Predicting Frontal Lobe H-MRS Metabolite Levels Using Obesity and Metabolic Markers in Individuals with Bipolar Disorder:

A Meta-Analysis and Cross-Sectional Study

by

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ABSTRACT

Obesity and related metabolic disorders, such as metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) impact the brain, particularly in the frontal lobe. Individuals with severe and persistent mental illness (SPMI), such as bipolar disorder (BD), are at greater risk for obesity, MetS, and T2DM, and show worse outcomes than individuals without SPMI. Structural and H-MRS data suggests brain changes in BD in addition to obesity, however, the findings in BD are more heterogenous, and this may be due to an interaction or additive effects between BD and metabolic conditions. In addition, considering metabolic markers in addition to obesity and BD may help predict and possibly prevent worse psychiatric and somatic outcomes.

We conducted a meta-analysis of previous research testing associations between NAA (N-Acetylaspartate, a biochemical metabolite found in the brain) and obesity in three regions of the brain (frontal lobe, hippocampus, and occipito-parietal lobe). NAA was significantly and negatively associated with BMI (body-mass index) in the frontal lobe only ($p = 0.00$).

Secondly, we ran several multiple regression models using age, sex, status (BD patient or control), BMI, waist-to-hip ratio, fasting high-density lipoprotein (HDL), fasting low-density lipoprotein (LDL), fasting triglycerides (TGC), fasting glucose, and fasting insulin measured in the frontal lobe as variables. Participants were either patients with BD ($n = 110$) or healthy controls ($n = 78$). BMI significantly predicted NAA ($p = 0.042$) and Cr ($p = 0.032$), and TGC also significantly predicted NAA ($p < 0.002$) and Cr ($p = 0.008$). TGC also significantly predicted GPC (glycophosphocholine; $p < 0.001$), but only when analyzed as the sole predictor.

This suggests that BMI and TGC, both easy to measure in an outpatient setting, may be an effective way of monitoring for brain-related changes in BD and obesity, in addition to replicating previous findings of brain alterations in obesity and BD.

LIST OF ABBREVIATIONS USED

BD - Bipolar disorder, a severe mental illness that typically consists of patterns of elevated (manic or hypomanic) and lowered (depression) moods over an individual's lifetime.

BMI - Body mass index, a measure of weight that takes height into account. Calculated using the formula = weight (kg)/height (meters)^2

dlPFC – dorsolateral pre-frontal cortex, an area in the frontal lobe.

Cr - Creatine.

GAF - Global assessment of functioning, psychiatric measure of global functioning.

GMV - Grey matter volume in the brain.

Glx - Glutamate.

Gln - Glutamine.

GPC - Glycophosphocholine.

H-MRS - Proton magnetic resonance spectroscopy.

HOMA-IR - Homeostatic model assessment for insulin resistance

HDL - High-density lipoprotein.

LDL - Low-density lipoprotein.

MetS - Metabolic Syndrome, inter-related symptoms linked to both obesity and insulin resistance and typically characterized by increased adiposity around the waist, hypertension, low levels of HDL, high levels of TGC, (known in combination with low HDL as dyslipidemia), and insulin resistance.

mI - *myo*-Inositol.

NAA - N-Acetylaspartate, a biochemical metabolite found in the brain thought to represent neuronal health.

NT - Neurotransmitter.

SES - Socioeconomic status.

SPMI - Severe and persistant mental illness, typically refers to individuals with bipolar disorder, schizophrenia, and major depression.

TCA - The citric acid cycle.

T2DM - Type 2 Diabetes Mellitus.

TGC - Triglycerides.

VIF - Variable inflation factors, a measure of multicollinearity.

WHR - Waist-to-hip ratio.

WMG - White matter volume in the brain.

CHAPTER 1 INTRODUCTION
1.1 INTRODUCTION

Obesity and overweight are among the biggest public health challenges of our time. Obesity is defined as having a BMI of over 30 kg/m^2, with severe obesity being a BMI over 35 kg/m^2. Overweight refers to individuals with a BMI of between 25 and 29.9 kg/m^2. BMI, the most common measure used to track and measure weight, is calculated as weight in kilograms divided by height in metres squared (kg/m^2; Twells et al., 2020). Worldwide, 650 million adults were obese in 2016, and the rate of obesity has nearly tripled since 1975 (WHO, 2021;b). As of 2021, 13% of the world's adult population has obesity, and 39% has overweight (WHO, 2021;b). Obesity and overweight have been steadily increasing over the last few decades, with the United States reaching prevalence rates of obesity of 41.8% for men and women combined (Stierman et al., 2021). Prevalence of obesity in Canada has reached 26.8% (StatsCan, 2019), and increased by 300% since 1985, with severe obesity increasing by 455% (Twells et al., 2020) and making the impacts of obesity and overweight a necessary focus of health research. In addition, cases of type 2 diabetes mellitus (T2DM) have drastically increased, causing 1.5 million deaths directly each year (WHO, 2021;a). Obesity is a risk factor for T2DM (WHO, 2021;b), and currently, approximately 422 million individuals worldwide are living with T2DM today (WHO, 2021;a). In Canada, 13.8% of individuals living with obesity also are diagnosed with T2DM, compared to 2.9% of individuals with weight falling in healthy BMI range (StatsCan, 2019), and overall, 2.2 million Canadians live with T2DM (MHCA, 2013).

Obesity prevalence in Canada and other countries varies by both population and location, and it is likely that prevalence of T2DM and metabolic syndrome (MetS; see below) vary with it.

For example, the proportion of adults with obesity differs by Canadian province, with Newfoundland and Labrador having the highest rate at 40.2%, and British Columbia the lowest at 25.0% (StatsCan, 2019). Nova Scotia, the site of our data collection, has a rate of 33.7%, higher than the national average (StatsCan, 2019). In addition, in many areas of Canada Indigenous Canadians (Inuit, Métis, and First Nations) experience higher rates of obesity in addition to higher rates of other health problems (PHAC & CIHI, 2011; StatsCan, 2019), and in the United States Black Americans also face higher rates (Stierman et al., 2021). These disparities underscore the importance of investigating the impacts of obesity and T2DM on at risk populations.

The presence of markers of metabolic syndrome (MetS) may be used to assess risk for obesity and T2DM. MetS is characterized by a core of inter-related symptoms linked to obesity and insulin resistance. First defined by the WHO in 1998 during attempts to re-examine criteria for T2DM (Alberti & Zimmet, 1998; Huang, 2009), MetS is typically characterized by increased adiposity around the waist, hypertension, low levels of high-density lipoprotein cholesterol (HDL) and high levels of triglycerides (TGC; known in combination with high HDL as dyslipidemia), and insulin resistance (Alberti & Zimmet, 1998; Cornier et al., 2008; Huang, 2009; Mitchell et al., 2013). Although exact definitions vary by source, increased abdominal fat and insulin resistance have typically been considered crucial (Huang, 2009).

1.2 SPMI AND OBESITY

The issue of obesity is particularly pronounced in individuals with SPMI (severe and persistent mental illness), including bipolar disorder (BD). SPMI typically includes BD, schizophrenia, and major depressive disorder. Bipolar disorder is a mood disorder characterized by recurrent episodes of depression and either mania and/or hypomania (Smith et al., 2012).

These episodes are often separated by periods of euthymia, or relatively stable and normal moods. Symptoms of mania include elevated or irritable mood, grandiosity, decreased need for sleep, increased talkativeness, increased sociability, agitation, increased risk-taking behaviours, flight of ideas, and increased distractibility (Smith et al., 2012).

Bipolar disorder is typically categorized as either bipolar disorder type 1 (BD-1) or bipolar disorder type 2 (BD-II), with the former defined by the presence of mania (as opposed to hypomania only, as in BD-I; (Smith et al., 2012). BD-I can sometimes include psychotic symptoms and individuals with BD-I are more likely to potentially require hospitalization at some point or points over their lifespan (Smith et al., 2012). Prevalence estimates range from around 1% of the population for BD-I to around 4% for the more loosely defined bipolar spectrum (Bijl et al., 1998; Judd et al., 2014).

Individuals with severe mental illness (SPMI) can also face discrimination and difficulties accessing medical care (De Hert et al., 2011; Knaak et al., 2017) in addition to the inherent risks associated with their illness and treatment (Brown et al., 2010; Chen et al., 2021; Lawrence et al., 2013; Osborn et al., 2007a; Reininghaus et al., 2015; Saha et al., 2007), which may make accessing care for living with obesity and related conditions more difficult. SPMI typically refers to bipolar disorder, schizophrenia, and major depression, although definitions differ, and here will be used to refer to bipolar disorder and schizophrenia (Zumstein & Riese, 2020). Specifically, evidence suggesting that individuals with SPMI receive inadequate care and monitoring for T2DM in comparison to other patients without mental illness (Frayne et al., 2005; Goldberg et al., 2007) should raise concerns about unmet needs of this population. The cost in Canada of providing treatment and support for individuals with severe and persistent mental illness was estimated at 42.3 billion dollars in 2011, with an additional loss of 50 billion dollars

to the economy, numbers that are predicted to increase over the next few decades (MHCA, 2013). While measures of the economic cost of mental illness typically measure costs directly incurred by mental illness, the high level of comorbidity in this population (De Hert et al., 2009; Mitchell et al., 2013; Vancampfort et al., 2016) suggests that there may be additional costs of somatic illness and disability in this population that go unaccounted for. The focus of our research, BD, is in particular often highly disabling, costly on both individual and systemic levels, and has a prevalence estimated at approximately 45 million individuals worldwide (Bessonova et al., 2020; James et al., 2018; Merikangas et al., 2011). Targeting obesity and metabolic risk factors in individuals with SPMI may be one way of decreasing both cost and risk of mental illness while improving quality of life.

Individuals with SPMI are also at increased risk of living with obesity and other metabolic disturbances (Vancampfort et al., 2016). For example, a meta-analysis by Afzal et al. found the pooled prevalence of obesity in individuals with SPMI to be 25.9%, and 60.1% when combining obesity and overweight (Afzal et al., 2021). Notably, prevalence rates of obesity in the SPMI population were highest in high-income countries (Afzal et al., 2021). In BD specifically, rates of obesity have been found to reach nearly 40% in a Canadian sample (Calkin et al., 2009) and just over 30% in a South Korean one (Kim et al., 2009). Obesity and overweight may also be an important precursor to MetS and T2DM in SPMI (Afzal et al., 2021; Osborn et al., 2007b).

MetS has also been shown to have increased prevalence and impact in individuals with schizophrenia (Bora et al., 2017; De Hert et al., 2008, 2009; Hagi et al., 2021; Mitchell et al., 2013), with around 33% of patients meeting criteria (Mitchell et al., 2013; Vancampfort et al., 2015), and up to three times higher rates than the general population (De Hert et al., 2008).

Increased rates of MetS are also found in BD patients (Bora et al., 2019; McIntyre et al., 2010), with rates of up to 30% in an American population (Fagiolini et al., 2005) and 19% in Canada meeting full criteria (unpublished data, as cited in McIntyre et al., 2010). Rates of MetS have also been found to occur at similar rates in individuals with both schizophrenia and BD (Correll et al., 2008). Higher rates of specific MetS-related criteria in this population have been found, and nearly 70% of BD patients meet HDL limit (Correll et al., 2008).

Individuals with SPMI also appear to have an increased risk of T2DM, with Kim et al. finding that 43.5% of BD inpatients were hyperglycemic, and 4.3% on anti-diabetic medications (2009) and Fagiolini finding that 8.6% of patients with BD had either high levels of fasting glucose or were on anti-diabetics (Fagiolini et al., 2005). Levels of T2DM were found to reach 11.3% in individuals with SPMI (Vancampfort et al., 2016), and individuals with BD, in particular, may have up to a threefold greater risk of T2DM (McIntyre et al., 2005).

Why are the rates of obesity and related metabolic alterations so high in SPMI? One reason could be obesogenic medications. The use of anti-psychotic medication is highly associated with weight gain, with the highest risk coming from clozapine (Mitchell et al., 2013). Individuals with SPMI are also more likely to be sedentary than the general population (Osborn et al., 2007b; Vancampfort et al., 2012), have greatly increased rates of smoking (Dickerson et al., 2013), and have less healthy diets (Osborn et al., 2007b), all of which may also contribute to risks associated with T2DM, MetS, and obesity. Individuals with SPMI may also have lower levels of health literacy with regards to healthy exercise, obesity prevention, and cardiovascular risks (Osborn et al., 2007b). Regardless of the reasons for high co-occurrence of SPMI and obesity, it is highly relevant to look at the marked consequences of this comorbidity.

Obesity, MetS, and T2DM all carry health risks, but those health risks may be amplified in individuals with SPMI. Individuals with SPMI have a mortality rate approximately two and a half to three and a half times higher than the general population (Brown et al., 2010; Osborn et al., 2007a; Reininghaus et al., 2015; Saha et al., 2007), and life expectancy shortened by ten to twenty years in comparison to individuals without SPMI (Chen et al., 2021; Lawrence et al., 2013). Among the many causes of this premature and excessive mortality the foremost for individuals with SPMI are cardiovascular causes, which can be directly related to obesity, metabolic syndrome, and diabetes. For example, nearly 80% of excess deaths in individuals with SPMI appear to be caused by physical comorbidities, with cardiovascular problems being one of the more significant factors in mortalities (Brown et al., 2010; Lawrence et al., 2013). Obesity and T2DM are also associated with increased mortality rates in SPMI (Brown et al., 2010; Chen et al., 2021; Saha et al., 2007), with the standardized mortality ratio (SMR) for T2DM being six-times higher in individuals with SPMI (Brown et al., 2010), and a Taiwanese study finding that the presence of comorbid T2DM in inpatients with BD increased mortality risk by 40%. In addition to increased mortality, obesity and related conditions have also been linked to worsened psychiatric symptoms and outcomes in individuals with SPMI. Higher BMI has been linked to lower scores on the global assessment of functioning (GAF) scale in individuals with BD (Calkin et al., 2009). Reduced GAF scores were also found in BD patients with T2DM-related metabolic markers (such as increased insulin resistance) in comparison to patients with only BD (Hajek et al., 2015). Importantly, MetS appears to be associated with greater depressive symptoms and suicidality (McIntyre et al., 2010), reduced treatment response (Fagiolini et al., 2003; McIntyre et al., 2010), increased disability (Calkin et al., 2009; Hajek et al., 2005), more manic and

depressive episodes (Fagiolini et al., 2003), increased chronicity (Calkin et al., 2009; Hajek et al., 2005) and decreased time from treatment to recurrence (Fagiolini et al., 2003).

