Mitochondrial Function and Inflammation in Individuals at Clinical High Risk for Psychosis and Patients with Early Stage Schizophrenia

by

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

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2019

Abstract

Schizophrenia (SCZ) is a chronic brain disease. Early detection and treatment are associated with improved disease outcome; and while there are interview-based methods of identifying clinical high risk for psychosis (CHR) individuals, there are yet to be reliable biomarkers to identify at-risk population.

Several lines of evidence implicate mitochondrial function and inflammation in the etiology and pathology of SCZ. Here, we examine changes in peripheral mitochondrial function, measured by mitochondrial electron transport chain expression and levels of lactate and pyruvate, and changes in levels of inflammatory cytokines in CHR and early stage SCZ individuals; and compare these to clinical symptoms. We did not detect differences in peripheral mitochondrial function and levels of inflammatory cytokines between our non-psychiatric control, CHR, and early stage SCZ groups; however mitochondrial function may be associated with prodromal symptom severity. Additional studies are warranted to further elucidate mitochondrial changes during the start of disease development.
Acknowledgements

I would like to thank my supervisor Dr. Ana Andrezza, and my advisor Dr. Romina Mizrahi for their guidance and the opportunity they provided me to work on this wonderful project. Thank you, Ana for your constant support and enthusiasm for mitochondria throughout my masters. Thank you, Romina for making this project and collaboration possible. I would also like to thank Tania Da Silva, who was so patient and understanding with me throughout our collaboration. Thank you, Tania for all your help and guidance; especially when I was first starting out on the project.

Additionally, I would like to thank everyone in the Andrezza lab for sitting through my presentations and helping me through the last two years. Without you I would not have been able to do it. I'm especially grateful to Isabelle and Wendy for helping me as I adjusted to the lab, to Angela for taking me under her wing and teaching me all things cell culture, and to David for repeatedly helping me with statistics and R. Thank you, Jine, Young, Erika, Kassandra, Thiago, Nayara, Alencar, Francine, and all our wonderful past and present undergrads for helping me when I needed it, and for brightening up the lab. You have all helped me tremendously. Thank you to everyone in the Pharmacology and Toxicology Department for making it enjoyable to come in every day. I'd like to especially thank Dr. Rebecca Laposa, who was the one who greatly inspired me to pursue research.

I would also like to thank Betty, David, and Emma for helping me through the toughest parts of the past two years, and for always having my back. You guys are the best!

Lastly, I would like to thank my family. Thank you, mom, dad, Lianne, and Wesley, for making sure I slept and ate enough to function; and to my children Linus, Jack, Snow, Tangerine, and Lemon who have made this masters worth it.
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List of Abbreviations

ATP       Adenosine triphosphate
B2M       Beta-2-microglobulin
BMI       Body mass index
BP        Bipolar disorder
CAMH      Centre for Addiction and Mental Health
CHR       Clinical high risk for psychosis
COPS      Criteria of Prodromal Syndromes
CTL       Non-psychiatric controls
DLPFC     Dorsolateral prefrontal cortex
DSM       Diagnostic and Statistical Manual of Mental Disorders
FEP       First Episode of psychosis
Fxr1      Fragile X mental retardation syndrome-related protein 1
FYPP      Focus on Youth Psychosis Prevention
GLUT1     Glucose transporter 1
GLUT3     Glucose transporter 3
HXK1      Hexokinase 1
ICD       International Statistical Classification of Diseases and Related Health Problems
KO        Knockout
MRS       Magnetic resonance spectroscopy
mtDNA     Mitochondrial DNA
N2a cells Neuro2a cells
NAPLS     The North American Prodrome Longitudinal Study
nDNA      Nuclear DNA
NMDA      N-methyl-D-aspartate
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<tr>
<td>NNT</td>
<td>Nicotinamide nucleotide transhydrogenase</td>
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<td>OXPHOS</td>
<td>Oxidative phosphorylation</td>
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<td>PACE</td>
<td>Personal Assessment and Crisis Evaluation Clinic</td>
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<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<td>PFK1</td>
<td>Phosphofructokinase 1</td>
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<tr>
<td>PGC-1α</td>
<td>Proliferator-activated receptor-gamma coactivator 1-alpha</td>
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<td>PMI</td>
<td>Post-mortem interval</td>
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<td>PRIME</td>
<td>Prevention through Risk Identification, Management, and Education Clinic</td>
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<td>RBANS</td>
<td>Repeatable Battery for the Assessment of Neuropsychological Status</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SCID-IV</td>
<td>Structured Clinical Interview for DSM-IV Axis I disorders</td>
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<td>SCZ</td>
<td>Schizophrenia</td>
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<tr>
<td>SIPS</td>
<td>Structured Interview for Prodromal Syndromes</td>
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<tr>
<td>SNPs</td>
<td>Single-nucleotide polymorphisms</td>
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<tr>
<td>SOPS</td>
<td>Scale of psychosis-risk symptoms</td>
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<tr>
<td>TSPO</td>
<td>Translocator protein</td>
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<tr>
<td>UHR</td>
<td>Ultra high risk state</td>
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<td>WT</td>
<td>Wildtype</td>
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1. INTRODUCTION

1.1. Clinical High Risk for Psychosis and First Episode of Psychosis

Schizophrenia (SCZ) is a chronic disease of the brain that is estimated to have a prevalence of 0.5% to 1.6% depending on the population that is studied, and usually has an onset in an individual’s late teens to early twenties (van Os and Kapur 2009, Rössler et al. 2005, Jablensky 1995). SCZ is a psychotic disorder, and individuals with SCZ experience periods of psychosis which consist of positive symptoms such as hallucinations and delusions; and negative symptoms such as avolition and social withdrawal, that affects an individual’s beliefs and sense of reality. (Picchioni and Murray 2007). In addition to psychosis, cognitive dysfunction is a classic symptom of SCZ. Schizophrenic individuals are deficient in areas including attention, memory, verbal learning, language, and executive functioning (Bowie and Harvey 2006, Heinrichs and Zakzanis 1998, Tripathi, Kar and Shukla 2018). These symptoms can negatively impact an individual's quality of life by affecting an individual’s relationships, ability to live independently, education, and careers. While there is no cure for SCZ, it can be successfully managed with the use of medication and psychological treatment (van Os and Kapur 2009).

Before an individual experiences a full-blown psychotic episode, also known as their first episode of psychosis (FEP), some individuals experience sub-clinical psychosis symptoms, often referred to as the prodrome of SCZ (Keith and Matthews 1991, Cornblatt et al. 2003, Rössler et al. 2011). However, not all individuals displaying symptoms of a prodromal state of SCZ go on to experience psychosis (Yung et al. 2003). Additionally, even if an individual were to go on to develop psychosis, it is not guaranteed that it will manifest as SCZ, as SCZ is just one of many psychosis disorders (van Os and Kapur 2009). Furthermore, many prodromal symptoms are non-
specific to psychosis, making it difficult to determine and accurately predict whether an individual will go on to develop psychosis and if they are truly in the prodromal phase of SCZ (Phillips, Yung and McGorry 2000). This difficulty has been reported in the literature, with a meta-analysis conducted in 2012 that found there to be a 30% transition rate from at risk for psychosis to psychosis within two years of follow-up with individuals at risk (Fusar-Poli et al.). Similarly, a systematic review performed by van Os et al. found that there was a 5% prevalence rate in the general population of individuals that experience prodromal symptoms, compared to the 0.5% to 1.6% prevalence of SCZ (2009).

Individuals that display these prodromal symptoms have been referred to by many terms including: being at clinical high risk for psychosis (CHR), being in an ultra high risk state (UHR), having psychosis risk syndrome, and being in an at-risk mental state (Lepock et al. 2018). In this study, patients were recruited by Dr. Romina Mizrahi’s group following the definition of CHR as defined by the Prevention through Risk Identification, Management, and Education (PRIME) prodromal research team at Yale University (Miller et al. 2003).

1.1.1. Diagnosis, Signs, and Symptoms

Both SCZ and CHR are clinically assessed by mental health professionals by observing an individual's behaviour and by considering an individual's self-reported experiences. SCZ is most commonly diagnosed using the criteria set by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM) or the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD) (van Os and Kapur 2009). Symptoms of SCZ includes positive symptoms such as hallucinations, delusional ideas, and thought disorders that manifest as illogical speech; negative symptoms such as social withdrawal, self-neglect, and avolition (Picchioni and Murray 2007); and cognitive
deficits in areas such as attention, memory, verbal learning, language, and executive functioning (Bowie and Harvey 2006, Heinrichs and Zakzanis 1998, Tripathi et al. 2018). Some individuals experience episodes of psychosis followed by periods of remission, while others experience continuous, chronic psychosis symptoms (Harrow et al. 2005). The cognition of these individuals has been previously assessed using various tests, including the DSM-5 which incorporates cognitive testing in the diagnosis of SCZ (Mattila et al. 2014). In our study, the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used to assess participant neurocognitive performance.

