see Appendix 4 for number of individuals in each BMI category) and mental health. Out of the 48 HC participants, only 4 (8.3%) met BMI cut-off to be
considered OW or OB (as per CDC guidelines for BMI-for-age and sex percentiles), which is far lower than the general population prevalence of approximately 30% (Ogden et al. 2006). Moreover, the National Comorbidity Survey has reported that nearly one in four adolescents in the US meet the criteria for a mental disorder with severe impairment (Merikangas et al. 2010). In contrast, our HC sample excluded any individuals with SUD and psychotic disorder, and only 10% of our HC sample had ADHD and 4% had an anxiety disorder. One can speculate that the lower-than-population rate of OW/OB, combined with the lower-than-population rate of psychopathology, in our HC sample may have collectively contributed to the null findings in this group.

It is worth noting that the clinical characteristics of our BD sample were representative of another study with a larger sample of 75 treatment-seeking BD youth from our group (Shapiro et al. 2016). For example, the proportion of BD youth with psychiatric comorbid diagnoses in our sample were similar to those reported in the previous study where there were 43% individuals with ADHD, 80% with anxiety, 36% with SUD. In addition, the proportions of BD youth who were medicated with specific psychotropic medications were also similar to the other study where they reported 52% who were on SGAs, 19% on stimulants, 35% on SSRIs, and 15% using lithium. As such the current sample is a good reflection of treatment-seeking youth with BD.

4.2.1 Whole Brain Exploratory Results: The Role of the ACC in BD Neuropathology

The whole brain vertex-wise analysis of BMI-to-volume correlations between groups resolved two fairly large clusters predominantly in the ACC, and also in the OFC. Convergent with our ROI findings, BMI-volume correlations in those regions were more
negative in BD than in HC. Consistent with previous findings, the ACC is another structure that may be related to the neuropathology of BD. Most notably, one study found reduced subgenual ACC volumes following the onset of BD in at-risk youth (Gogtay et al. 2007). This finding would imply that neurostructural changes take place after the onset of BD symptoms. Taken together with previous evidence that suggests that obese youth with BD experience earlier age of BD onset (Goldstein et al. 2008), we can speculate that elevated BMI and its corresponding reduction in ACC volume could be a consequence of BD-onset and therefore related to pathophysiology of BD. However, future longitudinal studies would be needed to confirm this.

Furthermore, there are two functionally distinct areas in the ACC: the ventral ACC, which is functionally responsive to emotional stimuli, and the dorsal, which is responsive to cognitive stimuli. Numerous fMRI studies have shown abnormal activity in the ACC in both emotional and cognitive domains across varying mood states in BD (Strakowski et al. 2004; Strakowski et al. 2012). Given that the ACC plays an integral role in regulating emotion and cognition in BD, its negative correlation with BMI in adolescents with BD is a finding with potential clinical applications. Intervention studies are warranted to determine whether optimization of BMI yields volumetric normalization, and in turn improvements in emotional regulation, among adolescents with BD.

4.2.2. Sensitivity Analyses

In our study, we acknowledged that there were a number of potential confounds, and it was unrealistic to fully mitigate that concern as there are inherent differences
between BD and HC that we cannot control. Nonetheless, we controlled for the most important covariates in our primary analyses (age, sex, and ICV) and then interrogated additional covariates (medication and psychiatric comorbidities) in sensitivity analyses and the findings remained similar for everything except for the effect of comorbid ADHD. Since our sensitivity analyses were exploratory, and as such intended to provide heuristics for future research, they were not subject to correction for multiple comparisons.

Our GLM results showed that the effect of BMI in predicting FL and OFC volumes in BD differs on the basis of ADHD diagnosis. We observed a stronger negative correlation in the adolescents with both BD and ADHD diagnosis compared to the BD-only and the HC groups. Present findings converge in part with prior studies that examined the neurostructural correlates of ADHD. A meta-analysis has shown that there are total brain volume deficits in patients with ADHD compared to psychiatrically normal controls (Valera et al. 2007). One study specifically found cortical thinning in frontal regions and cingulate cortex in children with ADHD (Qiu et al. 2011). Similar to BD, adolescents with ADHD are also more likely to be OW or OB (Fliers et al. 2013). Part of this association may be due to the fact that ADHD individuals eat impulsively and therefore are more likely to binge-eat (Pagoto et al. 2009).