The risks of obesity in SPMI also appear to differ by sex. Afzal et al. found increased rates of obesity in women with schizophrenia in comparison to men, but did not find a difference between rates of obesity in men and women with BD (2021). Elias et al., investigating cognitive functioning in the presence of obesity and hypertension in individuals without SPMI, found significant impairment in men but not in women (2003). Men with BD are also more likely to have high blood pressure and high TGC than women (McIntyre et al., 2010), and a Dutch study found an eightfold higher rate of MetS in men with BD in comparison to women (de Jong et al., 2018), however, women with BD have been found to have a higher chance of having obesity in comparison to men (Baskaran et al., 2014). These results suggest that obesity may have differing impacts on and outcomes from obesity in individuals with SPMI.

Finally, evidence suggests that obesity and MetS are also negatively associated with cognitive functioning in general and especially in SPMI populations. Bora et al. found that BD patients with obesity or overweight had significantly worse executive functioning and processing speed, in addition to global impairment (2017). In patients with schizophrenia and MetS, memory, attention, processing speed, and verbal learning tasks, in addition to global functioning, showed impairment in comparison to individuals with schizophrenia but no MetS (Bora et al., 2019; Hagi et al., 2021). T2DM in individuals with schizophrenia was associated with global cognitive functioning as well, but only in male patients (Bora et al., 2019), and obesity was found to be associated with visual learning impairment (Hagi et al., 2021). Executive functioning impairment has also been found in non-SPMI elderly individuals with T2DM and

increased BMI as well, and severity and length of illness of T2DM had a dose-dependent response on that impairment (Mallorquí-Bagué et al., 2018).

Why is it that a mostly peripheral disorder, such as obesity, has such an impact on psychiatric and cognitive outcomes? Potentially this is related to the brain being one of the target organs for obesity-related damage. Evidence of associations between BMI and brain structure, including decreased grey matter volume (GMV) in particular, has been well replicated including large studies and meta-analyses. Regions of interest implicated in research on obesity included frontal regions and in particular the prefrontal-cortex (PFC; Kolenič et al., 2020; Willette & Kapogiannis, 2015; Willeumier et al., 2011) and medial orbitofrontal-cortex (OFC; Marqués-Iturria et al., 2013), the cerebellum (Kolenič et al., 2020), the temporal lobe (García-García et al., 2019; Janowitz et al., 2015; Willette & Kapogiannis, 2015), lower GMV in sub-cortical structures (Dekkers et al., 2019; Janowitz et al., 2015; Willette & Kapogiannis, 2015), and overall GMV (García-García et al., 2019; Janowitz et al., 2015; Willette & Kapogiannis, 2015). In addition, altered WM microstructure was found, if less consistently (Dekkers et al., 2019), along with alternations in WM tracts (Mazza et al., 2017). Many of these regions, including the subcortical regions in particular (Hajek et al., 2009; Hajek et al., 2012; Hibar et al., 2016), are also implicated in BP (Hallahan et al., 2011; Hibar et al., 2017), however, heterogeneity in the results of structural differences in BD complicates interpretation.

More research is now focusing specifically on the interplay between obesity, SPMI and brain structure. For example, Mazza et al. (2017) and Kuswanto et al. (2014) found more frequent alterations in white matter (WM) tracts in BD patients with higher BMIs in diffusion tensor imaging (DTI;(Kuswanto et al., 2014; Mazza et al., 2017). Several studies found decreased white matter volume (WMV) in BD and obesity (David J. Bond et al., 2011, 2014) and

mixed findings for GMV in BD and obesity, but only found decreased GMV and not WMV in participants without BD and obesity (David J. Bond et al., 2011, 2014). Reduced cortical thickness was negatively correlated with BMI in BD patients in the frontal lobe and PFC, OFC, and caudal anterior cingulate cortex (ACC) (Islam et al., 2018; Soares & Law, 2009). Finally, research directly investigating brain changes in obesity and BD has found that one-fifth of the association between BD and increased ventricle size was moderated by BMI (McWhinney et al., 2021), but that sub-cortical areas associated with BD such as the basal ganglia, hippocampus, and thalamus did not appear to be affected (McWhinney et al., 2021). Increased ventricular size has also been found in obesity (Isaac et al., 2011). Hippocampal volumes, already reduced in individuals with BD, were found to be even further reduced in individuals with BD and comorbid insulin resistance (IR), glucose intolerance (GI), or T2DM (Hajek et al., 2014), as well as obesity (Bond et al., 2017). There is, however, another brain-imaging modality that has been given less attention than structural imaging and may help provide some insight into changes in both obesity and BD. While there have been studies on BD using this modality (Cecil et al., 2002, 2003; Hajek et al., 2008; Hajek et al., 2012; Liu et al., 2017; Moore et al., 2000; Yildiz-Yesiloglu & Ankerst, 2006), the intersection between BD and obesity has been studied less using proton magnetic resonance spectroscopy, which I will introduce below.

1.3 - H-MRS AND METABOLITES OF INTEREST

Structural imaging research in obesity and BD-related investigations is commonly cited, but another form of imaging evidence pertinent to this area is proton magnetic resonance spectroscopy (H-MRS) evidence. Rather than taking high-resolution images of the brain as in *magnetic resonance imaging (MRI), H-MRS measures the levels of metabolites in a defined voxel in the brain, and evidence is biochemical (Buonocore & Maddock, 2015; Ross & Bluml,

2001). This measurement is achieved through a phenomenon known as nuclear magnetic resonance (NMR; Buonocore & Maddock, 2015; Ross & Bluml, 2001).

Metabolites, biochemicals created by the breakdown or usage of resources by the body, are measurable using H-MRS and related MRS imaging such as P-MRS (phosphorus MRS). The high specificity of voxel placement in MRS means that very small biochemicals (smaller than 0.1 mM; Ross & Bluml, 2001) can be readily measured *in vivo* with relative precision to region. While at least 70 neurochemicals can be measured using MRS, only small molecules that can be manipulated by the magnetic spin created by NMR can be studied with this technique currently (Ross & Bluml, 2001). Since metabolic rates in the brain change with dysfunction and damage, in addition to the processes of both healthy aging and dementia, measuring metabolite levels can provide insight into both the structure of the brain on a cellular level and the functions of those cells and interactions between them (Lin & Rothman, 2014).

In H-MRS the presence of a net magnetic moment, or a "dipole", is what is being measured. The net magnetic moment is the energy difference between nuclei aligned with or against an external magnetic field, and that dipole is what MRS is measuring (Buonocore & Maddock, 2015). Nuclei have intrinsic spin due to the spin of protons and neutrons in the nucleus, which creates overall spin as long as there is an odd number of protons, neutrons, or both. If the numbers are even, the spin cancels out, since protons tend to pair up with protons spinning in the opposite direction if they are present (Buonocore & Maddock, 2015).

Measuring the dipole of nuclei in biological molecules allows for the creation of a magnetic resonance spectrum, the outcome of H-MRS imaging. MR spectra are images generated based on the signals of protons (or whichever molecule is being used to measure the dipoles) and represent temporal frequencies of the biological molecules being imaged on the

horizontal axis, with amplitude on the vertical (Buonocore & Maddock, 2015). To compare frequencies between metabolites, a zero frequency (the Lamor frequency of a reference molecule at the correct field strength) is established, and other metabolites are represented on spectra in terms of the chemical shift (typically in parts per million, ppm) from this zero frequency (Buonocore & Maddock, 2015). The Lamor frequency of the same atom changes based on the molecule it is in. H-MRS spectra create distinctive peaks representing the interaction between metabolite frequency and amplitude generation that can be interpreted and compared across multiple sessions of data collection, with consistent methods. For example, NAA typically has its major peak found at around 2.0ppm (Ross et al., 2011).

While MRS can be performed using molecules other than hydrogen (e.g., $^{1}H_{1}$), the use of protons is most common because they are present in higher concentrations in biological substances. Alternatives include $^{23}NA_{11}$ and $^{39}K_{19}$, however, the nuclei must have intrinsic spin, and lower concentrations than hydrogen are present for every other atom. Low concentration means that scans require bigger voxels and longer scans, and therefore H-MRS is the most frequently used (Buonocore & Maddock, 2015). Specific sequences of excitation and rotation are also followed during imaging, and while others exist, the most frequently used is the PRESS sequence (Point REsolved Spectroscopy; Bottomly, 1984, 1987; Buonocore & Maddock, 2015).

Imaging done with rodents reveals around 20 different measurable metabolites in the brain, and human research suggests that with high-field tomography the neurochemicals possible to detect is similar (Duarte et al., 2014). These metabolites include creatine (Cr/Cre), y-aminobutyrate (GABA), glutamine (Gln), glutamate (Glu), glucose (Glc), *myo*-inositol (mI/Ins), *N*-acetylaspartate and *N*-acetylaspartyl glutamate (NAA and NAAG), choline-compounds such as glycophosphocholine (GPC; Buonocore & Maddock, 2015; Duarte et al., 2014). Metabolite

levels vary by region of the brain (e.g. NAA decreases with age in the hippocampus and striatum, but not the cortex), by sex, and, as previously mentioned, during the aging process (Duarte et al., 2014). For example, creatine decreases with age and levels vary by gender (Duarte et al., 2014).

One of the most studied metabolites is N-acetylaspartate (NAA), an accepted marker of neuronal health reduced in significant brain injury such as strokes as well as other brain traumas (Ross & Bluml, 2001; Ross et al., 2011). NAA is the acetylated version of the amino acid aspartic acid (i.e., an acetyl group is added to aspartic acid), and is represented chemically by $C_6H_9NO_5$. NAA is also an osmolyte, a substance that controls osmotic pressure in biological materials such as human cells, and also plays a role in the glutamate/glutamine cycle (Bak et al., 2006; Clark et al., 2006). Evidence suggests that NAA is a source of acetyl-CoA, a molecule that provides an acetyl group in the citric acid cycle (TCA; Bak et al., 2006; Clark et al., 2006). The citric acid cyle produces α-ketoglutarate, which is used for the synthesis of glutamate, the main excitatory neurotransmitter (NT; Bak et al., 2006; Clark et al., 2006). NAA has been considered a neuronal marker of viability, however, recent evidence shows that NAA is also found in the precursor cells of oligodendrocytes, a type of glial cells that specialize in myelination (Ross & Bluml, 2001; Ross et al., 2011). This finding is supported by the presence of NAA in both WM and GM in the brain, and by the finding that NAA is reduced with neuronal loss, but also axonal loss (Ross & Bluml, 2001; Ross et al., 2011).

Levels of NAA are also reduced by high glucose levels in T2DM and are increased with surgical weight loss (Daniele et al., 2020). Adults doing endurance training have also been found to have higher levels of NAA (Gonzales et al., 2013). While existing evidence has suggested a negative relationship between BMI and NAA in some areas of the brain (Gazdzinski

et al., 2008; Gazdzinski et al., 2010), not all studies have shown a relationship (Caravaggio et al., 2018; Lee et al., 2020a). Decreased NAA is also associated with increased HbA1C (glycated hemoglobin, or blood sugar) levels in the PFC in individuals with T2DM (Sahin et al., 2008). A recent meta-analysis also found lowered levels of NAA in individuals with schizophrenia, and even in those only at high-risk for schizophrenia (Whitehurst et al., 2020). NAA can also be affected by diet (Auer et al., 2015; Setkowicz et al., 2015). Due to relative ease of imaging (in comparison to other metabolites) and the association with neuronal loss, NAA has been the frequent subject of neuroscientific studies. The association with SPMI and BMI makes it highly relevant to this study. Notably, decreased levels of NAA in individuals with schizophrenia were found in the frontal lobe, temporal lobe, hippocampus, and thalamus (Whitehurst et al., 2020), similar to evidence of structural changes in both SPMI and obesity. In addition to the association between decreased NAA and increased A1C levels in the PFC in T2DM (Sahin et al., 2008), lower NAA has also been found to be associated with frontal, parietal, and temporal areas (Gazdzinski et al., 2008).

However, NAA isn't the only metabolite of interest in SPMI and obesity. Compounds including choline, for example, such as glycophosphocholine (GPC), are involved in the breakdown and synthesis of biological membranes and have been found to be elevated in T2DM (Gazdzinski et al., 2018; Wu et al., 2017) and in individuals with insulin sensitivity (Caravaggio et al., 2018). Choline is very common (present in 50%) of phospholipids, which make up cell membranes and are involved in the breakdown and creation of cells (Jao et al., 2009; Ross et al., 2011). However, phospholipids are too bulky to be visualized in H-MRS except in the case of trauma or extreme duress such as that caused by brain tumours, in which case it is hypothesized that choline may separate from the larger molecules (Jao et al., 2009; Ross et al., 2011).

Increased choline levels are used with neuroinflammation, tumours, and post-stroke. Choline also plays an important role as part of myelin sheaths in the brain as sphingomyelin, and increased levels of choline may also represent damage to myelin (Jao et al., 2009).