As previously mentioned, it is hard to predict whether an individual that displays prodromal symptoms will transition to psychosis (Phillips et al. 2000, Yung et al. 2003). Because of this, there have been many teams that have created sets of criteria to identify predictive symptoms. This includes criteria put forth by the Personal Assessment and Crisis Evaluation (PACE) Clinic in Melbourne, Australia (Phillips et al. 2000). This group focused on three different criteria to form three groups. This included a group that presented with “low-grade psychotic symptoms” several times a week for at least one week within the last year, but not longer than five years; a second group that presented with transient psychotic symptoms; and a group with risk factors such as having a first-degree relative with either a psychotic disorder or schizotypal personality disorder, or significant decrease in mental state or functioning for at least a month (Phillips et al. 2000). The Prevention through Risk Identification, Management, and Education (PRIME) prodromal research team at Yale University also created a set of criteria called the Criteria of Prodromal Syndromes (COPS), which is part of the Structured Interview for Prodromal Syndromes (SIPS) (Miller et al. 2003). Their criteria for identifying CHR individuals focuses on family history and psychosis symptoms; including hallucinations, delusional ideas, persecutory
ideas or suspiciousness, grandiose ideas, and disorganized communication. COPS also assesses the severity of the prodromal symptoms with the scale of psychosis-risk symptoms (SOPS); including positive symptoms, negative symptoms, disorganization symptoms, and general symptoms (Miller et al. 2003). Negative symptoms include social anhedonia, avolition, decreased expression and experience of emotions, decreased experience of emotions and self, decreased ideational richness, and decreased occupational functioning; disorganization symptoms include odd behaviour/appearance, bizarre thinking, trouble focusing, and impaired personal hygiene; and general symptoms include sleep disturbances, dysphoric moods, motor disturbances, and impaired tolerance to normal stress. In our study, COPS was used to identify CHR individuals; as adopted by our collaborator (Miller et al. 2003, Barron et al. 2017, Lepock et al. 2018).

It is worth noting that all the tests mentioned above for the identification of SCZ and CHR are based on observed behaviours and individual experiences. Diseases of the brain such as SCZ and psychosis, like any other disease, consists of underlying biological changes. Despite efforts made to date to advance the understanding of the biological changes underlying the pathophysiology of SCZ, the scientific community has yet to identify the biological cause of SCZ and reliable biomarkers to diagnose SCZ and to identify the at-risk population.

1.1.2. Medical Burden of SCZ

Individuals with SCZ present with symptoms that interfere with day-to-day life, and these individuals have a decreased life expectancy compared to the general population (Laursen, Nordentoft and Mortensen 2014).

A review conducted by Laursen et al. found that mortality rates were two to three times higher across all age groups in individuals with SCZ compared to the general population, causing the
life expectancy of an individual with SCZ to decrease by 10 to 25 years compared to the general population (2014). This decrease in life expectancy is the result of many factors. The risk of suicide has been shown to be increased in individuals with SCZ. A meta-analysis in 2005 estimated that 5.6% of individuals with SCZ would commit suicide in their lifetime (Palmer, Pankratz and Bostwick 2005). A retrospective cohort focusing on FEP individuals conducted in the United Kingdom found that in the mean 11.5 years that patients were followed, the rate of suicide was 1.9%, with the greatest number of suicides occurring within the first year after the initial episode of psychosis, and a median time to suicide of 5.6 years. This was found to be four times the suicide risk of the general population (Dutta et al. 2010). It has also been reported in numerous papers that many individuals with SCZ suffer from comorbid psychiatric illnesses such as depression, substance abuse, anxiety and panic disorders; and physical illnesses such as cardiovascular disease, diabetes, obesity, and lung disorders related to smoking (Buckley et al. 2008, Smith et al. 2013). These comorbidities have been shown to play a role in the mortality rate of individuals with SCZ; with cardiovascular diseases often being cited as a leading cause of natural deaths in these individuals (Smith et al. 2013, Crump et al. 2013, Newcomer and Hennekens 2007, Ringen et al. 2014). The current literature also suggests that comorbidities in SCZ are under diagnosed in the population with SCZ, delaying or preventing treatment, and thereby contributing to increased mortality rates (Smith et al. 2013, Crump et al. 2013, Laursen, Munk-Olsen and Gasse 2011). Furthermore, prolonged duration of illness and prolonged duration between the initial psychotic episode and start of treatment is associated in decreased response to treatment and poorer disease outcome (Perkins et al. 2004, Brousse et al. 2010, Bottlender et al. 2003). There is also evidence suggesting that early intervention and adherence to treatment while in the prodromal stage results
in a better disease outcome; with a greater risk in conversion to psychosis the longer the duration and the more severe an individual’s symptoms are (Cornblatt et al. 2003, Fusar-Poli et al. 2012). As such, early detection and treatment are imperative to alleviating the medical burden of SCZ. To improve treatment, diagnosis and discovery of novel drugs it’s necessary to understand the underlying biological causes associated with the pathophysiology of SCZ. Next, we discuss the molecular alterations in SCZ and CHR, with the focus on bioenergetics and inflammatory changes.

1.2. Molecular Alterations in SCZ and CHR

1.2.1. Classical Biological Hypotheses of SCZ

To date, the etiology of SCZ is still unknown, but there have been many theories proposed and extensively studied in the scientific community. Neurotransmitter abnormalities have the basis of many of these theories; with the dopamine theory of SCZ being one of the most studied hypotheses (Benson and Feinberg 2011, Guillin, Abi-Dargham and Laruelle 2007). One of the first observations to support this theory was the correlation seen in dopamine receptor binding and the potency of antipsychotic drugs (Creese, Burt and Snyder 1976, Seeman and Lee 1975). Additionally, the use of psychostimulants like amphetamine induce psychosis at high doses and can exacerbate symptoms in SCZ patients (Lieberman, Kane and Alvir 1987, Angrist and Van Kammen 1984, Benson and Feinberg 2011). Many studies have supported this hypothesis, with a positron emission tomography (PET) study reporting increased D2 dopamine receptor density in the caudate nucleus of both antipsychotic-naïve SCZ patients and antipsychotic-treated SCZ patients compared to healthy volunteers (Wong et al. 1986). Additionally, a study in 2012 using PET to measure stress-induced dopamine release reported sensitization to dopamine in CHR and
SCZ patients compared to matched controls when participants where completing a psychosocial stress task, suggesting dysregulation of the dopaminergic system in patients with SCZ (Mizrahi et al. 2012). Another focus in the literature of SCZ has been on the glutaminergic system; with dysfunction of the N-methyl-D-aspartate (NMDA) receptor as the most prevalent hypothesis (Howes, McCutcheon and Stone 2015, Javitt 2010). There have been many lines of evidence to support this hypothesis. It was initially observed that the use of non-competitive antagonists of the NMDA receptor such as ketamine resulted in symptoms resembling the positive and negative symptoms of SCZ (Krystal et al. 1994). Additionally, post-mortem studies have reported reduced expression of the NMDA receptor subunits in the superior frontal cortex and superior temporal cortex of SCZ patients (Sokolov 1998, Humphries et al. 1996) and decreased expression of proteins associated with the NMDA receptor in the frontal cortex of patients with SCZ (Funk et al. 2009). There have also been reports of genes coding for a NDMA receptor subunit and genes coding for proteins involved in NMDA receptor activation that are associated with SCZ (Schizophrenia Working Group of the Psychiatric Genomics Consortium et al. 2014, Howes et al. 2015). Furthermore, many proton magnetic resonance spectroscopy studies have reported both increased and decreased glutamine and glutamate levels in different brain regions of patients with SCZ as well as in CHR individuals when compared to non-psychiatric controls (CTL) (Bartha et al. 1997, Stone et al. 2009, Tibbo et al. 2004, Tandon et al. 2013, Shakory et al. 2018). Other neurotransmitter systems implicated in the etiology of SCZ include serotonin and norepinephrine, both of which have been reported to be targets of second-generation antipsychotics (Benson and Feinberg 2011, Macdonald and Bartolomé 2010).
Proper neuronal signaling requires a balanced production of adenosine triphosphate (ATP), therefore it is hypothesized that mitochondrial dysfunction could be involved in SCZ. Next, we discuss the evidence supporting this hypothesis.

1.2.2. Evidence of Mitochondrial Dysfunction in SCZ and CHR

Altered brain energy metabolism and mitochondrial dysfunction have been implicated in the etiology and pathology of SCZ for a while (Prabakaran et al. 2004). Mitochondria are involved in cell redox signaling, oxidative phosphorylation, calcium homeostasis, apoptotic cell death, and neuronal development and synaptic plasticity (Collins et al. 2012, D’Autreaux and Toledano 2007, Duchen 2004, Clay, Sillivan and Konradi 2011). The mitochondria house the electron transport chain; a group of protein complexes responsible for oxidative phosphorylation. Briefly, the electron transport chain consists of five complexes (I-V) which lie within the inner mitochondrial membrane, and creates ATP with the use of NADH and FADH$_2$ (Zhao et al. 2019). Complex I and II transfer electrons from NADH and FADH$_2$ to Coenzyme Q$_{10}$ respectively; and the transfer of electrons across complex I generates energy to allows for the transportation of protons through the complex from the mitochondrial matrix into the intermembrane space (Zhao et al. 2019). Complex III then transfers the electrons from Coenzyme Q$_{10}$ to cytochrome C, and this transfer of electrons generates energy to transport more protons into the intermembrane space (Zhao et al. 2019). Complex IV then transfers the electrons from cytochrome C to O$_2$, generating H$_2$O and transporting protons into the intermembrane space (Zhao et al. 2019). Lastly, complex V is an ATP synthase which uses the electrochemical gradient created by the pumping of protons into the intermembrane space to produce ATP. The protons travel down the electrochemical gradient through the complex, producing energy to phosphorylate ADP to ATP (Zhao et al. 2019). Compromised mitochondrial function or electron
transport chain function can result in increased ROS production, impaired calcium buffering, apoptosis, and impaired oxidative phosphorylation (Duchen 2004, Clay et al. 2011). Additionally, mitochondrial dysfunction may alter critical neuronal processes underlying abnormal brain development and cognitive impairment in psychosis (Da Silva et al. 2018).