Since the distribution of BMI in the whole sample was skewed to the right with only a few HC participants who were classified as OW/OB, we repeated our multiple regression analyses only using participants with BMI of 30 or less and found that the diagnosis x BMI interaction effects in predicting FL and OFC volumes were more significant (p=0.015 and p=0.005 respectively). When rerunning the analyses with participants with BMI of 25 or less, we observed the loss of significant diagnosis x BMI
interaction effects in predicting FL and OFC volumes (p=0.339 and p=0.221 respectively).

After controlling for family history of MDD in our HC sample we saw that the diagnosis x BMI interaction effect remained significant in predicting OFC volumes (p=0.016) but not FL volume (p=0.086).

4.2.3 Obesity and Neurostructure: Mechanistic Underpinnings

Present findings demonstrate an important association between BMI and brain structure in BD. The important question that remains is: what are the potential mechanisms that mediate the relationship between BMI/WC and neurostructural reductions in adolescents with BD? One theory to explain this stems from the fact that both BD and obesity phenotypes share common pathogenic processes.

As discussed before, obesity and BD both represent conditions of low-grade inflammation. Adipose tissue in obese individuals has been known to produce proinflammatory cytokines such as interleukin-6 (IL-6) (Johnson, Milner, and Makowski 2012). Several studies have shown a relationship between IL-6 levels and smaller brain volumes in the general population, including smaller hippocampi (Marsland et al. 2008; Satizabal et al. 2012). Proinflammatory markers such as IL-6 have been known to interfere with glucose homeostasis and thus are believed to compromise neuronal viability (Marsland et al. 2008; Willette et al. 2010). In adolescents, inflammatory markers are markedly increased in individuals with BD, and these markers are related to the course and symptoms of BD (Goldstein, Collinger, et al. 2011). It therefore could be
speculated that elevated BMI, along with the BD phenotype, could be synergistically related to brain volumes.

A second conceivable mechanism is the dysregulation of the hypothalamic-pituitary-adrenal axis, which impacts immune function. Some studies have suggested that cortisol may be associated with brain atrophy and poor cognitive performance (Lupien et al. 1998). Among adolescents specifically, BMI is known to mediate the association between cortisol awakening response and reduced hippocampal and frontal volumes (Ursache et al. 2012). Furthermore, abnormalities in the HPA axis, similar to those seen in obese individuals, have been observed in BD as well.

There is also considerable body of evidence supporting the link between brain-derived neurotrophic factor (BDNF) and obesity (Rios 2013). Early studies in rodents showed that intracerebroventricular BDNF delivery led to weight loss and reduced feeding (Pelleymounter, Cullen, and Wellman 1995). Other studies have found that mice deficient in BDNF (e.g. via conditional knockout) presented with overeating and weight gain along with a higher incidence of metabolic syndrome components (Kernie, Liebl, and Parada 2000; Rios et al. 2001). Human studies have also highlighted the link between obesity and BDNF. A subset of children afflicted by a rare genetic syndrome that causes large deletions in chromosome 11 are consistently more obese when the deletion encompasses the BDNF gene (Han et al. 2008). Consistent with these findings, clinical studies have found an inverse relationship between BMI and BDNF (Lommatzsch et al. 2005) and circulating BDNF concentrations have also been reported in humans with obesity (Krabbe et al. 2007). Collectively, those studies indicate that BDNF plays a role in maintaining energy homeostasis and that its dysregulation is associated with obese phenotypes. Not surprisingly, adolescent and adult patients with BD have shown
decreased BDNF gene expression (through mRNA levels) as well as lowered BDNF protein levels (Seitz et al. 2014; Pandey et al. 2008). Moreover, a recent meta-analysis has suggested that peripheral BDNF levels are lower during symptomatic intervals and therefore can serve as a potential biomarker for disease activity in BD (Fernandes et al. 2015). In addition to this, BDNF may also be a marker of illness progression in BD since its levels have been found to be inversely correlated with length of BD illness (Kauer-Sant'Anna et al. 2009)

Adipokines comprise another potential mediator of the association between BMI and GM volume in adolescents with BD. Leptin is an adipocyte-derived hormone produced in proportion to body fat percentage, and it is a major regulator of food intake and energy homeostasis (Kenny 2011). The primary role of leptin is to communicate to the brain the abundance of energy storage and to limit food consumption. In most cases, obesity is associated with higher circulating leptin levels (i.e. hyperleptinemia) and a state of disrupted leptin signalling due to leptin resistance (Yang and Barouch 2007). In adults, elevated circulating leptin has been found to be negatively associated with GM volumes left interior frontal operculum, left postcentral gyrus, and right putamen and positively correlated with GM volumes in the left cerebellum and left inferior temporal gyrus (Pannacciulli et al. 2007). Although studies of adipokines in BD are limited, one study found high circulating levels of serum leptin in depressed BD patients (Lee et al. 2014).