Creatine is another metabolite of note - in particular, because it is often used in a ratio with the metabolite of interest, with the assumption that creatine is "neutral". However, creatine levels in the brain may change with trauma, hypoxia, stroke, and other neurological injuries. It also has been shown to increase with age (Chang et al., 2002; Tibbo et al., 2013) and change with illness (Chang et al., 1996), and levels of creatine (Cr) differ between neurons and glial cells in the brain (Brand et al., 1993). In addition, Tibbo et al. found that creatine levels were significantly associated with age only in individuals with schizophrenia, but not in healthy controls (2013), which means age could potentially be a confounding factor in H-MRS studies that do not control for it. Since evidence suggests that creatine levels are associated with factors such as age and health, and are not, in fact, neutral, alternatives to measuring metabolites using a ratio to creatine, such as measuring levels using water suppression, are often used (Buonocore & Maddock, 2015; Jansen et al., 2006).

Glutamate, the main excitatory NT of the brain, can also be measured using H-MRS. Unlike most NTs, which are too bulky to be measured by H-RMS, both glutamate and the main inhibitory NT, GABA, can be measured. However, measurement of glutamate can be complicated by overlap on spectrums between glutamate and glutamine when measured by machines with low-strength magnets, resulting in them frequently being reported together as Glx (Buonocore & Maddock, 2015; Ross & Bluml, 2001). Altered levels of glutamate have been reported in T2DM (Sinha et al., 2014) and may be correlated with insulin sensitivity (Caravaggio et al., 2018) and BMI (Gazdzinski et al., 2010;a). In addition, altered levels of glutamate,

glutamine, abnormalities in the glutamate/glutamine cycle have been documented in individuals with schizophrenia in H-MRS data (Marsman et al., 2013).

Finally, *myo*-insositol (mI) has also been associated with the breakdown of myelin in the brain, and is both an osmolyte (Ross & Bluml, 2001), and part of the phosphatidylinositol (PI) cycle. Dysfunction of the PI cycle has been hypothesized to be part of the mechanism of BD, and one potential theory for lithium's effectiveness in BD is the inositol-depletion theory, which suggests that part of this effectiveness is due to increased update of mI caused by lithium (Silverstone et al., 2005).Research utilizing magnetic resonance spectroscopy data (H-MRS) has found reduced levels of NAA in the brain with obesity and overweight individuals without SPMI in some (Bond et al., 2017; Gazdzinski et al., 2008; Gazdzinski et al., 2010b), but not all studies (Caravaggio et al., 2018; Heikkilä et al., 2008; Lee et al., 2020b), suggesting a need for analysis at the meta-analytic level. In addition, there is very little research specifically investigating MRS correlates of obesity/metabolic alterations and SPMI. Dysglycemia in BD patients is associated with reduced NAA levels (Hajek et al., 2015), as was increased BMI in BD patients, but only when analyzed independently of controls (Bond et al., 2017). Increased BMI in BD patients, but not controls, is associated with altered levels of glutamine/glutamate (Glx; Bond et al., 2016). Interestingly enough, *myo*-inositol (mI), phosphocreatine, and choline levels were reduced in individuals with BD, but increased with overweight or obesity (Bond et al., 2017). In a unique investigation, Gonzales et al. (2013) also found results suggesting that regular aerobic exercise was also associated with increased NAA levels.

Increased levels of Glx and glutamine (Gln), as well as choline, were also found in BD patients but not in HC by Scotti-Muzzi et al. (Scotti-Muzzi et al., 2021). Chitty et al. (2013), in a meta-analysis of H-MRS BD research, also found significantly increased Glx in frontal areas in

individuals with BD, as well as increased Glx in the ACC that was approaching significance (p = 0.064;). Chitty et al. also reported significant heterogeneity among studies, which could potentially affect effect sizes of findings (2013). Some of this heterogeneity could also be due to the impacts of differing medications on metabolites. The presence of mood stabilizers impacted levels of Glx and Glu (Scotti-Muzzi et al., 2021), and a study looking at the impact of lithium found that while BD patients initially had significantly increased mI and choline levels in the ACC in comparison to controls, neither metabolite was significant after six weeks on lithium (Soeiro-de-Souza et al., 2021). Furthermore, choline levels remained high in BD lithium non-responders (Soeiro-de-Souza et al., 2021). The considerable heterogeneity in results suggests the need for more investigation into metabolite levels in BD patients with obesity and related metabolic markers.

Research into brain metabolites is a non-invasive way of studying brain changes and differences in population and has the advantage of being spatially specific. Both obesity and SPMI are associated with brain changes, and H-MRS provides a method to measure biochemical metabolites in the at-risk population of individuals with SPMI and obesity *in vivo* and in areas of the brain. Improving our understanding of metabolic differences in obesity and SPMI may also help predict unwanted outcomes and potentially, even assist in prevention.

In light of heterogeneity and conflicting results in imaging studies investigating BD, we focused on important knowledge gaps and provided the following original contributions. Firstly, we meta-analyzed previously published MRS findings on the association between NAA and obesity. Secondly, we analyzed MRS data from a large sample of individuals with BD, who were phenotyped for obesity and related metabolic alterations and compared these findings with control subjects who were otherwise healthy.

CHAPTER 2 – ORIGINAL RESEACH: META-ANALYSIS

INTRODUCTION

While there are a large number of studies investigating brain metabolites in relation to obesity using H-RMS (Bond et al., 2011, 2016; Caravaggio et al., 2018; Chenji et al., 2021; Gazdzinski et al., 2010a; Kaur et al., 2017; Lee et al., 2020), a meta-analysis in this area has yet to be performed.

In addition, considering the small sample size in many of the included studies and the replication crisis in psychiatry and psychology, there is a need to quantitatively combine these results to understand if individual findings of associations between obesity and brain metabolites (such as NAA) are genuine findings. Meta-analysing previous data will also provide better understanding of the brain regions that might be more predictive of high BMI.

Finally, while our meta-analysis is not primarily about the effect of BD on biochemical brain metabolites (see: Chapter 3), understanding the impacts of obesity on brain chemistry will also help researchers and clinicians, as well as patients, understand the risks of having both BD and obesity.

2.1 METHODS

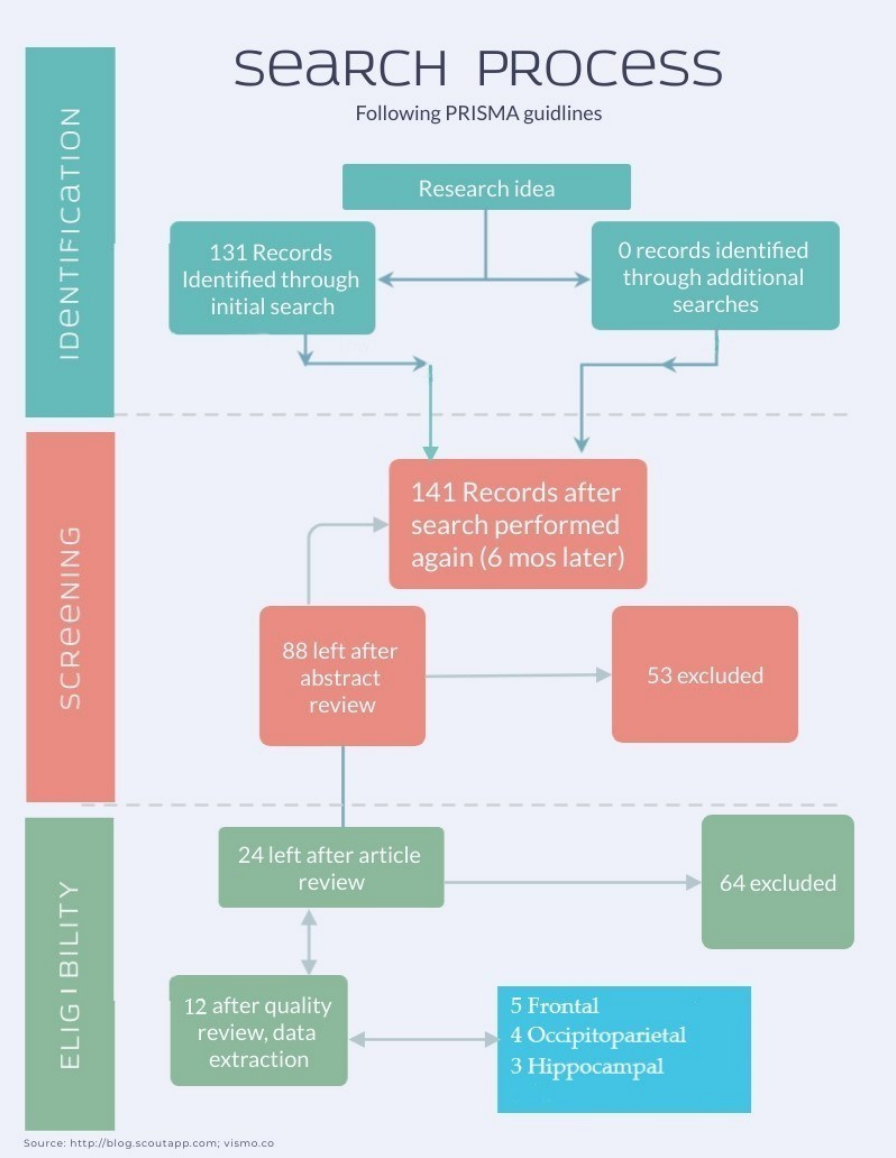
2.1 Study Selection

Studies were considered for inclusion if they 1) compared levels of NAA in an overweight or obese group to a group with normal weight, or 2) measured the correlation between levels of NAA in a sample that ranged in weight from normal to overweight or obese.

We included studies with a mixture of clinical and non-clinical populations if they met one of those two criteria. This inclusion was done for practicality given the small number of studies that met our criteria, and additionally to ascertain if a relationship existed between overweight or obesity and levels of NAA in the brain across populations.

Following the PRISMA guidelines (Page et al., 2021), the initial search was done on PubMed on December 12th, 2021, and consisted of 'obesity AND brain AND "spectroscopy," delivering 141 results. Several other searches were performed (including "H-MRS" and other terms) but only duplicate or clearly unrelated articles returned, so no articles were identified through original searches. In addition, informal searching for relevant citations was employed by investigators scanning articles found via the search process. This informal search process revealed no new relevant articles. Multiple reviewers were involved with the article review process to ensure the inclusion and exclusion criteria were applied correctly.

Figure 1 - Study Selection Process Illustrated



Note: Graphic illustrating the 3 stages of the search process.

2.2 Exclusion Criteria

Exclusion criteria included research on non-proton-MRS (such as P-MRS), MRS research on non-humans or other parts of the body, H-MRS research that did not include NAA as

a measure of interest, and studies that exclusively looked at metabolite levels in children since NAA levels are decreased in infancy (Brand et al., 1993). In contrast, NAA levels remain relatively stable with aging (Chang et al., 1996). Inclusion criteria were kept broad to capture as many potentially relevant studies as possible, given the lack of meta-analyses published in this area. We did not restrict the search by date, and the articles included in the meta-analysis ranged in publication date from 2008 (Gazdzinski et al., 2008) to 2021 (Chenji et al., 2021).

2.3 Statistical Collection

As previously mentioned, the data collected varied across clinical populations, e.g., patients with major depressive disorder, bipolar disorder. To compare varying groups across multiple studies we compared the overweight or obese group to the healthy weight group, regardless of other conditions represented in that population. For example, for Coplan et al., we compared NAA levels between the group with MDD and "normal"-weight to those with MDD with overweight or obesity (Coplan et al., 2014). Because of this, the total number of participants in the original paper is more for some data than the of number of participants included in the final meta-analysis. Papers that included only a "normal" weight group and an overweight/obese group were also included.

Studies included in the meta-analysis presented the statistics necessary for our analysis in varied ways. For example, one study gave NAA levels between groups only in graphical format (Heikkilä et al., 2008), and to obtain values for this data the graph was manually measured, and values calculated from those measurements. Other studies included provided only the statistic calculated (e.g. R^2, *p*-value, etc.). Those statistics we either included as-is or converted into a usable statistic, depending on the values initially provided. Some studies included raw metabolite values or ratios.

Statistics that had to be converted for inclusion in the meta-analysis were converted using Google Sheets, a free Microsoft-Excel like online program, and Microsoft Excel. Conversions performed include calculating t-inversions using *p*-values and degrees of freedom and calculating Cohen's *d* using *t*-values and number of participants. It was also sometimes necessary to convert the standard effect or standard deviation of a value from one to another, and to calculate confidence intervals. Whenever possible values were used in their original form and not converted.

When interpreting results, we used a random effects model (rather than a fixed effects model) to acknowledge that effect sizes found by this meta-analysis only represent the sample populations measured and not all potential populations.

2.4 Data Inclusion

Outcomes recorded for the meta-analysis were limited to the measurement of NAA in the frontal, occipito-parietal lobes, or the hippocampus. Previous investigations have found stronger evidence of volumetric differences in obesity or overweight in areas of the frontal lobe (such as the PFC) than in other areas of the brain (García-García et al., 2019; Marqués-Iturria et al., 2013; Willeumier et al., 2011), therefore, this was a logical region to concentrate on. There was also prior evidence that NAA levels and other metabolite levels are altered in T2DM (Sahin et al., 2008) in the frontal cortex. With regards to structural evidence, as previously mentioned, the hippocampus is another area that has been linked to volumetric changes in obesity and overweight (Dekkers et al., 2019). Finally, the occipital-parietal lobes were included as an outcome because of the availability of studies measuring NAA in obesity and overweight in those areas. While several other studies measured NAA in various subcortical areas, the data was too heterogenous to reliably compare.

Deliberation over which areas of the brain to include was also influenced by the paucity of research measuring NAA in individuals with overweight or obesity, that is to say, we initially included all data regardless of the area of the brain the analysis was done on. We eventually excluded sub-cortical areas during the process due to lack of sufficient data in corresponding areas to analyze in a meaningful way.