Changes in mitochondrial function in SCZ are supported by evidence from genetic, post-mortem, peripheral, and imaging studies (Rajasekaran et al. 2015, Clay et al. 2011). Genetic studies have identified single-nucleotide polymorphisms (SNPs) in mitochondrial DNA (mtDNA) and mitochondrial-related genes as risk factors for SCZ (Amar et al. 2007, Marchbanks et al. 2003, Rollins et al. 2009, Verge et al. 2011, Cuperfain et al. 2018, Goncalves et al. 2018). Furthermore, several post-mortem studies have reported reductions in the expression of mitochondrial-related genes, particularly genes encoding mitochondrial OXPHOS complexes (Prabakaran et al. 2004, Ben-Shachar and Karry 2008, Karry, Klein and Ben Shachar 2004). A post-mortem cortical tissue study also reported decreased mRNA expression of transcripts dependent on proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), a coactivator involved in mitochondrial biogenesis (McMeekin et al. 2016, Dorn, Vega and Kelly 2015). Related to mitochondrial bioenergetics, decreases in mRNA expression and activity of phosphofructokinase 1 (PFK1) in the dorsolateral prefrontal cortex (DLPFC) and decreased mRNA expression of hexokinase 1 (HXK1), PFK1, glucose transporter 1 (GLUT1), and GLUT3 in patients with SCZ was reported, illustrating abnormal bioenergetic function in SCZ patients compared to CTL individuals (Sullivan et al. 2018). Additionally, reduced electron transport chain activity in multiple brain regions has been reported (Maurer, Zierz and Moller 2001); including decreased complex IV activity in the frontal cortex and temporal cortex of patients with SCZ, and decreased complex I and III activity in the temporal cortex and basal ganglia of patients with SCZ (Maurer et al.
2001). However, others have failed to replicate these findings (Andreazza et al. 2010, Konradi et al. 2004).

Alterations in mitochondrial complex activity have also been consistently reported in blood cells of SCZ patients. Reduced mitochondrial complex I activity has been reported in lymphocytes and platelets of SCZ patients chronically treated with antipsychotics compared to healthy controls (Burkhardt et al. 1993, Whatley et al. 1998), with no differences in complex II and III activity (Gubert et al. 2013). Conversely, increased complex I activity was observed in platelets of medicated and unmedicated SCZ patients in acute exacerbation (Ben-Shachar et al. 1999, Dror et al. 2002). Research in mitochondrial alterations in the prodromal phase of SCZ are sparse, and to my knowledge, this is the first study to examine changes in mitochondria in CHR individuals.

1.2.3. Evidence of Mitochondrial Metabolite Changes in SCZ and CHR

Altered mitochondrial function results in changes in mitochondrial metabolites. Glucose is the main substrate used in energy metabolism, and oxygen is required to undergo oxidative phosphorylation to produce ATP (Hertz and Dienel 2002). Altered glycolysis enzyme expression has been reported in the post-mortem prefrontal cortex of individuals with SCZ, suggesting increased demand for glucose or increased hypoxia in the brains of SCZ individuals (Prabakaran et al. 2004). Anaerobic metabolism results in increased levels of lactate, which has been previously reported in the periphery of SCZ individuals (Halim et al. 2008, Rowland et al. 2016, Regenold et al. 2009). Magnetic resonance spectroscopy (MRS) studies have also reported increased lactate levels in brains of SCZ patients compared to CTL (Rowland et al. 2016, Rowland et al. 2018). Studies have also reported that increased levels of brain lactate correlated to poorer cognitive function and poorer functional capacity, indicating a relationship between
levels of lactate and disease symptom severity (Rowland et al. 2016, Rowland et al. 2018).

Levels of pyruvate have also been reported to be altered in SCZ (Yang et al. 2013, Dean et al. 2016). Serum samples from SCZ individuals showed increased levels of pyruvate, indicating changes of pyruvate in the periphery (Yang et al. 2013). Additionally, studies on post-mortem striatal tissue of SCZ patients revealed increased levels of pyruvate and lactate compared to CTL (Dean et al. 2016). This supports the evidence of mitochondrial dysfunction and an increase of anaerobic metabolism in SCZ. However, contrary to this evidence, Huang et al. have reported no changes in pyruvate levels in serum of SCZ patients compared to CTL (2016, 2018).

Additionally, decreased mitochondrial function produces excess ROS resulting in the depletion of antioxidants resulting in oxidative stress (Li et al. 2003, Lin and Beal 2006). During normal mitochondrial metabolism processes, a small portion of electrons escape the electron transport chain, which can result in the formation of ROS (Green and Kroemer 2004, Malkus, Tsika and Ischiropoulos 2009, Clay et al. 2011). Under regular conditions, ROS are reduced by antioxidant defence mechanisms including superoxide dismutase, glutathione peroxidase, and glutathione to maintain redox balance (Kankofer 2001). However, this balance is interrupted with impaired mitochondrial complex function resulting in increased ROS formation (Kankofer 2001). Levels of glutathione have been reportedly decreased in post-mortem prefrontal cortices of individuals with psychiatric disorders including bipolar disorder, major depressive disorder, and SCZ (Gawryluk et al. 2011). Additionally glutathione peroxidase has been shown to be reduced in individuals with SCZ and major depressive disorder (Gawryluk et al. 2011). However, it has been reported that there is no difference in glutathione levels in CHR compared to controls (Da Silva et al. 2017). In this study, we will focus on changes in levels of peripheral lactate and
pyruvate in conjunction with changes in peripheral mitochondrial electron transport chain expression as indirect indicators of mitochondrial function.

Mitochondrial dysfunction can cause proinflammatory signalling, and proinflammatory mediators alter mitochondrial function; which can lead to a cycle of inflammation increasing oxidative stress to mitochondria (Lopez-Armada et al. 2013). This cycle of inflammation and oxidative stress to mitochondria can result in the mitochondrial dysfunction present in SCZ disease pathology. In this study, we will focus on alterations in peripheral cytokines as an indicator of changes in inflammation in CHR and early stage SCZ. Next we discuss the evidence of alteration in inflammatory markers associated with SCZ and CHR.

1.2.4. Evidence of Inflammatory Changes in SCZ and CHR

Various studies have reported changes in inflammation and immune disturbances in SCZ (Hope et al. 2013, van Kesteren et al. 2017, Muller and Schwarz 2010). A significant increase in the expression of proinflammatory genes at the levels of RNA and protein has been reported in post-mortem studies (van Kesteren et al. 2017) and multiple studies have reported alterations of peripheral inflammatory cytokines as being linked to SCZ and psychosis (Rodrigues-Amorim et al. 2018, Hope et al. 2013, Wu et al. 2016). These studies found altered levels of pro-inflammatory cytokines and cytokine mRNAs in the blood as well as cytokine gene polymorphisms in SCZ individuals (Katila, Hänninen and Hurme 1999). Many specific cytokines have been implicated in SCZ pathology. A systematic review focusing on alterations in peripheral cytokines in SCZ and psychosis identified alterations in IL-6, TNFα, IL-10, IFNγ, IL-1β, IL-8, IL-2, and IL-1Ra to be the most associated with SCZ or psychosis in the 99 studies included in the analysis (Rodrigues-Amorim et al. 2018). These changes appear to be present very early on in the development of psychosis, with studies reporting altered levels of peripheral
cytokines in FEP individuals (Miller et al. 2011, Petrikis et al. 2015). Neuroinflammation has also previously been reported in SCZ (Marques et al. 2018). A meta-analysis studying in vivo microglial activation as a marker of neuroinflammation reported moderate elevations of TSPO tracer binding, indicative of increased microglial activation and neuroinflammation in SCZ (Marques et al. 2018, Suridjan et al. 2014). However, this increased neuroinflammation has not been detected in early stages of disease, with studies reporting no differences in microglial activation in untreated FEP patients and CHR individuals compared to CTL (Hafizi et al. 2017a, Hafizi et al. 2017b). Immune activation and inflammation are also tightly linked to mitochondrial dysfunction (Lopez-Armada et al. 2013).

1.3. Aim of the Thesis

1.3.1. Statement of the Problem

SCZ is a debilitating illness that significantly affects an individual’s quality of life and contributes to a great global medical burden. Both increased duration of illness and duration between start of psychosis and start of treatment are associated with decreased treatment response and worse disease outcome (Perkins et al. 2004, Brousse et al. 2010, Bottlender et al. 2003); and earlier intervention, especially in individuals displaying prodromal symptoms of SCZ results in better disease outcome (Cornblatt et al. 2003). It is therefore imperative that the development of SCZ is detected as early as possible. To date, both SCZ and CHR are diagnosed by assessing patient behaviour and relying on self-reported experiences and symptoms. While there have been sets of criteria put forth for the identification of individuals at risk for psychosis, they are not reliably predictive of the prodromal state of SCZ, and individuals often do not convert to a psychosis state (van Os et al. 2009). These assessments do not employ a biological
component to the diagnosis or detection of the disease. However, the current literature points to many noticeable changes in the biology of individuals with SCZ compared to CTL. In line with the interests of this study, the current literature suggests changes in mitochondrial function and inflammation to be associated with SCZ. Most studies published in the literature about SCZ and mitochondrial changes focus on patients where the disease is already established. To the best of our knowledge, this study is the first study that has examined alterations in mitochondrial function and inflammation in parallel, in a group at risk of developing psychosis and SCZ. The use of the currently employed interview-based diagnosing in addition to peripheral biomarkers in the CHR population would be invaluable to improve diagnosis at the early stages of the disease.

In this study, our focus is to identify whether the well-studied mitochondrial dysfunction and inflammation of SCZ are present in the CHR population, which could potentially offer a chance to develop biomarkers for the prodromal phase of psychosis and SCZ.