4.2.4 Neurocognitive Underpinnings

One plausible explanation for the negative associations between FL/OFC volumes and BMI in the BD sample is that deficits in these frontal regions may lead to disinhibited
eating and impulsive feeding behaviour, contributing to an obese phenotype. This is reported in Maayan et al. (2011), who showed that obese participants had lower OFC volumes, a higher propensity for disinhibition in feeding behaviour, as well as lower performance on executive-functioning cognitive tests. A number of other studies have reported that OW/OB individuals have poorer executive functioning, primarily in the domain of inhibitory control, compared to normal-weight counterparts (Chamberlain et al. 2015; Delgado-Rico et al. 2012; Grant et al. 2015; Koritzky et al. 2012). Similarly, BD in youth has also been associated with impairments in executive functioning, working memory, and attention (Pavuluri et al. 2006) as well as impulsivity (Nandagopal et al. 2011).

A number of other studies have reported that obesity is correlated with response inhibition at the neural level. These studies report lower neural activation in regions involved in response inhibition such as the frontal gyrus, dorsolateral and ventrolateral prefrontal cortex, and orbitofrontal cortex (Asahi et al. 2004; Batterink, Yokum, and Stice 2010; Horn et al. 2003; Leibenluft et al. 2007). The reduced inhibitory activity observed in obese individuals is further supported by functional neuroimaging studies that demonstrate that obese individuals show reduced activation in fronto-limbic reward circuits in response to visual food cues; the implication of this is that they may require more food to achieve the same reward response (Stice et al. 2010). Similarly, one study using positron-emission tomography found that obese adults have decreased prefrontal glucose metabolism and lower performance on the Stroop task, a test of executive functioning (Volkow et al. 2009).
4.2.5 Theoretical Framework: Obesity-Brain-BD Relationship

It is likely that the relationship between BMI and brain structure is bidirectional, especially in the context of BD. Perhaps behavioural disinhibition and heightened feeding behaviour, caused by FL and OFC reduction, can be putting adolescents at risk for weight-gain and increasing likelihood of assuming an obese phenotype. Additionally, reduced OFC/PFC, regions that are involved in emotion regulation and reward circuitry, are associated with depressed mood (Russo and Nestler 2013) which in itself is correlated with overeating and decreased energy expenditure (Macht 2008).

The resulting obese phenotype, in turn, may be causing reduction of aforementioned brain regions via the mechanisms outlined earlier such as increased inflammatory cytokines, decreased peripheral BDNF levels, disrupted cortisol levels, and abnormal leptin signalling.

![Figure 11. Bidirectional relationship between BMI and ROI volumes](image)

Lastly, there may be genetic factors underlying the link between obesity and brain structure. Meta-analytic findings support associations between the G allele polymorphism of the FTO gene with both higher total body fat and lower total brain volume in adolescents (Melka et al. 2013). In support of this, the FTO gene has been found to be
associated with decreased sensitivity to satiety and overfeeding (Wardle et al. 2008). As discussed earlier, the BDNF gene itself plays a putative role in regulating eating behaviour as well as in maintaining the structural integrity of the hippocampus and integrity of the WM. Perhaps a common genetic denominator in both obesity and BD is driving the changes observed in brain structure.

**4.2.6 Potential Treatments to Target Obesity in Youth BD**

According to the Canadian Network for Mood and Anxiety Treatments, the most effective pharmacological treatments for weight-gain in individuals with mood disorders include orlistat, topimirate, and metformin (McIntyre et al. 2012). Orlistat is a pancreatic lipase inhibitor that prevents fat absorption. This drug is particularly effective in individuals with mood disorders because it has no central activity and hence it is less likely to interfere with the effect of psychotropic medication (McClendon, Riche, and Uwaifo 2009). Topimirate, an anticonvulsant, has been shown to be associated with reduced binge-eating behaviour and has known weight-loss effects in individuals suffering from binge-eating disorders (McElroy et al. 2009). Metformin, an insulin sensitizer, has been proven to induce statistically significant, but modest reduction in BMI in children and adolescents (Ehret et al. 2010). In addition to pharmacotherapies, behavioural treatments like cognitive behavioural therapy with dietary modifications and exercise interventions have also been effective in weight-loss and weight-management (McElroy et al. 2009). Few studies have examined this topic in youth.
4.3 Limitations

There were several limitations to this study that are worth noting. Firstly, the cross-sectional and observational design of our study precluded us from making any inferences on directionality or causality on observed associations.