Other variables recorded included magnet strength (1.5T or 3T), inclusion of obese individuals in comparison to overweight individuals, ratio used to measure metabolite levels (creatine or water), matrix, voxel size, resolution of voxel, ratio of female to male participants, mean age of participants, and mean BMI. Demographic variables collected were heterogenous. However, because of the small number of studies included the heterogeneity of data among studies, and missing values in these variables for many studies, demographic analyses were not performed. Statistics included in the original article (*f*-value, *z*-score, etc.) are shown in the table detailing included studies.

2.5 Statistical Analysis

The statistical computation for the meta-analysis was performed using Comprehensive Meta-Analysis Lite v3 (*Comprehensive Meta-Analysis*, 2021). Statistical conversions were performed in either Microsoft Excel or Google Sheets, with the exception of confidence intervals that were calculated using Dr. Wuensch's add-on for SPSS (Wuensch, 2021) along with SPSS v.27. We recorded all statistics from the original manuscripts, and then all statistics were converted into Cohen's *d*, which were then used to complete a random effects meta-analysis comparing the differences between groups. We also collected other relevant information from the manuscripts such as confidence intervals, standard deviation and standard error, and demographic statistics, which were also used in meta-analysis. In addition, the RStudio build

2021.09.2 was used to calculate a funnel plot to check for publication bias (RStudio Team, 2020).

Table 1 Table 2 - Table of Included Articles

Reference	ROI	Program	Magnet strength	Ratio Used	Population Descriptions	N	% Female	Mean Age	Mean BMI	Original Stat
Caravaggio et al., 2018	DLPFC	LCModel	3T	Water	HC	17	0.53	28.35	23.81	raw metabolite values, *r*, *p*
Gazdzinski et al., 2008	Frontal	Research Systems (Boulder, CO)	1.5T		HC	50	0.34	41.7	24.84	ρ, % variance/R^2, *p*
Gazdzinski et al., 2010	Frontal		1.5T	Water	Alcohol-dependent males abstinent for 1 mo.	54	0	50.8	20.4-37.1	β, % variance/R^2, *p*
Heikkilä et al., 2008	Frontal cortex	Matlab 7.2	1.5T	Water	non-smoking HC men	9	0	35.9	26	raw metabolite values
Lee et al., 2020	MPFC	LCModel	1.5T	Water	T2DM-O and T2DM-N	100	0.5	49.15	25.45	raw metabolite values
Bond et al., 2017	Hippocampus	LCModel	3T	Water	BD patients	57	0.54	22.8	23.6	F; *p*
Chenji et al., 2021	Hippocampus	LCModel	1.5T		MDD-O and MDD-N	44	0.4	18.7	26.85	raw metabolite values
Coplan et al., 2014	Hippocampus	Interactive Data Language (Boulder, CO)	1.5T; 3T		GAD/HC-O and GAD/HC-N	51	0.63	35	24.89	*r*; *p*
Gazdzinski et al., 2018	L Parietal	LCModel	3T	Creatine	Obese and HC	23	0.55	40.55	37.55	raw metabolites
Haley et al., 2013	Occipito-parietal	LCModel	3T	Creatine	40-60 yr. metabolic symptoms	51	0.52	51	29.4	F, *r*, *p*, metabolite ratios
Kaur et al., 2017	Occipito-parietal	LCModel	3T	Creatine	40-60 yr. HC	73	0.51	49.55	27.88	β, *p*, adj. R^2
Gonzalez et al., 2012	Occipito-parietal	LCModel	3T	Creatine	40-60 yr. HC	55	0.56	50.7	29.6	F, *p*

Note: Citations for table are as follows:

Frontal: (Caravaggio et al., 2018; Gazdzinski et al., 2008; Gazdzinski et al., 2010a; Heikkilä et al., 2008; Lee et al., 2020)

Hippocampal: (Bond et al., 2017; Chenji et al., 2021; Coplan et al., 2014)

Occipito-Parietal:(Gazdzinski et al., 2018; Gonzales et al., 2012; Haley et al., 2013; Kaur et al., 2017)

ROI: refers to region of interest; MPFC, medial pre-frontal cortex; DLPLC, dorsolateral prefrontal cortex. MDD-O? MDD-N? HC?

2.6 HYPOTHESES

Our hypotheses were based on previous research showing decreased levels of NAA in the frontal lobe in individuals with both SPMI and T2DM (Hajek et al., 2015) and the implications of frontal lobe abnormalities in obesity research (Kolenič et al., 2020; Marqués-Iturria et al., 2013; Willette & Kapogiannis, 2015; Willeumier et al., 2011); temporal lobe (García-García et al., 2019; Janowitz et al., 2015; Willette & Kapogiannis, 2015); and subcortical structures (Dekkers et al., 2019; Janowitz et al., 2015; Willette & Kapogiannis, 2015).

1) NAA would be negatively associated with BMI (as BMI rose, NAA would fall) for the frontal lobe analysis.

2) NAA would be negatively associated with BMI in the hippocampus.

3) NAA would be negatively associated with BMI in the occipito-parietal lobes.

2.7 RESULTS

2.7.1 Frontal Lobe

Out of the five studies included in the frontal lobe analysis three used a sample of healthy controls (Caravaggio et al., 2018; Gazdzinski et al., 2008). Other studies included compared individuals with and without obesity with a particular condition or trait with one control group and obese group consisting of men only (Heikkilä et al., 2008), one using previously alcohol-dependent men after one-month of abstinence (Gazdzinski et al., 2010a), and the last using population of individuals with T2DM for both groups (Lee et al., 2020). In total these studies included 230 individuals (see: Table 1).

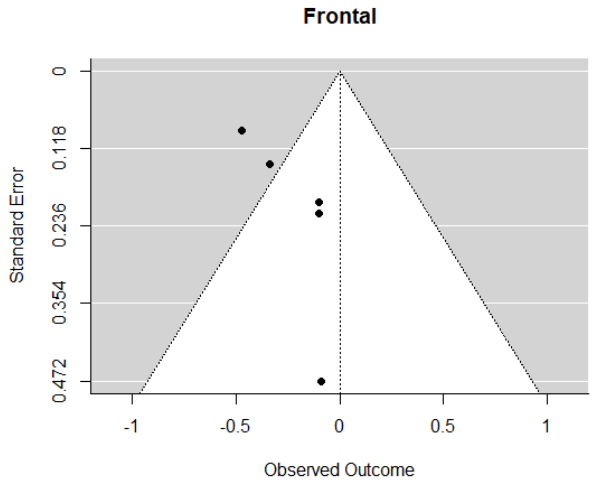
Results of the meta-analysis suggested that levels of NAA were significantly lower in the frontal lobes of individuals with overweight or obesity in comparison to those of normal weights. Five studies were included in this analysis, and the point estimate using a random effects model was -0.32 (*95% CI* -0.49 to -0.16) with $p < .001$. Since I^2 was not significant ($p = 0.16$) we could potentially interpret the results using a fixed effects model, however, erring on the side of caution and using a random effects model acknowledges that this data comes from samples representing a population and not the population itself.

While two of the five studies were published with the same first author only two years apart (Gazdzinski et al., 2008; Gazdzinski et al., 2010a), the sampling was unique, with one sample consisting of alcohol-abusing males (Gazdzinski et al., 2010a), and the other, healthy middle-aged adults (Gazdzinski et al., 2008).

There was a suggestion of publication bias (e.g. the Egger test was approaching statistical significance, $p = 0.06$) when we generated a funnel plot for our results. While this may partially

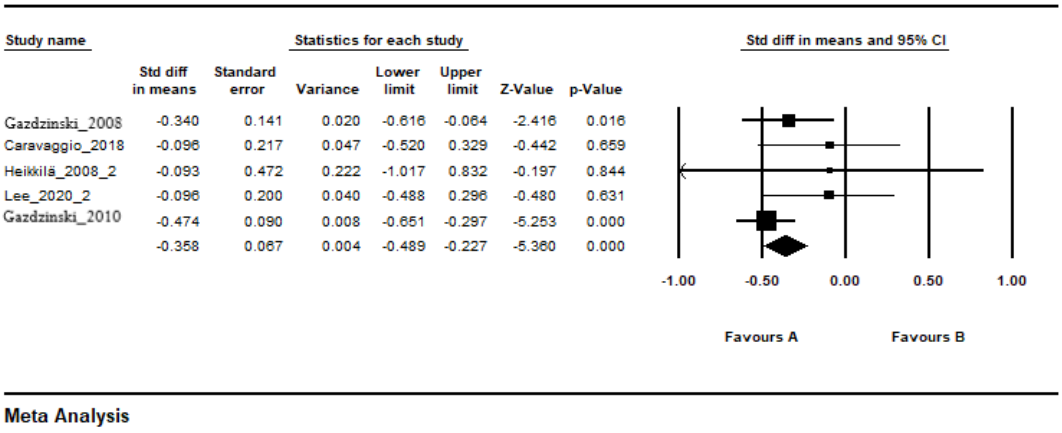
be due to the small number of studies available for inclusion in our meta-analysis, it should be taken into consideration when interpreting the results.

Figure 1 – Meta-Analysis Funnel Plot



Note: Funnel plot for the frontal region meta-analysis.

Figure 2 - Frontal Lobe



Note: Results of the meta-analysis of the studies looking at the frontal lobe. Analyzed in Comprehensive Meta-Analysis Lite v.3 (Borenstein et al., n.d.). *Favours "A" refers to significantly lower levels of NAA.*

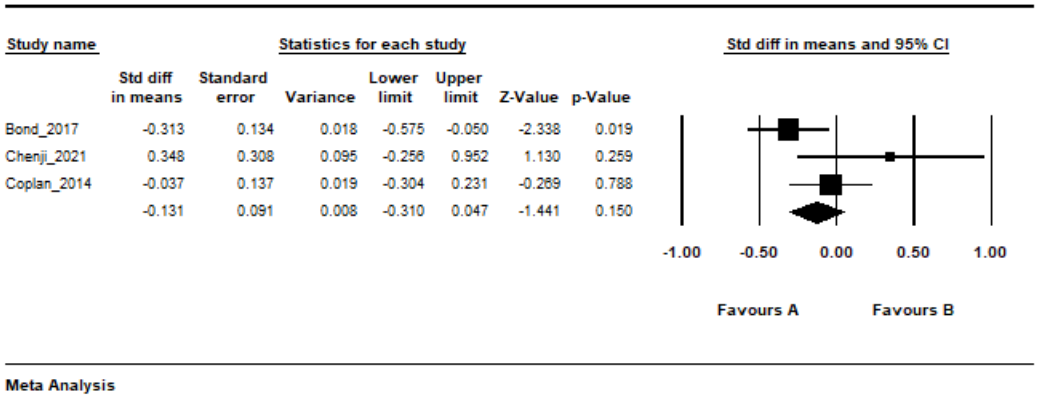
2.7.2 Hippocampus

Available studies compared individuals with obese or overweight with normal weight individuals from somewhat heterogenous groups, including BD patients (Bond et al., 2017), major depressive disorder patients (MDD; Chenji et al., 2021), and patients with generalized anxiety disorder (GAD; Coplan et al., 2014). In total these studies included 152 individuals (see: Table 1).

Results of the meta-analysis suggested that levels of NAA in hippocampus were comparable between individuals with overweight or obesity and normal weights. Three studies were included in this analysis, and the point estimate using a random effects model was - 0.08 (*95% CI* -0.39 to 0.22) with $p = 0.59$. Again, since I^2 is not significant ($p = 57.77$) we could potentially interpret the results using a fixed effects model, however, erring on the side of caution and using a random effects model acknowledges that this data comes from samples representing a population and not the population itself.

As there were no significant differences between the groups, there was no need to test for publication bias.

Figure 3 - Hippocampus



Note: Results of the meta-analysis of the studies looking at the hippocampus. Analyzed in Comprehensive Meta-Analysis Lite v.3 (Borenstein et al., n.d.). *Favours "A" refers to significantly lower levels of NAA.*

2.7.3 Occipital-Parietal Lobes

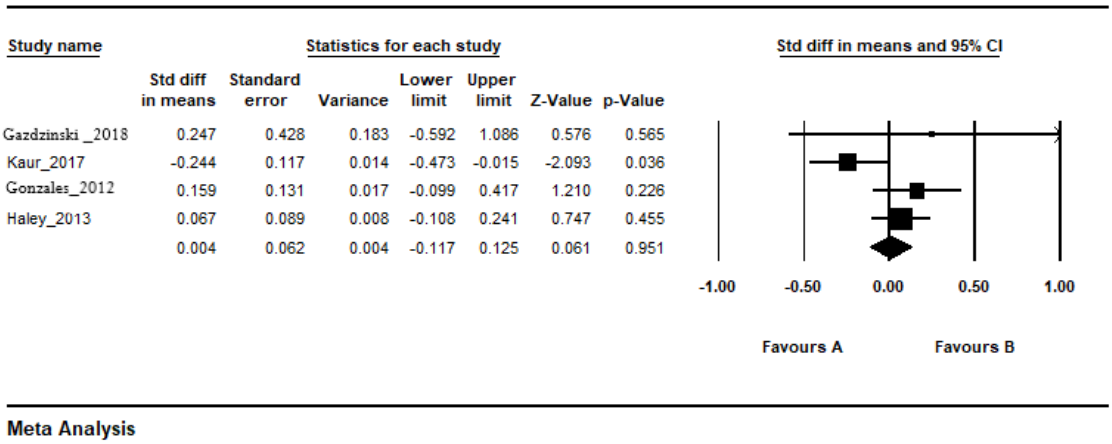
Sample populations were less heterogenous for this analysis, including mostly healthy controls (Gazdzinski et al., 2018; Gonzales et al., 2012; Kaur et al., 2017) and one sample including some individuals with components of metabolic dysfunction (such as hypertension; Haley et al., 2013). In addition, three out of the four samples only included adults from the age of 40 to 60 years old (Gonzales et al., 2012; Haley et al., 2013; Kaur et al., 2017). In total these studies included 202 individuals (see: Table 1).