1.3.2. **Purpose and Objectives of the Study**

The purpose of this study is to examine changes in peripheral mitochondrial function and inflammation in CHR and early stage SCZ patients and its correlation to clinical symptoms. The following objectives were analyzed in this study:

1. Characterize mitochondrial function, mitochondrial metabolites, and inflammatory cytokines in the CHR population
   a. Evaluate mitochondrial electron transport chain expression and levels of lactate and pyruvate in the periphery of CHR individuals as an indirect indicator of mitochondrial function
   b. Evaluate peripheral levels of proinflammatory cytokines IL-1β, IL-2, and IL-8; anti-inflammatory cytokine IL-10; and cytokines with both pro- and anti-inflammatory
properties IL-6 (Scheller et al. 2011, Xing et al. 1998, Luo and Zheng 2016, Schett 2018), IFNγ (Miller et al. 2015, Muhl and Pfeilschifter 2003), and TNFα (Zakharova and Ziegler 2005, Masli and Turpie 2009)

2. Explore the relationship between biomarkers and prodromal symptom severity
   a. Explore associations between peripheral mitochondrial electron transport chain expression and cognition and prodromal symptom scores
   b. Explore associations between levels of peripheral lactate and pyruvate and cognition and prodromal symptom scores
   c. Explore associations between peripheral mitochondrial electron transport chain expression and peripheral cytokine levels

3. Validate mitochondrial function and inflammation data in a new CHR population and investigate the extent of mitochondrial damage and inflammation in patients during early stage of schizophrenia

1.3.3. **Hypothesis and Rationale**

1.3.3.1. **Mitochondrial Function and Inflammation**

Mitochondrial function has been shown to be impacted in SCZ individuals, and if these changes are part of the etiology of SCZ, we should begin to see changes in individuals at high risk of developing the disease (i.e. CHR). Thus, we hypothesize that mitochondrial electron transport chain expression will be mildly decreased in the CHR group compared to the non-psychiatric control (CTL) group. Furthermore, we hypothesize that peripheral levels of mitochondrial metabolites – lactate and pyruvate will be mildly increased in the CHR group compared to the non-psychiatric control group. Together, this hypothesis supports a decrease in mitochondrial function that seem to be associated with disease progression.
Similarly, higher levels of inflammation have been shown to be associated with SCZ. Therefore, we expect to find slightly increased levels of proinflammatory cytokines in CHR individuals compared to CTL, suggesting an increased activation of immune responses and overall inflammation in the body. Alterations in levels of IFN$\gamma$, IL-10, IL-1$\beta$, IL-2, IL-6, IL-8 and TNF$\alpha$ have all been implicated in SCZ pathology. Furthermore, dysfunction of mitochondrial electron transport chain has been shown to increase ROS formation, thereby increasing immune activation and cytokine release. If mitochondrial dysfunction is a part of disease etiology, changes in cytokine levels should begin to be detectable in the prodromal and early stages of SCZ.

1.3.3.2. The Relationship Between Biomarkers and Cognition and Prodromal Symptom Severity

Decreased cognition and increased prodromal severity are often associated with worse disease prognosis. We therefore hypothesize that mitochondrial dysfunction (i.e. altered levels of electron transport expression and increased levels of lactate and pyruvate) will be associated with prodromal symptom severity and cognition ability.

1.3.3.3. Validation of Results in a New Set of Samples

If there is consistency in methodology between cohorts, we would expect the test results of our second sample set to validate the results on the first set. As such, we expect to see decreased mitochondrial electron transport chain expression in CHR compared to CTL consistently with our first sample set. To investigate whether mitochondrial dysfunction is involved in disease progression we evaluated the levels of mitochondrial function and inflammatory cytokines during early stages of the disease. Similarly, we hypothesize mildly decreased mitochondrial
electron transport chain expression and mildly increased levels of peripheral inflammatory cytokines in patients during early stages of schizophrenia when compared to CHR.

2. METHODS

2.1. Participants

Participants were recruited by Dr. Romina Mizrahi from the Focus on Youth Psychosis Prevention (FYPP) Clinic at the Centre for Addiction and Mental Health (CAMH), and through online postings. The first cohort consisted of 43 participants, which included 27 CHR individuals and 16 CTL. The second cohort (validation cohort) consisted of 33 participants, including 19 CHR individuals and 14 CTL. Also, as part of the second cohort, individuals in the early stages of schizophrenia disease progression were recruited. This group of patients (N=11) is characterized by either samples collected shortly after the first episode of psychosis or at early stage of the illness; less than four years after their first episode of psychosis.

CHR individuals were included into the study after diagnosis of prodromal risk syndrome as determined by the Criteria of Prodromal Syndromes (COPS) (Miller et al. 2003). CHR participants were excluded if they had an axis I disorder at the time of the study as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-IV) (Miller et al. 2002). CTL individuals were included in the study if they did not have a history of psychoactive drug use and if they had no first-degree relatives with a major mental disorder. CHR and CTL participants were excluded if they had a significant medical illness, if they were currently diagnosed with alcohol or substance abuse or dependence, or if they were currently pregnant or breastfeeding. Participants were administered a urine drug screen on the day of the tests. Patients
at early stages of schizophrenia were included into the study after diagnosis of schizophrenia, schizophreniaform disorder, delusional disorder, or psychosis not otherwise specified, as determined with the SCID (Shakory et al. 2018, First et al. 1995). Patients at early stages of schizophrenia were excluded from the study if they had a significant medical illness, or if they were currently pregnant or breastfeeding.  

Worth noting, my role in this thesis focuses on evaluation of biological markers and data analysis and interpretation. As noted above, clinical recruitment and assessment were performed by Dr. Romina Mizrahi’s group without my help. I am very thankful for the collaboration with Dr. Mizrahi’s group and for the participants that engaged in this study.

### 2.2. Blood Collection

Whole blood samples were collected from participants using EDTA-coated tubes on the day of their assessment at the Centre for Addiction and Mental Health. At Dr. Andreazza laboratory, whole blood was layered on Ficoll-Paque density gradient media before being centrifuged for 40 minutes at 400 g. This allows for the blood components to separate by density. Peripheral blood mononuclear cells (PBMCs) were extracted and suspended in 10% DMSO. Plasma was extracted and stored as neat samples. The samples were then slow-frozen and stored at -80 °C. PBMCs were used to measure levels of mitochondrial OXPHOS complex expression, and plasma was used measure levels of lactate, pyruvate, and inflammatory cytokines.

### 2.3. Assessment of Neurocognition and Prodromal Symptom Severity

The neurocognitive performance of participants was assessed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). This included five subscales including
immediate memory, visuospatial/constructional, attention, language, and delayed memory (Randolph et al. 1998). CHR participants were assessed on prodromal symptom severity using the Structured Interview for Psychosis-risk Syndromes (SIPS), a scale of psychosis-risk symptoms (SOPS). This included four symptom subscales including positive symptoms, negative symptoms, general symptoms, and disorganization symptoms.

2.4. Mitochondrial OXPHOS Complex Expression

A multiplex magnetic bead panel ELISA kit (EMD Millipore H0XPSMAG-16K) was used to evaluate levels of mitochondrial complex I-V and nicotinamide nucleotide transhydrogenase (NNT) expression in PBMCs. The assay was performed with the manufacturer’s protocol. Briefly, cells were partially thawed in a 37 °C water bath before being pelleted and washed with cold PBS. Mitochondrial lysis buffer (EMD Millipore 43-042) was used to lyse the cells, and 25 µL of 0.2 µg/µL was plated in triplicate in a 96-well plate, for a total of 5 µg/well of cell lysate. Lyophilized HepG2 cell lysate (EMD Millipore 47-239) was used as a positive control on each plate. Human OXPHOS Panel Pre-mixed Magnetic Beads (EMD Millipore H0XPSPMX6-MAG) were then added to each well and plates were incubated at room temperature on a rocker for 2 hours. The magnetic beads are coated with specific capture antibodies that bind to multiple subunits of each mitochondrial complex in order to detect “intact” mitochondrial complexes in the cell lysates (Zheng 2012). Human OXPHOS Panel Detection Antibodies (EMD Millipore H0XPS-1016) were then added to each well to detect and incubated at room temperature on a rocker for 1 hour before Streptavidin-Phycoerythrin (EMD Millipore MC-SAPE4) was added to each well and incubated for 30 minutes. Drive Fluid (EMD Millipore MPXDF-4PK) was then added to each well, and plates were read using the Luminex MAGPIX system (EMD Millipore).
Results were reported as a percentage of nicotinamide nucleotide transhydrogenase expression (%NNT); a protein closely related to oxidative phosphorylation, and encoded by the nuclear genome (Hoek and Rydström 1988). The assays were considered successful when magnetic bead count per well was greater than 35, and when there was a positive signal in wells with lyophilized HepG2 cell lysate. In the EMD Millipore white paper for the kit, a study was performed that compared the results of this assay to a mitochondrial complex IV (cytochrome c oxidase) functional assay (EMD Millipore AAMT004) (Zheng 2012). This was a colourimetric assay that detected the oxidation of reduced cytochrome c; and was used to show that the multiplex ELISA has a strong correlation with complex activity (Zheng 2012).