Secondly, there were a number of measures that were not included in this study but may have been relevant in improving our understanding of the brain-BD-obesity link. These include the addition of tests to measure peripheral biomarker such as inflammatory markers, C-reactive protein levels, fasting insulin, triglycerides, and cortisol. Understanding the associations of these peripheral biomarkers with brain structure in relation to BMI/WC could be informative from a biochemical mechanistic perspective. Additionally, given the putative mechanistic role of FL in impulsivity and executive functioning, our study could have benefited from cognitive tests such as the Stroop Test (Homack and Riccio 2004). Furthermore, our study could have benefitted from analyzing the frequency of genotypes, particularly at a priori single-nucleotide polymorphisms implicated in obesity and BD, in our sample. Genome-wide association studies could enable us to identify any novel genetic correlates common to both obesity and BD.

Another limitation was that the mean BMI of BD sample was significantly higher than the HC, and we had few HC participants (8% of HC sample) who were classified as either OW/OB. This may explain in part the absence of a negative correlation between BMI and brain volumes in our HC sample. Perhaps a larger sample with a wider-distribution of BMI could address this issue and enable us to do categorical comparisons between OW/OB vs. normal-weight individuals instead of examining BMI dimensionally.
A technical limitation in our study was the use of BMI as our primary proxy of adiposity. BMI has been criticized as a proxy for adiposity since it does not distinguish between fat mass and other tissue types (e.g. lean mass), between visceral and subcutaneous fat masses, or between different anthropometric fat distributions (Willette and Kapogiannis 2015). This has different implications for obesity-related risks of cardiovascular, cognitive, psychiatric and other complications in adults (Zhu et al. 2004; Luchsinger et al. 2007; Janssen, Katzmarzyk, and Ross 2004). As such, data are sparsely represented in children and adolescents; future studies might consider relationships between brain structure and additional measures of body composition such as visceral fat mass in youth.

Another limitation was that the BD group had varying mood states (i.e. euthymic, manic, depressed or mixed states). Studies have shown that there are apparent functional brain differences, as measured by fMRI, across mood episodes (Strakowski, Delbello, and Adler 2005). Future studies should aim to either recruit participants of one mood state, or a sufficiently large number of participants to examine between-state differences in brain structure.

Additionally, despite the fact that findings were robust to covariation for medications, we still recognize that medication is a potentially confounding variable since it has been associated with both weight-gain and neurostructural changes. The majority of BD adolescents in our sample were medicated, with over 50% on SGAs. Meta-analytic findings have shown that SGAs are associated with weight-gain in children and adolescents (Bak et al. 2014; Almandil et al. 2013). Psychotropic medication and mood stabilizers have also been associated with increased brain volumes in adult patients with BD (Hafeman et al. 2012; Atmaca et al. 2007) suggesting that there may be
neuroprotective effects of these drugs in patients with mood disorders. Although these patterns suggest that medications would bias the analyses toward the null hypothesis, the alternative bias remains possible.

Lastly, we did not have any information on the duration of overweight/obesity in the adolescents in our sample. This information could have been a useful explanatory variable in our GLMs since this could better inform us on the putative exposure-response relationship between obesity and brain structure.

### 4.4 Future Studies

**Longitudinal observational studies with multi-modal designs:** Future studies with longitudinal designs can better define the temporal sequencing of the associations observed in the current study. A prospective design with repeated measures of both BMI and brain structure will be particularly useful in understanding the relationship between putative bidirectional associations between these measures in BD, ideally alongside similar investigation in HC. Integrating fMRI, diffusion tensor imaging, and PET can further our understanding of the pathophysiology of BD. When combined with prospective study designs, we can elucidate the order in which changes occur in the brain. For example it can help answer the question: *does functional connectivity precede functional changes and do structural changes follow?* Moreover, understanding the brain changes associated with specific illness features (such as mania vs. depressive mood states) is relevant to a dynamic and progressive illness like BD. It would also be clinically meaningful to compare these neuroimaging correlates in individuals with different BD-subtypes across the spectrum (BD-I, II, and NOS). Lastly, future observational studies examining brain structure in relation to obesity should compare BD with other psychiatric
illnesses. This can tell us if our study has findings that are relevant to the neurobiology of general psychiatric illnesses or if they are specific to the neurobiology of BD.