Results of the meta-analysis suggested that levels of NAA in occipital-parietal lobes were comparable between individuals with overweight or obesity and normal weights. Four studies

were included in this analysis, and the point estimate using a random effects model was -0.170 (*95% CI* -.34 to .20) with $p = 0.95$. Again, since I^2 is not significant ($p = 51.36$) we could potentially interpret the results using a fixed effects model, however, erring on the side of caution and using a random effects model acknowledges that this data comes from samples representing a population and not the population itself.

As there were no significant differences between the groups, there was no need to test for publication bias.

Figure 4 - Occipital-Parietal



Note: Results of the meta-analysis of the studies looking at the occipital-parietal lobes. Analyzed in Comprehensive Meta-Analysis Lite v.3 (Borenstein et al., n.d.). *Favours "A" refers to significantly lower levels of NAA.*

2.8 DISCUSSION

The main finding of this meta-analysis was that, at least across the frontal lobe, NAA appears to be decreased with obesity. However, the results for the hippocampus and occipital-parietal lobes were both non-significant, suggesting that perhaps, as some of the structural evidence of brain changes in obesity suggests (Kolenič et al., 2020; Marqués-Iturria et al., 2013; Willette & Kapogiannis, 2015; Willeumier et al., 2011), the impact of obesity is higher in the frontal lobe than the other areas analyzed.

However, this interpretation should be made with the caveat that there was very little spatial consistency between locations within each of our three analyses. The frontal lobe is a large and heterogenous area of the brain, and further investigation may reveal sensitivities to obesity in particular areas, but not others. Still, the finding of significant decreases in frontal lobe NAA in association with obesity are an important contribution to the neurobiological impacts of obesity on the brain and should be followed up with more spatially precise research. Unfortunately, many of the studies included in this meta-analysis were nonspecific with regards to the voxel placement and specifying the location of the voxel used should be included in future research in this area.

Interestingly, the hippocampus and occipital-parietal lobes showed more heterogeneity in findings, with some studies reporting positive, and others, negative, associations between obesity and NAA. The meta-analyses revealed no significant result for either hippocampus or occipital-parietal lobes and understanding if this result was confounded in some way or truly representative is highly relevant. For example, the paper by Gonzalez et al. included in the meta-

analysis, which showed a trend for positive association between BMI and NAA, included participants with (treated) metabolic comorbidities (Gonzales et al., 2012). Chenji et al. had a younger population in comparison to Bond et al., with an average participant age of 18.5 years to Bond et al.'s approximately 23 years (Bond et al., 2017; Chenji et al., 2021); however, while at a period of rapid change this difference may or may not be significant. In addition, Chenji et al.'s sample had a 2:1 ratio of women to men in the high BMI condition, while Bond et al.'s sample was sex-matched (Bond et al., 2017; Chenji et al., 2021).

Finally, due to the limited number of published results in this area, some of the studies included in the meta-analysis compared NAA between individuals with obesity or overweight and normal weight in a particular group of interest, such as major depressive disorder (Coplan et al., 2014) and generalized anxiety disorder (Chenji et al., 2021). While this was also true of the studies included in the frontal lobe analysis, which did find a significant association between obesity and NAA, it is possible that the conditions included in the hippocampal and occipital-parietal analyses affected NAA in a manner that cancelled out a true effect. There may also have been a region by condition interaction. This suggestion is in keeping with mixed findings in structural research in sub-cortical areas such as the hippocampus (Bond et al., 2017; Hajek et al., 2014; McWhinney et al., 2021). Finally, it is also possible that obesity negatively predicts NAA in the frontal lobes but not the hippocampus or the occipito-parietal lobes. Obesity has been found to have structural effects in the PFC, the OFC, and reduce cortical thickness in the frontal lobe, supporting this possibility (Islam et al., 2018; Soares & Law, 2009). Whatever the source of this heterogeneity in findings, it should be further explored in future research.

Another source of heterogeneity came from the values used to calculate significance. Most studies included the raw metabolite values or raw metabolite ratio either in the published

article or in the supplemental data. In this case, we preferentially used the raw values, taking into consideration the method of normalization used. Typically, MRS data is normalized using creatine levels in the same voxel as a reference metabolite, expressed in a ratio with the metabolite of interest. Alternatively, metabolite levels can be normalized using the water suppression level in the same voxel (Buonocore & Maddock, 2015). While representing metabolite values in ratio with creatine is common, there are some potential problems associated with this practice. Using a ratio of creatine to the metabolite of interest means that if creatine levels are abnormal, the whole ratio will be inaccurate, but the presentation will not reveal which metabolite is responsible for the inaccuracy or even that an inaccuracy has occurred. Given evidence that creatine levels can change with illness and age (Chang et al., 1996, 2002) and aren't constant as had been assumed, makes this practice potentially problematic (Gruber et al., 2003; Öngür et al., 2009). Creatine levels also appear to be up to two to three times as high in astrocytes (a form of glial cells) than in neurons (Brand et al., 1993). Jansen and colleagues have suggested that using absolute metabolite values using water suppression instead of ratios may be a more accurate representation (Jansen et al., 2006).

One other potential source of heterogeneity was the discrepancy in magnet strengths between MRI machines used for imaging. Studies included used both 1.5T and 3T MRI machines, and one study included data measured by both 1.5 and 3T MRI machine (Coplan et al., 2014). Importantly, Coplan also found that hippocampal NAA levels were significantly greater in the 1.5T collection group in comparison to the 3T collection group (2014). While this could be an issue of study bias unique to these collection sites, it could also indicate a larger problem in comparing or statistically combining the data collected on 1.5T and 3T MRIs. We addressed this by using the random effect models and treating site as the random variable.

Another potential source of heterogeneity comes from the lack of statistical consideration for different ratios of volume between gray matter, white matter, and cerebral spinal fluid (CSF) across studies. Most studies did not include this in their analysis, with only two in the frontal meta-analysis correcting for this ratio (Caravaggio et al., 2018; Gazdzinski et al., 2010a), none in the occipital-parietal analysis, and one study in the hippocampal analysis (Bond et al., 2017). Therefore, there was some heterogeneity among included studies, so results could be affected by partial volume which would be a form of systematic bias. Finally, one last minor methodological inconsistency lies in the program used to analyze data. While most used LCModel, several studies (Coplan et al., 2014; Gazdzinski et al., 2008; Heikkilä et al., 2008) used other programs such as MATLAB. There were too few studies using alternative programs to specifically address these issues in the meta-analysis.

Several articles mentioned hypotheses that returned non-significant values and neglected to provided statistics or raw metabolite levels. For example, Bond et al. included an F-value and *p*-value for the significant negative association between NAA and obesity in the lower left hippocampus, but only mentioned that the lower right hippocampus did not meet significance without providing exact statistics (Bond et al., 2017). Not only does this potentially skew the results of this meta-analysis (although we only included left hippocampal data in the analysis for all three studies), but it also suggests the potential presence of publication bias in the research that has been published (Bradley et al., 2020). It is imperative to report results for all hypotheses even if they are not statistically significant, as this then allows others to meta-analyse the findings.

CHAPTER 3 ORIGINAL RESEARCH - HALIFAX STUDY

3.1 INTRODUCTION

Our meta-analysis showed that there was evidence obesity impacts NAA levels in the frontal lobe, validating the need for research specifically analysing the intersection between obesity, related metabolic markers (such as TGC, HDL, etc), and BD. Given the stigma, difficulties accessing healthcare, increased morbidity, and increased mortality rates discussed in BD, understanding how obesity affects this high-risk group is key to eventually predicting and preventing worse psychiatric and somatic complications in this population.

3.2 HYPOTHESES

Prior to beginning data analyses, we formed several hypotheses based on previous structural and H-MRS data covered in our literature-review and the results of the previously analyzed meta-analysis. Evidence considered includes the frequently found finding of decreased NAA with conditions that affect the brain (Ross & Bluml, 2001; Brian Ross et al., 2011), including T2DM (Daniele et al., 2020; Hajek et al., 2015; Sahin et al., 2008) and increased BMI (Gazdzinski et al., 2008; Gazdzinski et al., 2010a), structural evidence that obesity and related metabolic markers impacts the brain (Dekkers et al., 2019; García-García et al., 2019; Janowitz et al., 2015; Marqués-Iturria et al., 2013; McWhinney et al., 2021; Willette & Kapogiannis, 2015; Willeumier et al., 2011), and finally, evidence that the impact of MetS and obesity (Bora et al., 2017; Bora et al., 2019; Calkin et al., 2009; Hagi et al., 2021; Kolenic et al., 2018; McWhinney et al., 2021; Mitchell et al., 2013) may impact individuals with SPMI in unique or increased ways.

Our primary hypothesis was that higher BMI would be associated with 1) decreased NAA, 2) increased GPC, and 3) decreased Cr in both patients and controls, with stronger association in patients

We also wanted to explore which of the BMI-related biochemical metabolic changes would be associated with the MRS metabolites, and if waist-to-hip ratio was a more sensitive predictor than BMI. Therefore, our secondary analysis included the following:

1) Lower NAA in the frontal lobe will also be associated with higher BMI, HOMA-IR (homeostatic model assessment for insulin resistance; Wallace et al., 2004), fasting LDL, fasting triglycerides (TGC), and fasting glucose (glycosylated hemoglobin test; hbA1C), and with lower fasting HDL in patients with BD and controls. However, we hypothesize that there will be a stronger association in patients with BD than controls.

2) Higher GPC in the frontal lobe will be associated with higher HOMA-IR, fasting LDL, fasting triglycerides (TGC), and fasting glucose (glycosylated hemoglobin test; hbA1C), and with lower fasting HDL in both patients with BD and controls. However, again, we hypothesize that this association will be stronger in patients with BD than controls.

3) Lower Creatine in the frontal lobe will be associated with higher HOMA-IR, fasting LDL, fasting triglycerides (TGC), and fasting glucose (glycosylated hemoglobin test; hbA1C), and with lower fasting HDL in both patients with BD and controls. Again, we hypothesize that this association will be stronger in patients with BD than controls.

4) Effects of individual metabolic markers will be smaller than the effect of BMI.

5) Waist-to-hip ratios will show associations in the same directions as BMI.

3.3 METHODS

3.3.1 Recruitment

This was a retrospective study of BD patients (n = 110) and healthy controls (n = 78) recruited from the Nova Scotia Health Authority (NSHA) Mood Disorders Clinic and control subjects recruited through advertisement. This study was approved by the Ethics Committee of Nova Scotia Health Authority and all included participants signed informed consent.

3.3.2 Inclusion/Exclusion Criteria

Patients with BD were required to 1) have the diagnosis of bipolar I or II disorder made by a psychiatrist; and 2) be at least 18 years of age. Patients were excluded if they had 1) a diagnosis of an organic mood disorder; 2) a mood disorder not otherwise specified; or 3) more than one lifetime course of electroconvulsive therapy or electroconvulsive therapy within the last 6 months. The neuropsychiatrically healthy, euglycemic participants were excluded if they had 1) a personal history of psychiatric disorders; or 2) T2DM. Participants from any group were excluded if they 1) met any magnetic resonance imaging (MRI) exclusion criteria; 2) suffered from substance abuse disorder in the last 12 months; had a history of 3) neurodegenerative disorders; or 4) cerebrovascular disease/stroke, to avoiding possible confounding neuronal changes.

3.3.3 Data Collection

To measure body mass index (BMI), we measured weight and height and calculated BMI using the following formula: = weight (kg)/height (meters)^2. Waist circumference was also

measured, and waist-to-hip ratio calculated for all participants. All participants had bloodwork performed for measurement of metabolic markers (fasting plasma glucose, fasting serum insulin, fasting triglycerides, fasting HDL, and fasting LDL). Insulin resistance was calculated using the homeostatic model assessment-insulin resistance (HOMA-IR) equation: HOMA-IR = fasting plasma glucose (FPG;mmol/L) 3 fasting serum insulin (FSI; μU/mlL)/22.5 (Hajek et al., 2014). The HOMA-IR is a method of calculating insulin resistance, and was used because it has been shown to have a high degree of correlation with the euglycemic clamp method and is an accepted measure of insulin resistance (Hajek et al., 2014; Katsuki et al., 2001; Wallace et al., 2004).

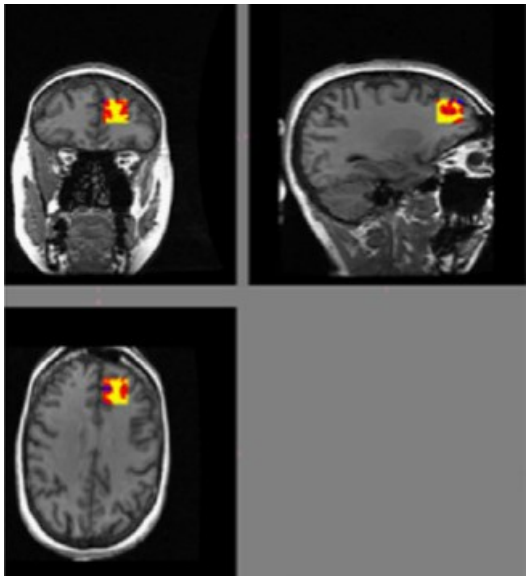
3.3.4 MRI Methods

All magnetic resonance acquisitions were performed with a 1.5 Tesla General Electric Signa scanner (General Electric Medical Systems, Fairfield, Connecticut) and a standard quadrature head coil. After a localizer scan, a T1-weighted spoiled gradient recalled scan was prescribed (flip angle = 40 °, echo time = 5 msec, repetition time = 25 msec, field of view = 24 cm x 18 cm, matrix = 256 x 160 pixels, number of excitations = 1, no interslice gap, 124 images, 1.5 mm thick), followed by one single volume (20 x 20 x 20 mm) proton magnetic resonance spectroscopy acquisition with a probe point resolved spectroscopy sequence and the whole gradient mode (echo time = 30 msec, repetition time = 2000 msec, 320 acquisitions, 2500 Hz spectral bandwidth, 2048 data points). We acquired unsuppressed water and water sup- pressed spectra from the same location. The unsuppressed water signal was used for eddy current compensation and for metabolite quantification.