### 2.5. Lactate Quantification

A colourimetric L-Lactate Assay kit (Abcam, ab65331) was used to measure levels of plasma lactate following the manufacturer’s protocol. To quantify lactate, 50uL of 1:25 diluted plasma was added to a 96-well plate in duplicate. A reaction mixture consisting of Lactate Assay Buffer, Lactate Substrate Mix, and Lactate Enzyme Mix was added to plates and incubated at room temperature for 30 minutes. During this incubation, lactate dehydrogenase in the reaction mixture converts lactate to pyruvate, resulting in the reduction of NAD⁺ to NADH, which in turn reduces water-soluble tetrazolium present in the reaction mix to formazan, which produces colour. Absorbance was then measured at 450 nm with a microplate reader and results were interpreted against a standard provided with the kit.
2.6. Pyruvate Quantification

A colourimetric Pyruvate Assay Kit (Abcam, ab65342) was used to measure levels of plasma pyruvate following the manufacturer’s protocol. To quantify pyruvate, 50µL of neat plasma was added to a 96-well plate in duplicate. A reaction mixture consisting of Pyruvate Assay Buffer, Pyruvate Probe, and Pyruvate Enzyme Mix was added to plates and incubated at room temperature for 30 minutes. During this incubation, pyruvate oxidase present in the reaction mix oxidizes pyruvate and forms hydrogen peroxide as a substrate. The produced hydrogen peroxide is then catalyzed by peroxidases in the reaction mix and reacts with 4-aminoantipyrine to produce a coloured dye. Absorbance was then measured at 570 nm with a microplate reader and results were interpreted against a standard provided with the kit.

2.7. Inflammatory Cytokines Quantification

Levels of plasma inflammatory cytokines were measured with the Human High Sensitivity T Cell Magnetic Bead Panel Kit (EMD Millipore HSTCMAG-28SK). The assay was performed with the manufacturer’s protocol. Briefly, levels of IFNγ, IL-10, IL-1β, IL-2, IL-6, IL-8 and TNFα were assessed against a standard made of Serum Matrix (EMD Millipore MXHSM-7) and Human High Sensitivity T Cell Standard (EMD Millipore HSTC-8028). Neat plasma samples were plated in duplicates in a 96-well plate. Two Human High Sensitivity T Cell Quality Controls (EMD Millipore HSTC-6028) were also plated to ensure that the assay results were reliable. Pre-mixed Human High Sensitivity T Cell Magnetic Beads were added to each well and incubated at 4 ºC on a rocker for 18 hours. Samples from the validation cohort (including early stage SCZ samples) were incubated for an additional 24 hours at this step because of equipment failure on the day of plate reading. Human High Sensitivity T Cell Detection Antibodies (EMD
Millipore HSTC-1028) were added to each well and incubated at room temperature on a rocker for 1 hour. Next, Streptavidin-Phycoerythrin (EMD Millipore MC-SAPE7) was added to each well and incubated for 30 minutes. Drive Fluid was added to each well and plates were read using the Luminex MAGPIX system (EMD Millipore). The assays were considered successful when the magnetic bead count per well was greater than 35, and when the quality controls were within the range provided by EMD Millipore.

2.8. Statistical Analysis

All statistical analyses were performed on SPSS (version 20.0, IBM), and all graphics were created with GraphPad Prism (version 7.0a, 2016). Aside from demographic measures, all non-normally distributed data was log-transformed before analysis.

2.8.1. Demographic Measures

Differences in demographic measures between CTL and CHR were analysed with chi-square tests for categorical variables, independent sample t-tests for normally distributed continuous variables, and Mann-Whitney U tests for non-normally distributed continuous variables. When early stage SCZ samples were added, differences in demographic measures were analysed with chi-square tests for categorical variables, one-way ANOVA for normally-distributed continuous variables and Kruskal-Wallis H-tests for non-normally distributed continuous variables.


For the both cohorts, a MANOVA and MANCOVA were performed to test the effect of group (CHR and CTL) on mitochondrial complex I-V expression. Body mass index (BMI), age, sex, acute cannabis use, acute tobacco use, and antipsychotic use were included as covariates in the
MANCOVA. BMI has been included as a covariate as it has been reported to be an important factor in energy metabolism and mitochondrial function and in SCZ symptomology; with studies reporting a negative correlation in the mitochondrial respiratory capacities in the adipose tissue of obese patients compared to BMI (Fischer et al. 2015), a negative correlation in leukocyte mitochondrial DNA copy number and BMI (Meng et al. 2016), and higher BMI to be associated with increased lipid peroxidation and fewer psychopathological symptoms in SCZ patients (An et al. 2018). Bivariate correlations were performed to explore associations between mitochondrial complex I-V expression, and cognition and prodromal symptom severity. The first and validation cohorts were then combined to increase sample size. The effect of group on mitochondrial complex I-V expression was assessed with independent t-tests and ANCOVAs for normally distributed data, and Mann-Whitney U tests for non-normally distributed data. BMI, age, sex, acute cannabis use, tobacco use, antipsychotic use, and study (first or validation study) were included as covariates in the ANCOVAs. Early stage SCZ samples were then added to analysis, and a MANOVA and a MANCOVA was performed to test the effects of group (early stage SCZ, CHR, and CTL) on mitochondrial complex I-V expression. BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use were included as covariates in the MANCOVA.

2.8.3. Peripheral Lactate and Pyruvate, Neurocognition, and Prodromal Symptom Severity

An ANOVA was performed to test the difference in plasma lactate and pyruvate levels between groups (CHR and CTL for the first cohort; and early stage SCZ, CHR, and CTL for the validation cohort). Bivariate correlations were performed to explore associations between levels of lactate and pyruvate, and cognition and prodromal symptom severity.
2.8.1. Levels of Peripheral Cytokines

For the first cohort and validation cohort, a MANOVA and MANCOVA were performed to test the differences between groups (CTL and CHR) on levels of peripheral proinflammatory cytokines IL-1β, IL-2, and IL-8; anti-inflammatory cytokine IL-10; and cytokines with both pro- and anti-inflammatory properties IL-6, IFNγ, and TNFα. BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use were included as covariates in the MANCOVA. BMI was included as a covariate because of the various studies that have indicated BMI playing a large role in inflammation. As previously mentioned, a study in 2018 reported an association between increased BMI and increased lipid peroxidation products in SCZ patients (An et al. 2018). Additionally, multiple studies have reported positive associations between BMI and peripheral pro-inflammatory cytokine levels, indicating increased systemic inflammation with increased BMI in obese individuals (Larsson et al. 2015, Borges et al. 2018) as well as in individuals who have experienced an episode of psychosis (Juncal-Ruiz et al. 2018). The cohorts were combined to increase sample size, and a MANOVA and MANCOVA were performed to test the differences between groups (CTL and CHR) on levels of peripheral proinflammatory cytokines IL-1β, IL-2, and IL-8; anti-inflammatory cytokine IL-10; and cytokines with both pro- and anti-inflammatory properties IL-6, IFNγ, and TNFα. BMI, age, sex, acute cannabis use, acute tobacco use, antipsychotic use and incubation time (18 hours (following manufacturer’s protocol for the first cohort) or 42 hours (the result of equipment failure requiring samples to be incubated for an additional 24 hours for the validation cohort)), were included as covariates in the MANCOVA. Partial correlations (controlling for incubation time) were performed to explore associations between levels of cytokines, mitochondrial electron transport chain expression, and levels of lactate and pyruvate.
Early stage SCZ samples were then added to analysis in the validation cohort, and a MANOVA and a MANCOVA was performed to test the effects of group (early stage SCZ, CHR, and CTL) on levels of peripheral proinflammatory cytokines IL-1β, IL-2, and IL-8; anti-inflammatory cytokine IL-10; and cytokines with both pro- and anti-inflammatory properties IL-6, IFNγ, and TNFα. BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use were included as covariates in the MANCOVA.

1.3.3.1. Inflammatory Cytokine Ratio

When the two cohorts were combined, a ratio of inflammatory cytokines was created by dividing the sum of levels of proinflammatory cytokines (IL-1β, IL-2, and IL-8) by levels of the anti-inflammatory cytokine IL-10. An ANOVA was performed to compare the ratio of proinflammatory to anti-inflammatory cytokine levels between groups (CTL, CHR, early stage SCZ).

3. RESULTS

The following section consists of portions adapted from Da Silva et al. 2018 published in Scientific Report (DOI: 10.1038/s41598-018-24355-6) and Wu et al. 2019 (submitted to Scientific Reports, November 19, 2018. Full manuscript can be found in Thesis attachment.)

3.1. Participants

3.1.1. Clinical High Risk for Psychosis

Demographic data for the first cohort and second cohort are shown in Table 1 and Table 2 respectively. Demographic data for the two cohorts combined is shown in Table 3. In the first and second cohort, there was no difference in age, sex, BMI, or NNT between CTL and CHR groups. This did not change even after combining the two cohorts. In the first cohort, there were
more tobacco users in the CHR group ($\chi^2(1, N = 43) = 4.96, p = 0.03$). When the cohorts were combined, there were significantly more tobacco ($\chi^2 (1, N = 75) = 5.97, p = 0.015$) and cannabis ($\chi^2 (1, N = 75) = 4.35, p = 0.037$) users. In the first cohort, five CHR individuals were currently on low-dose antipsychotic treatment with either Risperidone (one with 0.5 mg and two with 1 mg), Quetiapine (75 mg) or Aripiprazole (5 mg). In the second cohort, there were six CHR individuals currently using antipsychotics with two individuals taking Aripiprazole (2.0 mg and an unspecified amount), three individuals taking Quetiapine (50 mg, 200 mg, and 6.25 mg), and one individual taking Risperidone (0.5 mg).