**Studies with direct measures of adiposity:** Future neuroimaging studies should consider measures that reflect a more accurate representation of central obesity/adiposity as their proxy instead of using BMI or WC. For example, bioelectric impedance can give quantitative estimate of total visceral fat content (Dehghan and Merchant 2008). Alternatively, abdominal MRI scans can provide estimates for visceral adipose tissue (or VAT), an indicator of central obesity (Shuster et al. 2012). These measures will provide less noise in the data in contrast to BMI, since BMI does not distinguish between fat mass and muscle tissue.

**Clinical Treatment Studies:** Studies are warranted to examine the effect of BMI-reducing treatments on neurostructure. Anti-obesity interventions, such as caloric restriction, can not only help in establishing an association between brain volume reduction and obesity, but also assess the reversibility of underlying pathophysiological mechanisms. This can be especially relevant in the context of BD, where obesity can potentially be a treatment target to alleviate mood symptoms. Future studies can also examine the effects of pharmacotherapy on weight-loss such as Orlistat, a lipase inhibitor that prevents absorption of fat in the intestines (Bray and Greenway 1999). This drug is particularly useful in a psychiatric population because it does not have any known effects in the central nervous system and therefore is also less likely to confound the neuroanatomical correlates being measured (Taylor, Stonehocker, et al. 2012).

Moreover, it would be also interesting to examine the effect of BD medication-related BMI increases on neurostructure. As mentioned previously, specific pharmacotherapies commonly used in BD also confer risk for meaningful weight gain,
including lithium and second-generation antipsychotics (SGAs) (Nashed, Restivo, and Taylor 2011). It is also known that mood-stabilizing drugs may be neuroprotective as they are associated with neurostructural changes, i.e. typically normalising brain volumes. Therefore, these future studies could help us understand if psychotropic-induced weight gain is similar or different to natural weight-gain from a neuroanatomical standpoint.

**Preclinical Mechanistic Studies:** Preclinical studies can offer a valuable tool to further elucidate directionality of the observed associations in our study. Studying the brains of animal models of obesity, i.e. through induced transgenic animals, can help us understand if obesity leads to structural changes in the brain. The benefit of using transgenic mice is that one can induce different components of metabolic syndrome in a multitude of different ways. For instance, one can induce a leptin deficient mouse that can phenotypically express hyperphagia, decreased energy expenditure, and hyperglycemia (Lutz and Woods 2012). Similarly, studying animal models with defective or reduced brain volumes (particularly in areas involved in impulsivity/emotion regulation) and examining its downstream consequences can be useful as well.

**4.5 Conclusion**

In conclusion, this was the first study of its kind to examine the relationship between BMI/WC and neurostructural MRI measures in adolescents with BD compared to HC participants. Findings from this study suggest that there are unique neurostructural correlates of elevated BMI and WC in adolescents with BD, which are not observed in HC. In particular, BMI was associated reduced FL and OFC volumes in BD. As it stands, it is likely that the relationship between BMI and brain structure is bidirectional,
especially in the context of BD. Perhaps behavioural disinhibition and heightened feeding behaviour, caused by FL and OFC reduction, can be putting adolescents at risk for weight-gain and increasing likelihood of assuming an obese phenotype. Conversely, obesity and BD, through the mechanistic actions of increased inflammation, cortisol, leptin, and decreased BDNF could be synergistically leading to loss of brain volumes. Evidently, future longitudinal studies with larger samples and better proxies of obesity are warranted to understand the direction of observed associations.


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Chamberlain, Samuel R, Katherine L Derbyshire, Eric Leppink, and Jon E Grant. 2015. 'Obesity and dissociable forms of impulsivity in young adults', *CNS spectrums*: 1-8.


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Tsuang, M. T., R. F. Woolson, and J. A. Fleming. 1980. 'Premature deaths in schizophrenia and affective disorders. An analysis of survival curves and variables affecting the shortened survival', *Arch Gen Psychiatry*, 37: 979-83.