3.3.5 Voxel Placement

The spectroscopic region of interest (ROI) was prescribed blind to subject status in the left dorsolateral prefrontal cortex (PFC), using previously validated methods (Hajek et al., 2012; Hajek et al., 2008). The MRI sequences used to measure H-MRS data had a repetition time (TR) of 30 ms and a time-to-echo (TE) of 2000 ms. The matrix was 256 by 160 pixels, with a thickness of 1.5mm. The field of view (FOV) was 24 mm by 18 mm. Scans on the coronal plane were placed with the inferior border ending above the cingulate cortex (CC), and those on the medial-lateral plane were placed halfway from the border of the hemisphere, with care taken to avoid pockets of cerebral spinal fluid (CSF). As the meta-analysis previously covered suggested, in addition to the research cited showing alternations associated with obesity in the frontal lobe (Kolenič et al., 2020; Marqués-Iturria et al., 2013; Willette & Kapogiannis, 2015; Willeumier et al., 2011), the frontal lobe appears to be an area of interest in obesity in addition to BD.

Figure 5 - Sample Voxel Placement

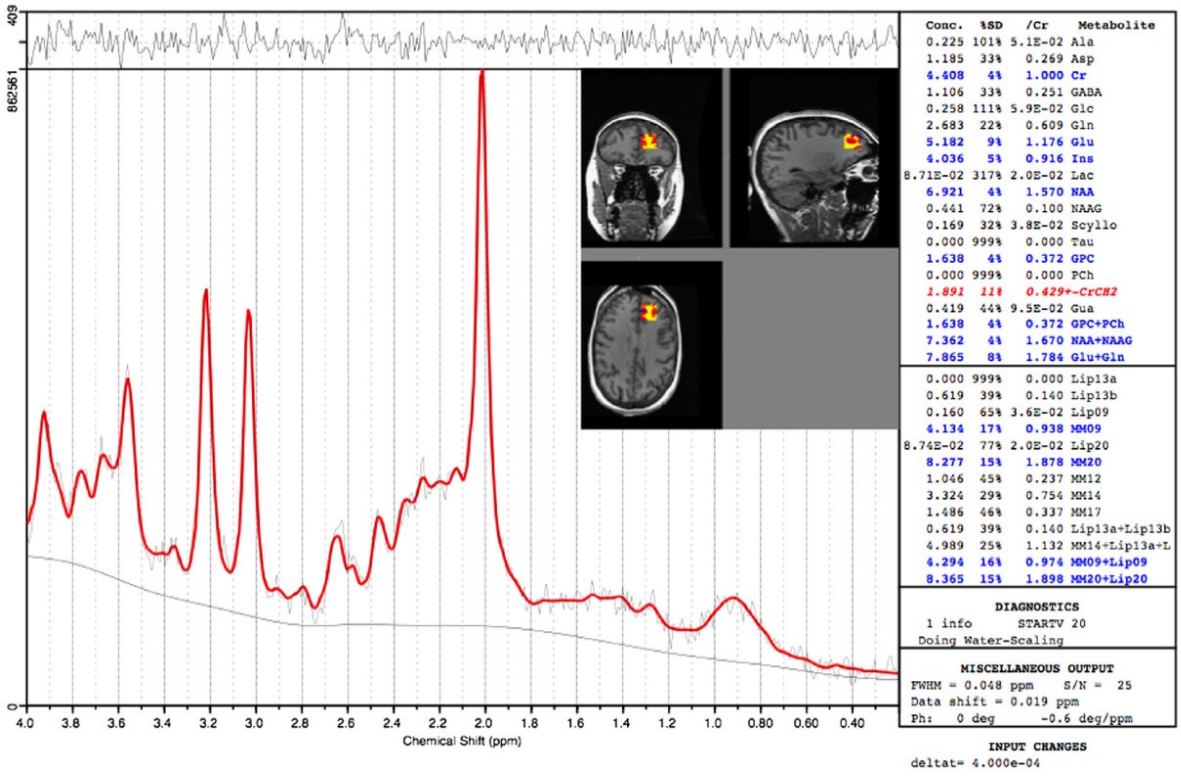


Note: Sample placement of the voxel in the dlPFC (dorsolateral prefrontal cortex) of a participant.

3.3.6 Spectral Analysis

We quantified metabolite levels with a linear combination model of in vitro spectra (LCModel version 6.1; http:// s-provencher.com/pages/lcmodel.shtml) using previously validated methods (Provencher, 2001)**.** The method employs a basis set of concentration-calibrated model spectra of individual metabolites including lipids and macromolecules, as listed below. As in our previous studies, we controlled for partial volume averaging effect by estimating the tissue type composition of the spectroscopic ROI. This was done by performing tissue-type segmentation using AFNI software (Cox, 1996), and using previously published criteria (Gispert et al., 2004). tringent quality criteria for assessment of spectral profiles were also adhered to (Hajek et al., 2008; Hajek et al., 2012)**.** These included full-width at half maximum (FWHM) < 08 ppm; signal-to-noise ratio (SNR) of the whole spectrum fit ≥ 8, as reported by LCModel; randomly distributed noise; and absence of artifacts, or signal splitting for NAA, GPC, Cr in the MRS spectra. To ensure reliability of measurement, the estimated standard deviations for NAA, GPC, and creatine expressed in percent of the estimated concentrations had to be less than 15%. As the use of ratios to other metabolites, especially Cr, has previously been questioned (Gruber et al., 2003; Öngür et al., 2009)**,** we reported metabolite levels in institutional units relative to water peak. The above-described methods meet all of the quality benchmarks suggested for MRS studies (Öngür, 2013)**.**

Figure 6 - Sample Spectrum and Quality Control



Note: Sample spectral analysis of a participant showing peaks for differing metabolites, with NAA at approximately 2.0ppm, Cr at approximately 3.0ppm, GPC at 3.2ppm, and mI at approximately 3.6ppm. Quality control measures are listed in the right-hand column.

3.3.7 Statistical Analyses

All statistical analyses were performed in RStudio 2021.09.2; Build 382 (RStudio Team, 2020).

Our analytical plan included the following steps, all performed as regression or multiple regression models: 1) in separate models we investigated associations between age, sex, status, BMI, and individual H-MRS metabolic markers, using each variable as the sole predictor. 2) To

test our main hypotheses (decreased NAA, increased GPC, decreased Cr in individuals with BD and obesity) we ran a model including BMI, status (BD patient or control), age and sex as predictors and individual MRS metabolites as dependent variables. 3) We investigated whether waist-to-hip ratio (WHR) on its own (as a sole predictor) or in combination with BMI predicted individual metabolites while controlling for age, sex, status.

For the metabolic markers we performed the following analyses. 4) First, we ran a multiple regression model controlling for age and sex and including all the biochemical measures, i.e., fasting TGC, LDL, HDL, Insulin, Glucose. 5) For any biochemical measures, which were associated with the MRS metabolites, we then ran a separate model including the specific biochemical measure, BMI and controlling for age and sex. This was done to evaluate whether BMI or the biochemical measures were more sensitive predictors. 6) Finally, we tested for interactions and included them where relevant. In line with our a-priori hypotheses, this approach allowed us to identify variables significantly associated with MRS metabolites and to test whether their effects were additive.

3.4 RESULTS

There were no differences between grey matter or white matter proportion in the ROI between the groups, furthermore, neither GMV nor WMV were associated with BMI, so we did not include either as covariates. Multicollinearity was not a concern, because the highest VIF (variable inflation factors, a measure of multicollinearity) was 1.70. Residuals were normally distributed, as confirmed by the Kolmogorov-Shapiro test.

Table 3 - Halifax Study Demographics

Demographics

	Status				
	Controls		BD		
N	78		110		
		SD		*SD*	*p*
Age (*M*)	39.16	13.85	48.54	14.54	**<0.001***
Sex (%F)	29	37.2	49	44.5	0.39
GAF (*M)*	90.84	7.08	74.81	12.48	**<0.001***
BP (Sys; *M*)	114.91	13.04	121.17	13.23	**0.002***
BP (Dia; *M*)	75.2	9.72	77.69	9.82	0.102
BMI (*M*)	26.28	4.74	29.22	5.58	**<0.001***
WTH (*M*)	0.82	0.09	0.93	0.07	**<0.001***
T2DM (%YES)	4	8.3	13	19.1	0.177
HBP (%YES)	2	4.2	21	31.3	**0.001***
THYROID (%YES)	3	7.5	24	38.1	**0.001***
IR (%YES)	24	30.8	46	41.8	
Education					0.067
Employment					**<0.001***
Marriage					0.057

Note*: M and SD are used to represent mean and standard deviation, respectively.* ***** *Indicates significant p (e.g., p < 0.05). GAF is the global assessment of functioning score; BP Sys refers to systolic blood pressure; BP diastolic blood pressure; WTH refers to waist-to-hip ratio in cm; T2DM specifies if the participant has been diagnosed with T2DM; thyroid (%YES) refers to the percentage of participants with a diagnosed thyroid disorder; IR (% YES) refers to the percentage of participants with insulin resistance; employment categorized participants as unemployed, disabled, full-time, part-time, retired, or student; education similarly categorized participants as having some high-school, less than high-school, a high-school diploma, bachelor's degree, community college, or a post-graduate degree based on highest level of*

education achieved; and finally marriage refers to the participants marriage status as either single, married, divorced, or widowed.

Table 4 - BD-Specific Demographics

	BD	(SD)
Age Onset (*M*)	23.51	9.31
Duration Illness (*M*)	22.86	12.31
Age 1st Adm. (*M*)	28.98	12.28
Episodes (*M*)	15.94	23.16
Psychotic Symptoms (%YES)	0.45	
Lifetime Suicide Attempt (%YES)	0.26	
Lifetime ECT (%YES)	0.1	

Note*: M and SD are used to represent mean and standard deviation, respectively.* ***** *indicates a significant p-value (e.g., $p < 0.05$). Age 1st Adm. refers to the age the patient was first admitted to hospital. Psychotic symptoms (%YES) is the percentage of patients who have experienced psychotic symptoms, lifetime suicide attempt (%YES) is the percentage of patients who have experienced a suicide attempt over their lifetime, and lifetime ECT (YES%; electroconvulsive therapy) is the percentage of patients who have experienced being treated with ECT over their lifetime.*

3.4.1 NAA Results

When analyzed independently, in separate models (e.g. as the sole predictors), we found a negative association ($\beta = -0.019$) between NAA and age ($F(1,186)=61.47$, $p < 0.001$), BMI ($\beta = -0.027$, $F(1,183)=13.09$, $p < 0.001$), status ($\beta = -0.319$, $F(1,186)=14.88$, $p < 0.001$), but not sex ($F(1,186)=2.30$, $p = 0.131$).

In a multiple regression model containing status, BMI, as well as age and sex as variables, NAA remained significantly and negatively associated (β = -0.014) with BMI ($F(1,180)=4.20$, $p = 0.042$). The status of the participant (i.e., either BD or control) was not found to be significant ($F(1,180)=2.29$, $p = 0.132$). Finally, age, again, remained significant and negatively associated (β = -0.017) with NAA ($F(1,180)=41.98$, $p < 0.001$). There was no significant interaction effect between status and BMI ($F(1,179)=0.01$, $p = 0.943$) so we did not include the interaction in our models.

Waist-to-hip ratio also significantly negatively (β = -0.936) predicted NAA on its own ($F(1,132)=22.26$, $p < 0.001$), but this effect was lost when controlling for status, age, and sex, unlike BMI ($F(1,129)=1.88$, $p = 0.173$).

With regards to individual biochemical markers, TGC was associated with NAA ($F(1,135)=8.73$, $p = 0.004$) when controlling for age and sex. Other metabolic markers included in the analysis such as LDL, HDL, Glucose, and Insulin were not significant. TGC remained a significant and negative predictor of NAA (β = -0.164) when controlling for age, sex, and BMI ($F(1,132)=4.11$, $p = 0.027$).