In the first cohort, PBMCs were not collected for sample FEPPA098, and was therefore not included into the OXPHOS analysis.
Table 1 (Da Silva et al. 2018): Demographic and clinical information of the first cohort

<table>
<thead>
<tr>
<th></th>
<th>Non-psychiatric Controls (n = 16)</th>
<th>Clinical High Risk (n = 27)</th>
<th>t</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (years), SD</td>
<td>21.25 ± 2.05</td>
<td>20.26 ± 1.72</td>
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<tr>
<td>Sex</td>
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<td></td>
<td>Female</td>
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<td>12</td>
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<tr>
<td>BMI, SD</td>
<td>23.99 ± 5.00</td>
<td>23.94 ± 5.66</td>
<td>U = 212</td>
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<tr>
<td>NNT expression (MFI), SD</td>
<td>6643.56 ± 2598.70</td>
<td>6719.50 ± 2560.16</td>
<td>U = 207</td>
<td>0.98</td>
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<td>Current Drug Use</td>
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<td></td>
<td>Cannabis</td>
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</tr>
<tr>
<td>Antipsychotic Use</td>
<td>Total</td>
<td>35.70 ± 9.64</td>
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<td></td>
<td>Positive</td>
<td>11.78 ± 3.38</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>10.78 ± 5.02</td>
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<td>SOPS, SD</td>
<td>Disorganization</td>
<td>3.63 ± 2.31</td>
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<td></td>
<td>General</td>
<td>8.48 ± 3.79</td>
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<td>RBANS, SD</td>
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<td>88.89 ± 14.28</td>
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<td>Immediate memory</td>
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<td>95.19 ± 15.36</td>
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<td>Visuospatial memory</td>
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<td>85.52 ± 12.97</td>
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<td></td>
<td>Language</td>
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<td>Attention</td>
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<td>Delayed memory</td>
<td>89.25 ± 13.48</td>
<td>93.81 ± 10.03</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; BMI, body mass index; NNT, nicotinamide nucleotide transhydrogenase; MFI, median fluorescence intensity; SOPS, Scale of Psychosis-risk Symptoms, RBANS, Repeatable Battery for the Assessment of Neuropsychological Status
### Table 2 (Wu et al. 2019): Demographic and clinical information of the validation cohort

<table>
<thead>
<tr>
<th></th>
<th>Non-psychiatric Controls (n = 14)</th>
<th>Clinical High Risk (n = 19)</th>
<th>(U)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), SD</td>
<td>21.29 ± 2.64</td>
<td>21.63 ± 3.50</td>
<td>131</td>
<td>0.95</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>15</td>
<td>(\chi^2 = 0.87)</td>
<td>0.35</td>
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<tr>
<td>Female</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td>BMI, SD</td>
<td>24.42 ± 4.82</td>
<td>23.32 ± 7.38</td>
<td>97.5</td>
<td>0.20</td>
</tr>
<tr>
<td>NNT expression (MFI), SD</td>
<td>1282 ± 878.8</td>
<td>1735 ± 1006</td>
<td>1.35</td>
<td>0.19</td>
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<tr>
<td>Current Drug Use</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Tobacco</td>
<td>0</td>
<td>1</td>
<td>(\chi^2 = 0.76)</td>
<td>0.38</td>
</tr>
<tr>
<td>Cannabis</td>
<td>0</td>
<td>4</td>
<td>(\chi^2 = 3.35)</td>
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<tr>
<td>Antipsychotic Use</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>6</td>
<td></td>
<td></td>
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<tr>
<td>SOPS, SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>88.55 ± 13.12</td>
<td>94.17 ± 12.45</td>
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<tr>
<td>Positive</td>
<td>95.00 ± 17.62</td>
<td>93.78 ± 11.55</td>
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<tr>
<td>Negative</td>
<td>13.16 ± 6.59</td>
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<tr>
<td>Disorganization</td>
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<tr>
<td>General</td>
<td>7.79 ± 4.35</td>
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<tr>
<td>RBANS, SD</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>83.09 ± 16.11</td>
<td>89.06 ± 15.23</td>
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<td></td>
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<tr>
<td>Immediate memory</td>
<td>95.00 ± 17.62</td>
<td>93.78 ± 11.55</td>
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<tr>
<td>Visuospatial memory</td>
<td>88.73 ± 20.53</td>
<td>99.06 ± 22.03</td>
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<tr>
<td>Language</td>
<td>99.73 ± 16.77</td>
<td>102.50 ± 15.11</td>
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<tr>
<td>Attention</td>
<td>91.91 ± 13.12</td>
<td>94.78 ± 10.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; BMI, body mass index; NNT, nicotinamide nucleotide transhydrogenase; MFI, median fluorescence intensity; SOPS, Scale of Psychosis-risk Symptoms, RBANS, Repeatable Battery for the Assessment of Neuropsychological Status
3.1.2. Early stage Schizophrenia

Demographic data for early stage SCZ individuals compared to the validation cohort are shown in Table 4. Between the three groups (CTL, CHR, early stage SCZ), there was no difference in sex, BMI, NNT, or cannabis use. There was a significant difference in age ($\chi^2(2) = 8.433$, $p = 0.015$), with a Dunn’s post-hoc test revealing a significant difference between early stage SCZ
and CTL (p = 0.033), and early stage SCZ and CHR (p = 0.025). There were five early stage SCZ individuals who were currently on low-dose antipsychotic treatment. One early stage SCZ participant had a brain tumor and this was considered in the analysis.

| Table 4: Demographic and clinical information of the validation cohort and early stage SCZ individuals |
|--------------------------------------------------|-------------------------------------------------|--------------------------------------------------|
| Non-psychiatric Controls (n = 14)               | Clinical High Risk (n = 19)                     | Early Stage Schizophrenia (n = 11)               |
| Age (years), SD                                 | 21.29 ± 2.64                                   | 21.63 ± 3.50                                    | 26.18 ± 5.02                                    | \( H = 8.433 \) | \( p = 0.015 \) |
| Sex                                              | Male: 9                                       | 15                                              | 10                                              | \( \chi^2 = 2.54 \) | \( p = 0.281 \) |
|                                                  | Female: 5                                     | 4                                               | 1                                               |                      |                  |
| BMI, SD                                          | 24.42 ± 4.82                                  | 23.32 ± 7.38                                    | 25.12 ± 4.88                                    | \( H = 3.191 \) | \( p = 0.203 \) |
| NNT expression (MFI), SD                        | 1282 ± 878.8                                  | 1735 ± 1006                                     | 1072 ± 954.6                                    | \( H = 3.174 \) | \( p = 0.156 \) |
| Current Drug Use                                 | Tobacco: 0                                    | 1                                               | 0                                               | \( \chi^2 = 1.346 \) | \( p = 0.51 \) |
|                                                  | Cannabis: 0                                   | 4                                               | 2                                               | \( \chi^2 = 3.29 \) | \( p = 0.19 \) |
| Antipsychotic Use                                | 0                                             | 6                                               | 5                                               |                      |                  |
| SOPS, SD                                         | Total: 38.74 ± 14.60                           |                                                   |                                                   |                      |                  |
|                                                  | Positive: 11.37 ± 3.86                         |                                                   |                                                   |                      |                  |
|                                                  | Negative: 13.16 ± 6.59                        |                                                   |                                                   |                      |                  |
|                                                  | Disorganization: 6.42 ± 3.78                   |                                                   |                                                   |                      |                  |
|                                                  | General: 7.79 ± 4.35                           |                                                   |                                                   |                      |                  |
| RBANS, SD                                        | Total: 88.55 ± 13.12                           | 94.17 ± 12.45                                   |                                                  |                      |                  |
|                                                  | Immediate memory: 95.00 ± 17.62               | 93.78 ± 11.55                                   |                                                  |                      |                  |
|                                                  | Visuospatial memory: 83.09 ± 16.11            | 89.06 ± 15.23                                   |                                                  |                      |                  |
|                                                  | Language: 88.73 ± 20.53                        | 99.06 ± 22.03                                   |                                                  |                      |                  |
|                                                  | Attention: 99.73 ± 16.77                       | 102.5 ± 15.11                                   |                                                  |                      |                  |
|                                                  | Delayed memory: 91.91 ± 13.12                 | 94.78 ± 10.11                                   |                                                  |                      |                  |

Abbreviations: SD, standard deviation; BMI, body mass index; NNT, nicotinamide nucleotide transhydrogenase; MFI, median fluorescence intensity; SOPS, Scale of Psychosis-risk Symptoms; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status
3.2. Mitochondrial OXPHOS Complex Expression and Prodromal Symptom Severity and Neurocognition

3.2.1. First Cohort

In the first cohort, there was no significant differences between CHR and CTL in the expression of mitochondrial complex I (F(1,40) = 0.36, p = 0.55), complex II (F(1,40) = 1.19, p = 0.28), complex III (F(1,40) = 0.74, p = 0.39), complex IV (F(1,40) = 0.45, p = 0.51), and complex V (F(1,40) = 0.6, p = 0.44) (Figure 1). After controlling for age, sex, BMI, antipsychotic use, tobacco use, and cannabis use there were still no significant differences between CTL and CHR in the expression of mitochondrial complex I (F(1,34) = 0.006, p = 0.94), complex II (F(1,34) = 0.48, p = 0.49), complex III (F(1,34) = 0.48, p = 0.49), complex IV (F(1,34) = 0.04, p = 0.85), and complex V (F(1,34) = 0.6, p = 0.44).