Appendices
Appendix 1

To: Dr. Benjamin Goldstein
Psychiatry
Room FG 53

From: Dr. Philip Hébert

Date: December 21, 2011

Subject: Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder

Project Identification Number: 408-2011
Approval Date: December 21, 2011
Expiry Date: December 21, 2012

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

The approval of this study includes the following documents:

- Protocol dated December 19, 2011
- Informed Consent Form for Adolescents 13-19 Years of Age Version 1 dated December 19, 2011
- Informed Consent Form for Parents of Adolescents 13-19 Years of Age Version 1 dated December 19, 2011
- Recruitment Poster (Must submit to Sunnybrook Communications & Stakeholder Relations for approval prior to posting)
- Study tools (received November 15, 2011):
  - General Information Sheet
  - Child and Adolescent Health Screening Report
  - Family History Score Sheet – First Degree Relatives
  - Family History Score Sheet – Second Degree Relatives
  - Family Medical History
  - K-SADS Mania Rating Scale
  - K-SADS-P Depression Section
  - Diagnostic Interview K-SADS-PL
  - K-SADS-PL Screen Interview
  - Exercise-Induced Feeling Inventory
  - PRETIE-Q
Appendix 2

- PAR-Q
- BORG'S Rating of Perceived Exertion The 10-Point Scale
- DUSI
- Wong-Baker Faces Pain Rating Scale
- WAVE Adults/Adolescents
- Menstrual History Interview
- Tobacco Use – Lifetime
- Sleep Quality Questionnaire
- Petersen Pubertal Development Scale
- Stressful Life Events Schedule (Adolescent Self-Report)
- Stressful Life Events Schedule (Parent about Child)
- EndoPAT Booklet
- Wechsler Abbreviated Scale of Intelligence

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

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

Philip C. Hebert, MD PhD FCFP
Chair, Research Ethics Board

OR

Miriam Shuchman, MD
Vice-Chair, Research Ethics Board
CONSENT TO PARTICIPATE IN A RESEARCH STUDY
For Adolescents 13-20 years of age

TITLE OF PROJECT:
Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without Bipolar Disorder

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

CO-INVESTIGATORS:
Dr. Daphne Korczak, MD, FRCP(C), FRCPC
Hospital for Sick Children
555 University Avenue
Toronto, Ontario M5G 1X8

Dr. Bradley MacIntosh, PhD
HSF Centre for Stroke Recovery
Sunnybrook Research Institute
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

Dr. Arron Metcalfe, PhD
Sunnybrook Research Institute
2075 Bayview Avenue
Toronto, Ontario M4N 3M5

SPONSOR:
Ontario Mental Health Foundation

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

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

WHAT IS THE USUAL TREATMENT?
Usually, bipolar disorder is treated by assessing symptom frequency and severity, safety and efficacy of medication therapy, and in some cases, psychosocial treatment. Height, weight, blood pressure, and, in some cases, waist circumference is collected. Non-invasive MRI scans of the brain can also be routine practice for some patients.

WHY IS THE STUDY BEING DONE?
This study aims to measure changes in brain activity and blood flow after aerobic exercise among adolescents with and without bipolar disorder, and to find out whether these changes are associated with performance on neurocognitive tests. Furthermore, this study aims to examine how these factors relate to blood vessel functioning, biomarkers, and certain genetic markers. By including these factors in the same study, we hope to learn about the mechanism behind these cognitive benefits of exercise, and how they relate to one another in adolescents with bipolar disorder and in healthy adolescents.

WHAT WILL HAPPEN DURING THIS STUDY?

Study Visit 1
Visit 1 involves taking part in a screening interview to see if you are eligible to participate in this study. The interview 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 illnesses or take these medications, you will be asked to complete a psychiatric interview and to answer questions regarding your medical history, eating habits, physical activity, lifestyle events including family conflict, and use of nicotine, alcohol and street drugs. In addition, an intelligence test will be completed with the interviewer. The interview will take about 3 hours to complete.

Study Visit 2
If you meet the study criteria for being a participant with bipolar disorder or a control participant, you will be asked to return to Sunnybrook for a second visit to complete the following tasks:

IMPORTANT: Before arriving for Visit 2, you will be asked to abstain from all food and drink (no caffeine and alcohol, water is permitted) for at least 8 hours prior. You must also not drink water, smoke or chew gum 30 minutes prior.