Table 5 - NAA - Variables of Interest (Basic Predictor Models)

Variable of Interest	Degrees of Freedom	F-Value	Estimate	*p*-Value
Model 1				
Status	1,186	14.88	-0.319	**< 0.001***
Age	1,186	61.47	-0.019	**< 0.001***
Sex	1,186	2.30	-0.130	0.131
BMI	1,183	13.09	-0.027	**< 0.001***
Waist-2-Hip	1,132	22.26	-2.408	**< 0.001***
Model 2				

Fasting HDL	1,133	1.33	0.18	0.253
Fasting LDL	1,133	0.10	-0.02	0.754
Fasting Glucose	1,150	0.11	-0.02	0.742
Fasting Insulin	1,141	0.83	0.00	0.363
Fasting TGC	1,134	9.61	-0.19	**< 0.002***

Note: ***** *indicates significant p-value. Status marks patient and control groups. Status, age, sex, BMI, and waist-2-hip are sole predictors. In Model 2, Fasting HDL, LDL, glucose, insulin, and TGC are corrected for age and sex. Bonferroni correction not performed for models, see: full models in Table 3.2. Coefficient estimate for sex is for "male", and for status is "patient".*

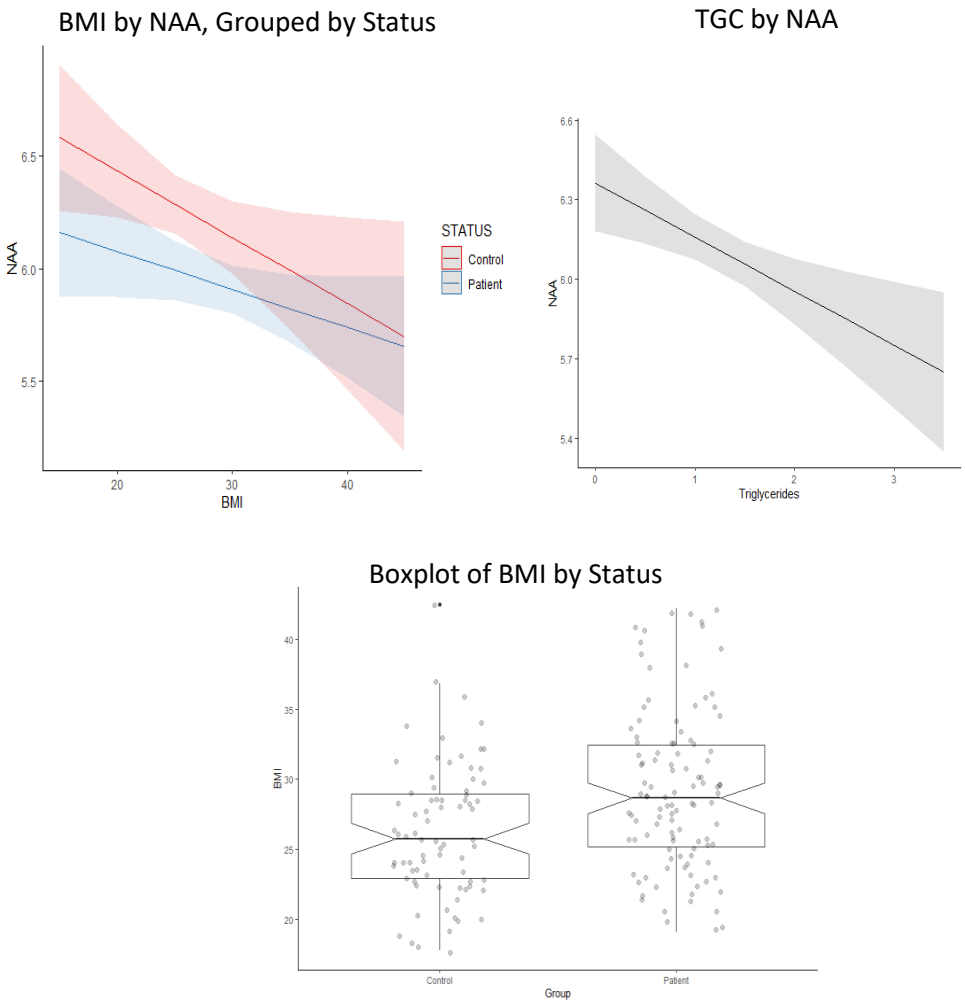
Table 6 NAA - Variables of Interest (Full Predictor Models)

Variable of Interest	Degrees of Freedom	F-Value	β	*p*-Value
Model 3				
Status	1,180	2.29	-0.120	0.132
Age	1,180	41.98	-0.017	**<0.001***
Sex	1,180	2.21	-0.110	0.138
BMI	1,180	4.20`	-0.014	**0.042***
Model 4				
Waist-to-Hip	1,129	1.88	-0.936	0.173
Model 5				
Fasting HDL	1,132	1.77	0.152	0.185
Fasting LDL	1,132	0.027	0.048	0.871
Fasting Glucose	1,1116	0.192	0.035	0.662
Fasting Insulin	1,140	0.0028	-0.000	0.929
Fasting TGC	1,132	4.11	-0.164	**0.027***

Note: ***** *indicates significant p-value. Model 3 contained status, age, BMI, and sex. Model 4 (only used to analyze waist-to-hip) contained waist-to-hip, while controlling for status,*

age, and sex. Model 5 contained either fasting HDL, LDL, glucose, insulin, or triglycerides, along with age, sex, and BMI. Coefficient estimate for sex is for "male", and for status is "patient".

Figure 7 – NAA Graphs



3.4.2 GPC Results

When analyzed independently, in separate models (e.g. as the sole predictors), we found a positive association (β = 0.059) between GPC and status ($F(1, 184)=4.82$, $p = 0.029$) indicating that individuals with BD had significantly higher GPC than controls, a positive association (β = 0.004) with age ($F(1,184)=16.56$, $p < 0.001$), with sex (β = 0.069, $F(1,184)=6.73$, $p = 0.01$), but not BMI.

In a multiple regression model containing status, BMI, as well as age and sex as variables, GPC remained significantly and positively associated (β = 0.003) with and age ($F(1,180)=13.61$ $p < 0.001$), and sex (β = 0.064, $F(1,180)=6.11$, $p = 0.014$), with males and older participants having higher GPC than females and younger participants. BMI remained non-significant, and status was also non-significant.

Waist-to-hip ratio did positively predict (β = 0.046) GPC in the sole predictor model ($F(1,132)=22.26$, $p < 0.001$), but this effect was lost when controlling for status, age, sex, and BMI.

With regards to individual biochemical markers, TGC was positively associated (β = 0.036) with GPC ($F(1,134)=27.19$, $p < 0.001$) when controlling for age and sex. However, no metabolites significantly predicted GPC when analyzed in a full model controlling for age, sex, and BMI.

Table 7 - GPC - Variables of Interest (Basic Predictor Models)

Variable of Interest	Degrees of Freedom	F-Value	β	*p*-Value
Model 1				
Status	1,184	4.82	0.059	**0.029***
Age	1,184	16.56	0.004	**<0.0001***
Sex	1,184	6.73	0.069	**0.010***
BMI	1,181	1.79	0.003	0.182
Model 2				
Waist-2-Hip	1,130	8.85	0.046	**0.003**
Fasting HDL	1,131	0.00	-0.011	0.993
Fasting LDL	1,137	0.78	0.014	0.378
Fasting Glucose	1,148	0.71	0.011	0.401
Fasting Insulin	1,139	0.68	0.000	0.411
FastingTriglyceride	1,134	27.19	0.036	**< 0.001***

Note: ***** *indicates significant p-value. Status marks patient and control groups. Status, age, sex, BMI, and waist-2-hip are sole predictors. In Model 2, Fasting HDL, LDL, glucose, insulin, and TGC are corrected for age and sex. Bonferroni correction not performed for models, see: full models in Table 4.2. Coefficient estimate for sex is for "male", and for status is "patient".*

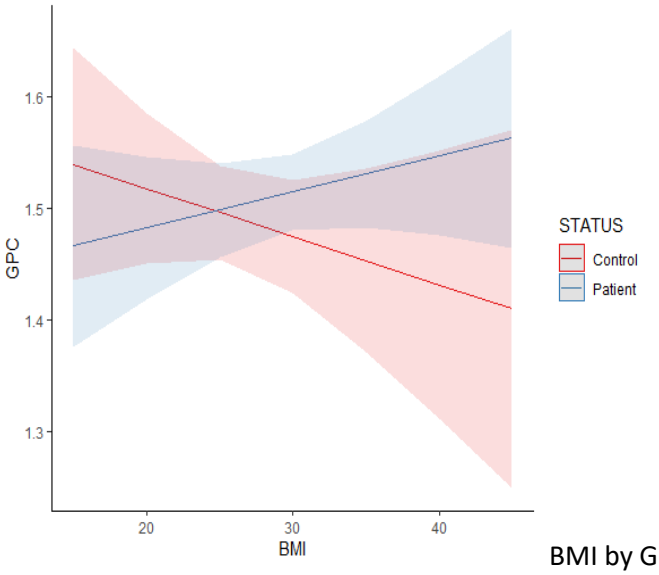
Table 8 - GPC - Variables of Interest (Full Predictor Models)

Variable of Interest	Degrees of Freedom	F-Value	β	*p*-Value
Model 3				
Status	1,178	0.55	0.021	0.461
Age	1,178	13.61	**0.003**	**<0.001***
Sex	1,178	6.11	**0.064**	**0.014***
BMI	1,178	0.09	0.001	0.763
Model 4				
Waist-to-Hip	1,127	0.00	-0.003	0.990
Model 5				
Fasting HDL	1,130	0.00	0.000	0.978
Fasting LDL	1,134	0.80	0.000	0.371
Fasting Glucose	1,147	0.02	-0.000	0.893
Fasting Insulin	1,138	0.43	0.000	0.514
Fasting Triglyceride	1,130	0.06	0.000	0.815

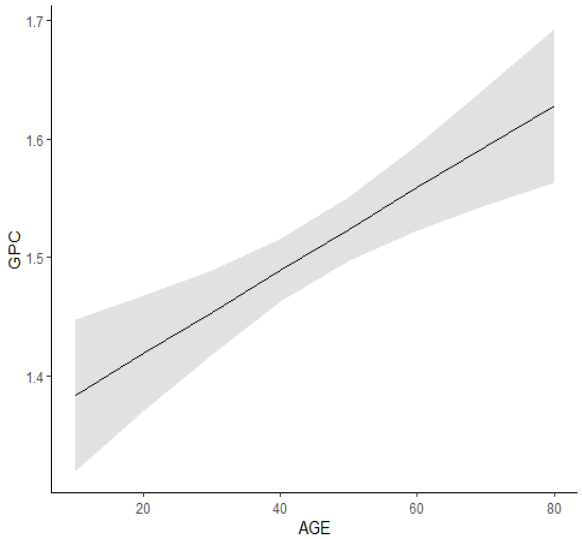
Note: ***** *indicates significant p-value. Model 3 contained status, age, BMI, and sex. Model 4 (only used to analyze waist-to-hip) contained waist-to-hip, while controlling for status, age, and sex. Model 5 contained either fasting HDL, LDL, glucose, insulin, or triglycerides, along with age, sex, and BMI. Coefficient estimate for sex is for "male", and for status is "patient".*

Figure 8 - GPC Graphs

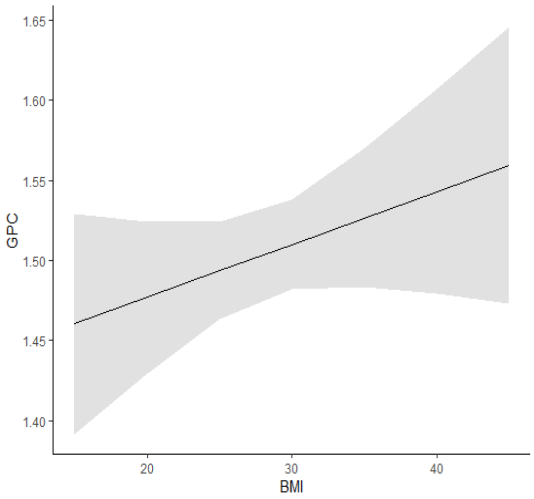
BMI by GPC, Grouped by Status



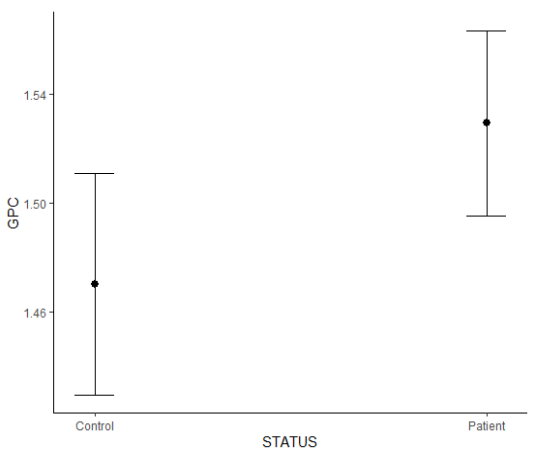
Age by GPC



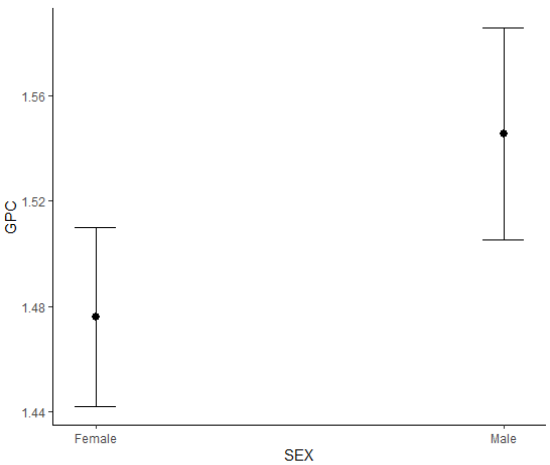
BMI by GPC



GPC BY STATUS



GPC BY SEX



3.4.3 Cr Results

When analyzed independently, in separate models (e.g. as the sole predictors), we found a positive association ($\beta = 0.006$) between Cr and age ($F(1,186)=12.46$, $p < 0.001$), but no association between Cr and status, sex, BMI, or waist-to-hip ratio.

In a multiple regression model containing status and BMI as well as age and sex as variables, Cr remained significantly and positively associated ($\beta = 0.008$) with age ($F(1,180)=17.01$, $p < 0.001$). In addition, BMI was significantly negatively associated ($\beta = -0.011$) with Cr ($F(1,180)=4.68$, $p = 0.032$),. Status and sex were not significantly associated with Cr.

Waist-to-hip ratio still failed to predict Cr in a model controlling for status, age, sex, and BMI.

With regards to individual biochemical markers, Cr was negatively associated ($\beta = -0.188$) with TGC ($F(1,135)=19.19$, $p < 0.001$) when controlling for age and sex and other biochemical variables. Only TGC predicted Cr, with a negative association ($\beta = -0.135$), when analyzed in a full model controlling for age, sex, and BMI ($F(1,132)=14.91$, $p < 0.001$).