![Figure 1](Da Silva et al. 2018): Mitochondrial complex I–V expression in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the first cohort. Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.
The following is an excerpt from Da Silva et al. 2018:

“Within the CHR group, mitochondrial complex III expression was inversely related to SOPS total symptom severity score ($r = -0.49$, $p = 0.01$) (Figure 2a), which survived correction for multiple comparisons. Follow up analysis revealed a significant contribution of SOPS negative symptom severity score ($r = -0.51$, $p = 0.008$) (Figure 2b). There were no significant correlations between mitochondrial complex (I-V) expression and SOPS positive symptom severity score ($p > 0.05$). In addition, mitochondrial complex V expression was inversely related with the RBANS attention subscale in the sample as a whole ($n = 42$; $r = -0.44$, $p = 0.004$) (Figure 3), suggesting that increased mitochondrial expression may be related to poorer cognitive performance. The [CTL] group appeared to drive this correlation ($r = -0.73$, $p = 0.001$) (Figure 3), which survived correction for multiple comparisons. There were no other significant correlations between mitochondrial electron transport chain expression and cognition ($p > 0.05$).”
Figure 2 (Da Silva et al. 2018): Association between peripheral mitochondrial complex III expression and (a) total SOPS symptom severity score ($r = -0.49$, $p = 0.01$) and (b) SOPS negative symptom severity score ($r = -0.51$, $p = 0.008$) in clinical high risk (CHR). Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.
Figure 3 (Da Silva et al. 2018): Association between mitochondrial complex V expression (%NNT) and RBANS attention subscale scores in non-psychiatric controls (r= -0.73, p=0.001) and in the sample as a whole (r= -0.44, p=0.003).

3.2.2. Validation Cohort

In the validation cohort, we were able to confirm that there are no significant differences between CTL and CHR in the expression of mitochondrial complex I (F(1,27) = 0.17, p = 0.68), complex II (F(1,27) = 0.06, p = 0.81), complex III (F(1,27) = 1.51, p = 0.23), complex IV (F(1,27) = 0.18, p = 0.68), and complex V (F(1,27) = 0.21, p = 0.65 (Figure 4). After controlling for age, sex, BMI, antipsychotic use, tobacco use, and cannabis use, there were still no significant differences between CTL and CHR in the expression of mitochondrial complex I (F(1,21) = 2.31, p = 0.14), complex II (F(1,21) = 2.46, p = 0.13), complex III (F(1,21) = 2.11, p = 0.16), complex IV (F(1,21) = 1.62, p = 0.22), and complex V (F(1,21) = 2.11, p = 0.16). In the
validation cohort, there were no significant correlations between mitochondrial complex I-V expression and SOPS in the CHR group (p > 0.05). There were also no significant correlations between complex I-V expression and RBANS assessments in both CTL and CHR groups (p > 0.05).

Figure 4 (Wu et al. 2019): Mitochondrial complex I–V expression in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.

3.2.3. Combining First Cohort and Validation Cohort

The following is an excerpt from Wu et al. 2019 (submitted to Scientific Reports):

“When the first cohort and validation cohort were combined, we were able to further validate that there were no significant differences between CTL and CHR in the expression of mitochondrial complex I (t(73) = 0.090, p = 0.93), complex II (U = 554, p
complex III \( t(73) = 0.019, p = 0.99 \), complex IV \( U = 591, p = 0.78 \), and complex V \( t(73) = 1.22, p = 0.23 \) (Figure 5). An ANCOVA could not be performed on the expression of mitochondrial complex II and IV as the data were non-normally distributed. In the CHR group, complex III was inversely correlated with SOPS total symptom severity score \( r(43) = -0.30, p = 0.047 \) (Figure 6a), which similar to the first cohort, seemed to be caused by a significant contribution of SOPS negative symptom severity score \( r(43) = -0.38, p = 0.009 \) (Figure 6b). Complex V was positively correlated with SOPS disorganization severity score \( r(43) = 0.50, p = 0.001 \) (Figure 7). Aside from the correlation between complex III and SOPS total symptom severity, results survived after Bonferroni correction for multiple comparisons.”

**Figure 5 (Wu et al. 2019):** Mitochondrial complex I–V expression in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) when the first and validation cohorts are combined. Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.
Figure 6 (Wu et al. 2019): Association between peripheral mitochondrial complex III expression and (a) total SOPS symptom severity score in the first cohort ($r = -0.49$, $p = 0.01$) and the cohorts combined ($r = -0.30$, $p = 0.047$), and (b) SOPS negative symptom severity score in the first cohort ($r = -0.51$, $p = 0.008$) and the cohorts combined ($r = -0.38$, $p = 0.009$) in clinical high risk (CHR). Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.
Figure 7 (Wu et al. 2019): Association between peripheral mitochondrial complex V expression and SOPS disorganization symptom severity score in the combined cohorts ($r = 0.50, p = 0.001$) in clinical high risk (CHR). Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.

3.2.4. Comparing CHR and Early Stage SCZ

When early stage SCZ individuals were added to the analysis, there were still no difference in mitochondrial complex I ($F(2,36) = 0.55, p = 0.58$), complex II ($F(2,36) = 0.02, p = 0.98$), complex III ($F(2,36) = 2.99, p = 0.63$), complex IV ($F(2,36) = 0.34, p = 0.71$), and complex V ($F(2,36) = 0.89, p = 0.42$) expression between the groups (CTL, CHR, early stage SCZ) (Figure 8). After controlling for age, sex, BMI, antipsychotic use, tobacco use, and cannabis use, there were still no significant differences between CTL, CHR, and early stage SCZ in the expression
of mitochondrial complex I ($F(2,21) = 1.42, p = 0.25$), complex II ($F(2,21) = 2.23, p = 0.69$), complex III ($F(2,21) = 1.45, p = 0.24$), complex IV ($F(2,21) = 1.33, p = 0.29$), and complex V ($F(2,21) = 1.63, p = 0.18$). Removing the individual with a brain tumor from the analysis did not change these results.

Figure 8: Mitochondrial complex I–V expression in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) when the first and validation cohorts are combined. Complex expression is reported as a percentage against each subject’s individual nicotinamide nucleotide transhydrogenase expression levels (%NNT). NNT is a nucleus-encoded protein present in the inner mitochondrial membrane that is closely related to mitochondrial oxidative phosphorylation.
3.3. Peripheral Lactate and Pyruvate

3.3.1. First Cohort

The following is an excerpt from Da Silva et al. 2018:

“There were no significant differences found in levels of peripheral lactate
\( F(1,31) = 0.17, p = 0.69 \) (Figure 9a) or pyruvate \( F(1,31) = 1.31, p = 0.26 \) (Figure 9b)
between CHR and CTL. Similar results were obtained after excluding CHR individuals
currently on antipsychotic medication \( n = 1 \), or positive for cannabis use \( n = 2 \).”

Figure 9 (Da Silva et al. 2018): (a) Peripheral lactate and (b) pyruvate levels in clinical high risk (CHR) and non-psychiatric controls (CTL) of the first cohort. There were no significant differences in lactate or pyruvate levels between CHR and CTL (lactate: \( F(1,31) = 0.17, p = 0.69 \); pyruvate: \( F(1,31) = 1.31, p = 0.26 \)).

However, in CHR, there was a significant effect of tobacco use on lactate \( F(1,18) = 5.18, p = 0.04 \) and pyruvate levels \( F(1,18) = 5.87, p = 0.03 \), such that CHR individuals who smoked tobacco \( n = 5 \) had significantly lower pyruvate \( (53.78\%) \) and lactate \( (42.06\%) \) levels compared to those who did not smoke \( n = 13 \) (Figure 10).
Figure 10 (Da Silva et al. 2018): (a) Peripheral lactate \( F(1,18) = 5.18, p = 0.04 \) and (b) pyruvate \( F(1,18) = 5.87, p = 0.03 \) levels in clinical high risk (CHR) tobacco users and non-users of the first cohort.

Within the CHR group, lactate levels were positively correlated with SOPS total symptom severity score \( r = 0.54, p = 0.01 \) (Figure 11a). Follow up analysis revealed a significant contribution from SOPS negative symptom severity score \( r = 0.61, p = 0.004 \) (Figure 11b), which survived correction for number of SOPS subscales. There were no significant correlations
between lactate and pyruvate levels and SOPS positive symptom severity score (p > 0.05). No significant correlations were found between lactate or pyruvate levels and cognition in the sample as a whole (p > 0.05). However, in the CTL group, pyruvate levels were inversely related to RBANS language subscale (r = −0.72, p = 0.006) (Figure 12), which survived correction for number of RBANS subscales. This was not seen in the CHR group (p > 0.05).”

**Figure 11 (Da Silva et al. 2018):** Associations between peripheral lactate and (a) SOPS total symptom severity score (r = 0.54, p = 0.01), and (b) SOPS negative symptom severity score (r = 0.61, p = 0.004) in the first cohort.
Figure 12 (Da Silva et al. 2018): Associations between peripheral pyruvate levels and RBANS language subscale in non-psychiatric controls (CTL) in the first cohort.
### 3.3.2. Validation Cohort

In the validation cohort, we were able to validate the result of no significant differences found in peripheral levels of lactate ($F(1,30) = 2.777, p = 0.106$) (Figure 13a) and pyruvate ($F(1,28) = 0.004, p = 0.948$) (Figure 13b) between CTL and CHR groups. Similar results were found after excluding participants currently on antipsychotics ($n = 5$) or positive for cannabis use ($n = 4$). There were too few tobacco users ($n = 1$) to test for effect of tobacco on peripheral lactate and pyruvate levels in this cohort.