Saliva Collection: We will first ask you to provide us with a 4mL sample of your saliva (about 1 teaspoonful) by spitting into a special tube. This will take approximately 10-15 minutes. Additionally, we will ask you to provide us with a sample of your saliva at 5 time points during the course of the study visit by asking you to place a cotton swab in your mouth for 60 seconds. Altogether, this additional saliva collection may take up to 10 minutes.
Blood Vessel Functioning: Next, we will measure your blood vessel functioning using a device called the EndoPAT. This will involve gently placing non-invasive probes on the index fingers of your hands while you are lying on your back. The EndoPAT will gather information for 10 minutes while you are resting. Then a blood pressure cuff will be tightly inflated on your arm for 5 minutes to prevent blood flow. The ultrasound will again gather information for 10 minutes after the blood pressure cuff is released. This will take up to about 60 minutes to complete.

Break: After the completion of these two tasks, you will be given a 30 minute break. Food and drink (non-caffeinated) will be provided.

Questionnaires: After returning from your break, you will be asked to complete questionnaires regarding your medical history, eating habits, physical activity, life events including family conflict, and use of nicotine, alcohol and street drugs. This should take about 30 minutes.

Aerobic Exercise and MRI Scans: Finally, you will be asked to complete a task that assesses brain changes while you perform a cognitive test. This will include a practice of the test, a pre-exercise assessment, a bout of aerobic exercise, and a post-exercise assessment. You will practice the cognitive test for 10 minutes so that you are familiar with it, and complete it two more times both before and after the exercise session. The test gathers information on cognitive function (thinking and memory) by using a reaction test, and may require you to press an appropriate button quickly after a stimulus appears.

After the practice, your brain will be imaged using non-invasive magnetic resonance imaging (MRI) at rest and while you complete the cognitive test. This will take approximately 1 hour. This scan assesses changes in activity and blood flow in the brain, and involves lying stationary on a bed that moves into the centre of the main magnetic field. MRI technologists will perform all MRI scans and are trained to address participant needs and maximize comfort. You will have constant communication with the MRI technologists and study staff while undergoing the MRI and you are free to withdraw at any time.

During one of the MRI scans, there will also be a breath hold task that will require your active participation. This task measures how breath holding may affect blood flow to your brain. You will be asked to hold your breath six separate times for 15 seconds each. You will see instructions on the screen that will switch from “rest” for 30 seconds to “breathe out” for 5 seconds followed by “hold breath” for 15 seconds.

After the MRI, you will be asked to ride a stationary bike for 25 minutes just outside of the MRI scanning room. This will include a five minute warm-up period and 20 minutes of exercise that will increase your breathing and heart rate. The goal is to maintain a constant rate and workload such that your heart rate stays between 60-80% of your age calculated maximum (208-0.7*AGE). You will be monitored for safety and are free to stop exercising at any time. After the exercise, your brain will be imaged again while at rest and during the cognitive test. This will take approximately 30 minutes. In total, this study phase will take about 2.5 hours to complete.
Your parent can accompany you to the MRI scan and wait just outside the testing room. Since the procedures must be the same for all participants, parents may not be inside the testing room.

<table>
<thead>
<tr>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL TIME:</strong></td>
<td><strong>Approximately 4.5 hours</strong></td>
</tr>
<tr>
<td>1 – 4 hours</td>
<td></td>
</tr>
<tr>
<td>Informed Consent = 45 minutes</td>
<td>Saliva Collection = 10 minutes</td>
</tr>
<tr>
<td>Screening = 10 – 15 minutes</td>
<td>Blood Vessel Assessment = 60 minutes</td>
</tr>
<tr>
<td>Psychiatric Interview / Complete self – report forms = 3 hours</td>
<td>Break = 30 minutes</td>
</tr>
<tr>
<td></td>
<td>Questionnaires = 30 minutes</td>
</tr>
<tr>
<td></td>
<td>Cognitive Practice Test = 10 minutes</td>
</tr>
<tr>
<td></td>
<td>Aerobic Exercise = 25 minutes</td>
</tr>
<tr>
<td></td>
<td>MRI Scans = 1.5 hours</td>
</tr>
</tbody>
</table>

**HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?**
It is expected that about 120 adolescents and their parents will take part in this study at Sunnybrook. The length of this study for participants include 2 separate sessions lasting approximately 8.5 hours total. The entire study is expected to take about 4 years to complete and the results should be known in 1 year following the completion of study procedures.