Table 9 - Cr - Variables of Interest (Basic Predictor Models)

Variable of Interest	Degrees of Freedom	F-Value	β	*p*-Value
Model 1				
Status	1,186	0.25	-0.028	0.621
Age	1,186	12.46	0.006	**<0.001**
Sex	1,183	0.00	-0.030	0.59
BMI	1,183	2.49	-0.008	0.121
Waist-2-Hip	1,132	0.601	0.278	0.439
Model 2				
Fasting HDL	1,133	2.75	0.079	0.111
Fasting LDL	1,139	0.37	-0.019	0.565
Fasting Glucose	1,150	1.33	-0.042	0.250
Fasting Insulin	1,143	3.97	-0.001	**0.048***
Fasting Triglyceride	1,135	19.19	-0.188	**<0.001***

Note: ***** *indicates significant p-value. Status marks patient and control groups. Bonferroni correction not performed, see Table 5.2.. Coefficient estimate for sex is for "male", and for status is "patient".*

Figure 9 - Cr - Variables of Interest (Full Predictor Models)

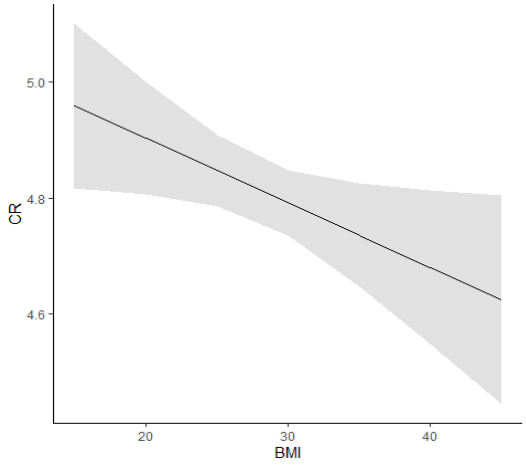
Variable of Interest	Degrees of Freedom	F-Value	β	P-Value
Model 3				
Status	1,180	1.13	-0.063	0.288
Age	1,180	17.01	0.008	**<0.001***
Sex	1,180	0.01	-0.018	0.753
BMI	1,180	4.68	-0.011	**0.032***
Model 4				
Waist-to-Hip	1,129	1.56	-0603	0.213
Model 5				
Fasting HDL	1,132	0.55	-0.011	0.461
Fasting LDL	1,136	0.10	0.027	0.748
Fasting Glucose	1,149	0.44	-0.030	0.506
Fasting Insulin	1,140	2.63	-0.000	0.107

Fasting Triglyceride	1,132	14.91	-0.135	**<0.001***

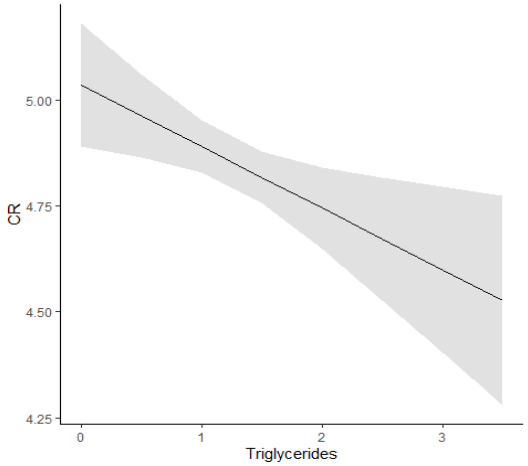
Note: ** indicates significant p-value. Model 3 contained status, age, BMI, and sex. Model 4 (only used to analyze waist-to-hip) contained waist-to-hip, while controlling for status, age, and sex. Model 5 contained either fasting HDL, LDL, glucose, insulin, or triglycerides, along with age, sex, and BMI. Coefficient estimate for sex is for "male", and for status is "patient".*

Figure 10 - Cr Graphs

TGC by Cr



BMI by Cr



3.5 DISCUSSION

While high BMI did significantly predict lower levels of NAA, it did not predict lower levels of GPC, and only predicted lower levels of creatine when correcting for age, sex, and status. In addition, despite our hypotheses and the possibility that waist-to-hip ratio may be a more robust predictor than BMI, when analyzed in the full models and not only as a sole predictor, waist-to-hip did not predict NAA, GPC, or Cr levels.

We found that BMI was associated with NAA and Cr in the frontal lobe. This was in keeping with previous studies that found volumetric differences in frontal regions in obesity (Kolenič et al., 2020; Marqués-Iturria et al., 2013; Willette & Kapogiannis, 2015; Willeumier et al., 2011). While there is greater heterogeneity found in previous structural imaging research in BD in comparison to obesity, the combination of increased psychiatric and somatic complications found in BD that is comorbid with obesity, along with the evidence implicating similar areas of brain change between BD and obesity, confirms that further research into this connection is necessary.

We did not find an association between GPC and BMI, which is in contrast with Bond (2017), however, this may be partially explained by the fact that Bond et al. found opposing evidence with regards to the association of BMI and GPC in individuals with BD and obesity. They found a positive relationship between BMI and GPC in that higher BMI predicted higher levels of GPC, but they also found a negative relationship between BD and GPC with GPC predicting lower levels of GPC (Bond et al., 2017). This may partially explain our non-significant findings; however, we did control for status in our model. The finding may be genuinely non-significant, and replication of this analysis in future studies may help clarify the discrepancy.

In light of the associations between Cr and sex and NAA and age, is the importance of controlling for these variables is a further consideration. Failing to control for age and sex in previous research may also explain some of the heterogeneity of previous H-MRS findings in obesity and BD. There may be an interaction between sex or age and BMI as well, which itself may be different in people with SPMI (as suggested by Bond et al., 2017 results) .

Interestingly enough, the blood metabolic markers analyzed did not predict any of our MRS metabolites, with the exception of fasting triglycerides. TGC was associated, as expected, negatively with NAA, positively with GPC in the sole predictor model only, and negatively with creatine. TGC may well be a more sensitive measure of the potential impact of MetS on the brain than other metabolic markers based on our findings and should be further investigated for specificity and sensitivity. Based on our results it is not, however, a stronger predictor than BMI. Measuring TGC levels and BMI among individuals with SPMI could also potentially identify patients at higher risk of brain-related changes due to obesity and MetS. Previous research has also found that dyslipidemia or obesity in BD predicted decreased NAA (Bond et al., 2017; Hajek et al., 2015). In addition, non-MRS research shows associations between dyslipidemia and obesity (Correll et al., 2008), dyslipidemia and MetS (De Hert et al., 2009), and dyslipidemia and T2DM (Bora et al., 2019; Hajek et al., 2015) in BD, so TGC may be a simple way to measure risk.

4.1 CHAPTER 4

4.1 CONCLUSIONS

Our MRS analysis of a sample of patients with BD and obesity also serves to replicate previous findings of alterations in brain-imaging in these populations in comparison to healthy controls. This additional replication of brain changes makes previous results more robust, and also potentially makes BMI another easily measurable way to predict brain changes in both BD and obesity. The suggestion that BMI may predict brain changes in obesity was also further replicated by our meta-analysis, which revealed a significant association between obesity and decreased levels of NAA in the frontal lobe.

In addition, the implication of significance in the frontal lobe could potentially suggest that changes to the brain preceded obesity, and the resulting impact on cognitive functioning (e.g. planning, impulse control) contributed to or caused weight gain. However, we cannot determine causality from our study.

Changes to the frontal lobe preceding obesity could also be a potential mechanism for obesity, which then impacts the frontal lobe in return. Another potential mechanism could be T2DM-related and obesity-related damage to the brain caused by insulin resistance (Hajek et al., 2014). Insulin resistance appears to contribute to systemic inflammation and increased levels of cytokines (Wisse, 2004), and systemic inflammation, in turn, is associated with increased mortality from cardiovascular causes (Jefferson et al., 2007). In individuals with SPMI medications that impact weight and insulin resistance those factors likely also contribute to obesity (Mitchell et al., 2013), in addition to lifestyle factors such as lack of exercise, smoking, and unhealthy diets (Dickerson et al., 2013; Nazareth, et al., 2007; Osborn et al., 2007b;

Vancampfort et al., 2015). Because of the importance of the frontal lobe in cognitive functioning, these potential mechanisms could also be bidirectional as well, causing a positive feedback loop. Overall, our findings suggest that BMI and TGC may be an effective way of measuring obesity-related brain changes in both populations with SPMI and in the general population. Both BMI and TGC are relatively easy to measure on an outpatient basis and might be used to alert clinicians to potentially brain-altering effects of obesity and MetS on their patients. Increasing BMI and TGC levels may also be an objective way for clinicians to communicate the potential health risks of obesity and individual blood metabolic markers to patients before more severe outcomes occur, given the evidence of worsened psychiatric, cognitive, and somatic outcomes, in addition to increased mortality rates associated. This early warning could enable the implementation of strategies to manage those risks early. Measuring BMI and blood metabolic markers is both easy to do in outpatient treatment and cost-effective.

Early alerts of worsening physical health are an especially important consideration given the many potential health impacts of obesity, health impacts that appear carry a higher burden in individuals with BP and other forms of SPMI with regards to both physical and mental health. In addition to facing greater physical and mental health challenges with obesity, individuals with SPMI also may difficulty in accessing healthcare for obesity and related findings of T2DM and MeTs. These factors all suggest that early intervention is key, and effective predictors of brain damage may be one way of providing that early intervention. Prevention may help mitigate some potential risk factors associated with BD and obesity, such as poor health literacy around diet and exercise, barriers to accessing tools to assist with healthy eating and exercise, less monitoring by clinicians for somatic issues, and medication effects, by addressing increasing BMI or TGC early on.

This is especially relevant because some research suggests the brain changes suggested by our evidence may be reversible. For example, regular aerobic exercise has been found to increase levels of NAA (Gonzales et al., 2013), and NAA can also be affected by dietary changes (Auer et al., 2015; Setkowicz et al., 2015). However, some of the most important research examines direct interventions on obesity. For example, intra-gastric balloon surgery has been found to normalize levels of *myo*-inositol in obese individuals (Gazdzinski et al., 2018). Levels of NAA can also be lowered with surgical weight loss (Daniele et al., 2020). And most relevant of all to our second study is the finding that lithium is neuroprotective in BD, with individuals on it showing higher levels of NAA than those not on it (Hajek et al., 2012; Moore et al., 2000). These initial findings suggest that interventions could be clinically useful and a valuable use of resources in treating individuals with BD and obesity.

Despite our robust results, our MRS analysis had some limitations. For example, our data was collected on a 1.5T scanner, and a stronger signal may have been possible to collect with a 3T MRI machine. However, when data collection began for this project 1.5T was the most typical magnet strength. In light of our prospective data analysis presented in this paper, we do not want to switch to a stronger magnet in the middle of ongoing data collection. In addition, 1.5T was sufficient to measure the metabolites analysed, and our results showed several relevant findings with good face validity. However, since 3T has better accuracy and few drawbacks for conducting H-MRS research, use of a 3T machine in future studies may be helpful.

Our data also included a broad age range, which may be especially relevant given the significant associations we found between all three MRS metabolites and age. However, we did

account for the effects of age in most of our analyses, with the exception of the sex, status, BMI, and waist-to-hip sole predictor models.

Lastly, unfortunately, we were unable to measure glutamate reliably in the imaging available. While this would have been interesting, considering previous findings linking alternations in glutamate to BD (Bond et al., 2016), and can hopefully be addressed by future studies, it would have made our study less feasible. 3T imaging should be considered, when possible, for future research.

In the future, longitudinal research into obesity, BD, and related biochemical blood markers and MRS metabolites should be conducted. Unfortunately, because our data was cross-sectional and not longitudinal, we were unable to provide evidence about causality or directionality in any of our results. This may be an important area of investigation given the potential to predict future brain changes in obesity and BD. If obesity and increased TGC precede worse outcomes in brain changes as obesity often precedes development of MetS and T2DM, that knowledge could provide valuable tools to mitigate future risks via preventative measures. If this is possible, it would present the opportunity to potentially prevent further brain changes before they occur, which is rare in psychiatry. However, reverse causality is also a possibility, and MRS metabolite levels may be found to precede obesity and related conditions in some or all circumstances. Finally, as obesity is associated with many brain imaging outcomes in spectroscopy, it also should be controlled for in future H-MRS research to provide a clear understanding of the mechanisms at play in this relationship. Previous investigations may also be interpreted in light of this association.

The results of both the meta-analysis and the MRS analysis both suggest that working jointly on physiological symptoms in SPMI in addition to psychiatric ones is of great

importance. Individuals with SPMI face increased physical health problems and higher mortality rates than the general population, however, they also face significant problems accessing care for physical health problems, including T2DM. Increasing access to resources such as registered dieticians and occupational therapists or recreation therapists may also help increase the ability of individuals with SPMI to prevent increasing BMI or developing MetS or T2DM.

Psychiatrists and other clinicians working with individuals with SPMI may also be able to pass on benefits of this research and related findings, especially once further replication has been undergone, by sharing the conclusions with their patients. Health literacy was an area Osborn noted as a potential risk for outcomes such as obesity in SPMI populations (Osborn et al., 2007b). Regular exercise may also prevent decreases in NAA (Gonzales et al., 2013). Knowing the potential risks of increasing BMI on physiological brain health and long-term psychiatric outcomes may provide motivation to patients to lose weight or prevent further weight gain, if presented in a non-judgemental manner. Increasing evidence of those risks may also help physicians and other health professionals gain funding to address obesity, MetS, and T2DM in individuals with SPMI. Likelihood of brain changes could potentially be addressed through simple bloodwork and calculation of BMI, which is much less resource-intensive than MRI scans.

In conclusion, our meta-analysis found that obesity was related to decreased NAA in the general population in frontal regions, and our MRS analysis confirmed that obesity was related to altered levels of NAA, GPC, and Cr, again in frontal regions, in a population of patients with BD. Both analyses were highly novel and in an area of psychiatric research that has the potential to provide great benefits to our scientific understanding of obesity, especially in SPMI, and effective interventions for negative outcomes in this population.

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