**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 saliva sample you will provide at visit 2:

**Duration of Storage of Information**

All saliva samples will be stored at Sunnybrook Health Sciences Centre. Your individual results of genetic markers and other results pertaining to cognitive test performance will not be reported to you because, at this point in time, these are research measurements, and they do not currently have any clear relevance to your medical health. Any samples obtained from you will be destroyed once analysis is complete. If the research study is extended beyond this time, you will be asked once again to give consent to extend the storage period for a specified amount of time. If you cannot be reached, your samples will be destroyed at that time.

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

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

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

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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<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
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<tr>
<td></td>
<td>Very Likely (30-100%)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Likely (10-30%)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Less Likely (1-10%)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Rare (0-1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Severe</td>
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</tr>
<tr>
<td>Muscle Fatigue/Soreness</td>
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<tr>
<td>Heart Trouble</td>
<td></td>
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<tr>
<td>Heart or Attack</td>
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<td>Light Headedness</td>
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<tr>
<td>Eye Strain or Headache</td>
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<tr>
<td>Hunger</td>
<td></td>
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</table>
There is a chance you may experience temporary muscle fatigue or soreness from the exercise. There are no known risks associated with magnetic resonance imaging other than discomfort while remaining still for the scanning period. You may experience temporary light headedness from the breath hold task. You may experience eye strain or headaches while concentrating on the computerized cognitive test. You may experience emotional discomfort when completing the psychiatric interview and questionnaires. You may experience hunger and/or hunger pains while fasting.

There is a minimal risk of heart trouble with exercise which could make you feel short of breath, pain or pressure in your chest, or pain down your arm. The risk includes the rare possibility of a heart attack. We will minimize the risk by monitoring your heart rate and having appropriate emergency services on hand.

You may discontinue any of the procedures at any time.
You will be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to study staff.

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

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

**CAN PARTICIPATION IN THIS STUDY END EARLY?**

The investigator(s) may decide to remove you from this study without your consent for any of the following reasons:
- You are unable or unwilling to follow the study procedures
- If you are disruptive to the study

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

**WHAT ARE THE COSTS FOR PARTICIPATING IN THIS STUDY?**

There is no cost for participation.

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

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

ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?
Parents will be compensated $50 for travel expenses and parking. Adolescents will be compensated $20 for completing study screening procedures. Eligible participants will also receive $90 at the completion of Visit 2.

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.

**DOES (DO) THE INVESTIGATOR(S) HAVE ANY CONFLICTS OF INTEREST?**
The study doctors do not have any conflicts of interest regarding this study.

**WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?**
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 Chair of the Sunnybrook Research Ethics Board at 416-480-6100 ext. 88144.

Assessing Changes in Cerebral Perfusion and Neuropsychological Function in Response to Aerobic Exercise among Adolescents with versus without 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 research study, or agree to allow the person I am responsible for, to participate in this research study
I understand that my family doctor may be informed of my participation in this research study
This informed consent document may be placed in my medical records

Name of Adolescent (print)                Signature              Date

Assistance Declaration

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

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

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

Name of Person Assisting (print)       Signature       Date

Person Obtaining Consent
By signing this form, I confirm that:

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

___________________________      _________________________________
Name of Person Obtaining                  Signature                                                        Date
Consent (print)
### Appendix 3

**Tests of Between-Subjects Effects**

#### Dependent Variable: Frontal Volume

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<tr>
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<th>Mean Square</th>
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<td>139528280.102</td>
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*a. R Squared = .708 (Adjusted R Squared = .690)*

#### Tests of Between-Subjects Effects

**Dependent Variable: PFC Volume**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
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<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<tr>
<td>Corrected Total</td>
<td>887527227.71</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .510 (Adjusted R Squared = .480)

**Tests of Between-Subjects Effects**

Dependent Variable: OFC Volume
Histogram showing WC and BMI distribution in whole sample

Number of OB, OW, and normal-weight individuals in each group
<table>
<thead>
<tr>
<th></th>
<th>BD</th>
<th>HC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal-weight</td>
<td>27</td>
<td>43</td>
<td>70</td>
</tr>
<tr>
<td>Overweight/Obese</td>
<td>12</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>