![Figure 13: Peripheral (a) lactate and (b) pyruvate levels in in clinical high risk (CHR) and non-psychiatric controls (CTL) of the validation cohort. There were no significant differences in lactate or pyruvate levels between CHR and CTL (lactate: $F(1,30) = 2.777, p = 0.106$; pyruvate: $F(1,28) = 0.004, p = 0.948$).](image)

In the validation cohort there were no significant associations between levels of peripheral lactate or pyruvate and prodromal symptom severity ($p > 0.05$). Additionally, there were no significant associations between lactate or pyruvate and the RBANS cognition subscales in both CTL and CHR groups ($p > 0.05$).
3.3.3. *Combining First Cohort and Validation Cohort*

When the two cohorts were combined, we were again able to validate the result of no differences in levels of peripheral lactate ($F(1,62) = 2.511, p = 0.118$) (Figure 14a) and pyruvate ($F(1,60) = 0.920, p = 0.341$) (Figure 14b) between CTL and CHR groups. Similar results were found after excluding participants currently on antipsychotics ($n = 11$) or positive for cannabis use ($n = 6$).

![Figure 14: Peripheral (a) lactate and (b) pyruvate levels in in clinical high risk (CHR) and non-psychiatric controls (CTL) when the cohorts are combined. There were no significant differences in lactate or pyruvate levels between CHR and CTL (lactate $F(1,62) = 2.511, p = 0.118, p = 0.106$; pyruvate: $F(1,60) = 0.920, p = 0.341$).](image)

In the CHR group when the cohorts were combined, there was a significant association between levels of peripheral lactate and SOPS total symptom severity scores ($r = 0.339, p = 0.037$) (Figure 15). There were no associations found between levels of peripheral pyruvate and prodromal symptom severity ($p>0.05$). In the sample as a whole, lactate was significantly associated with the RBANS total scale ($r = -0.271, p = 0.036$) (Figure 16a); which seemed to be
driven by the immediate memory subscale ($r = -0.410$, $p = 0.001$) (Figure 16b). There were no associations between levels of peripheral pyruvate and the RBANS scales in the sample as a whole ($p>0.05$). In the CTL group there was a significant inverse association between levels of peripheral lactate and the RBANS immediate memory subscale ($r = -0.506$, $p = 0.14$) (Figure 16a). In the CHR group, there was a significant inverse association between levels of peripheral lactate and the RBANS total scale ($r = -0.356$, $p = 0.031$) (Figure 16a). The immediate memory subscale seemed to drive this correlation, as there was a significant inverse correlation between levels of peripheral lactate and the RBANS immediate memory subscale ($r = -0.378$, $p = 0.021$) (Figure 16b). There were no associations between pyruvate and the RBANS scale in the CHR group ($p>0.05$).

Figure 15: Associations between peripheral lactate and SOPS total symptom severity score ($r = 0.339$, $p = 0.037$) when cohorts were combined.
Figure 16: Associations between peripheral lactate and (a) RBANS total scale (both groups: $r = -0.271$, $p = 0.036$; CHR: $r = -0.356$, $p = 0.031$), and (b) RBANS immediate memory subscale (both groups: $r = -0.410$, $p = 0.001$; CTL: $r = -0.506$, $p = 0.14$; CHR: $r = -0.378$, $p = 0.021$).
3.3.4. Comparing CHR and Early Stage SCZ

When early stage SCZ patients were added to analysis, there were no differences in levels of peripheral lactate (F(2,40) = 1.666, p = 0.202) (Figure 17a) and pyruvate (F(2,38) = 0.010, p = 0.990) (Figure 17b) between groups (CTL, CHR, early stage SCZ). Similar results were found after excluding individuals currently using antipsychotics (n = 11) or positive for cannabis use (n = 6). There were too few tobacco users (n = 1) to test for effect of tobacco on peripheral lactate and pyruvate levels in this cohort.

**Figure 17**: Peripheral (a) lactate and (b) pyruvate levels in in clinical high risk (CHR), non-psychiatric controls (CTL), and early stage SCZ. There were no significant differences in lactate or pyruvate levels between CHR and CTL (lactate F(2,40) = 1.666, p = 0.202, p = 0.106; pyruvate: F(2,38) = 0.010, p = 0.990.
3.4. Levels of Peripheral Cytokines

3.4.1. First Cohort

There were no differences in levels of pro-inflammatory cytokines IL-1β (F(1,32) = 0.786, p = 0.382), IL-2 (F(1,32) = 2.108, p = 0.156), and IL-8 (F(1,32) = 2.339, p = 0.136) (Figure 18); anti-inflammatory cytokine IL-10 (F(1,39) = 1.544, p = 0.221) (Figure 19); or cytokines with both pro- and anti-inflammatory properties IFNγ (1,39) = 0.540, p = 0.467), IL-6 (F(1,39) = 0.830, p = 0.368), and TNFα (F(1,39) = 0.013, p = 0.909) (Figure 20). After controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use, there were similar non-significant results (p>0.05).

![Figure 18: Peripheral levels of proinflammatory cytokines in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the first cohort. Levels of cytokines are reported as the log₁₀ of plasma cytokine concentration (pg/mL)](image-url)
Figure 19: Peripheral levels of anti-inflammatory cytokine IL-10 in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the first cohort. Levels of cytokines are reported as the log\(_{10}\) of plasma cytokine concentration (pg/mL)

Figure 20: Peripheral levels of cytokines with both pro- and anti-inflammatory properties in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the first cohort. Levels of cytokines are reported as the log\(_{10}\) of plasma cytokine concentration (pg/mL)
3.4.2. Validation Cohort

There were no differences in levels of pro-inflammatory cytokines IL-1β (F(1,15) = 0.348, p = 0.564), IL-2 (F(1,15) = 0.052, p = 0.823), and IL-8 (F(1,15) = 1.232, p = 0.284) (Figure 21); anti-inflammatory cytokine IL-10 (F(1,23) = 1.482, p = 0.236) (Figure 22); or cytokines with both pro- and anti-inflammatory properties IFNγ (1,25) = 0.085, p = 0.773), IL-6 (F(1,25) = 3.623, p = 0.069), and TNFα (F(1,25) = 2.165, p = 0.154) (Figure 23). After controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use, there were similar non-significant results (p>0.05).

Figure 21: Peripheral levels of proinflammatory cytokines in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL)
Figure 22: Peripheral levels of anti-inflammatory cytokine IL-10 in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL).

Figure 23: Peripheral levels of cytokines with both pro- and anti-inflammatory properties in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL).
3.4.3. Combining First Cohort and Validation Cohort

There were no differences in levels of pro-inflammatory cytokines IL-1β ($F(1,49) = 0.522, p = 0.473$), IL-2 ($F(1,49) = 1.839, p = 0.181$), and IL-8 ($F(1,49) = 1.249, p = 0.269$) (Figure 24); anti-inflammatory cytokine IL-10 ($F(1,64) = 0.703, p = 0.405$) (Figure 25); or cytokines with both pro- and anti-inflammatory properties IFNγ ($F(1,65) = 0.137, p = 0.712$), IL-6 ($F(1,65) = 0.291, p = 0.591$), and TNFα ($F(1,65) = 0.059, p = 0.809$) (Figure 26). After controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use, there were similar non-significant results ($p>0.05$).

![Figure 24](image-url)

*Figure 24:* Peripheral levels of proinflammatory cytokines in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) when cohorts are combined. Levels of cytokines are reported as the log$_{10}$ of plasma cytokine concentration (pg/mL)
Figure 25: Peripheral levels of anti-inflammatory cytokines in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) when cohorts are combined. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL).

Figure 26: Peripheral levels of cytokines with both pro- and anti-inflammatory properties in clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) when cohorts are combined. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL).
3.5. Relationship Between Inflammatory Cytokines and the Mitochondrial Electron Transport Chain

We have evaluated the relationship between inflammatory cytokines and mitochondrial electrons transport chain complexes using first cohort and validation samples combined to increase power of analysis. Table 5 shows all the CHR correlations. After Bonferroni correction, correlations are significant if the p-value is less than 0.0014. Notably, mitochondrial complex IV demonstrated a negative relationship with IFNγ \((r = -0.598, p < 0.001)\), IL-1β \((r = -0.591, p < 0.001)\), and TNFα \((r = -0.574, p < 0.001)\) in CHR individuals.

Table 5: Partial correlations exploring the association between mitochondrial complex I-V expression and levels of peripheral inflammatory cytokines in the CHR group when the first and validation cohorts are combined. Correlations are significant if \(p < 0.0014\) (Created with SPSS)
3.5.1. Comparing CHR and Early stage SCZ

There were no differences in levels of pro-inflammatory cytokines IL-1β (F(2,19) = 0.288, p = 0.753), IL-2 (F(2,19) = 0.115, p = 0.892), and IL-8 (F(2,19) = 1.411, p = 0.0268) (Figure 27); or cytokines with both pro- and anti-inflammatory properties IFNγ (2,30) = 0.046, p = 0.955), IL-6 (F(2,30) = 1.552, p = 0.228) (Figure 28). Levels of the anti-inflammatory cytokine IL-10 was significant between groups (F(2,28) = 8.722, p = 0.001) (Figure 29); with pairwise comparisons revealing a significant difference between early stage SCZ patients and both CTL (p = 0.018) and CHR (p = 0.001) groups. However, after controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use, the difference was no longer significant (p = 0.383). There were no specific covariates that significantly altered this result (Table 6). In addition, peripheral levels of TNFα were significantly different between groups (F(2,30) = 5.169, p = 0.012) (Figure 29); with pairwise comparisons revealing a significant difference between CHR and early stage SCZ groups (p = 0.010). However, after controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use, there was no longer any significant differences between groups (p = 0.813). There were no specific covariates that significantly altered this result (Table 7).
Figure 27: Peripheral levels of proinflammatory cytokines in early stage SCZ, clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL)
Figure 28: Peripheral levels of anti-inflammatory cytokines in early stage SCZ, clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL).

Figure 29: Peripheral levels of cytokines with both pro- and anti-inflammatory properties in early stage SCZ, clinical high risk (CHR) for psychosis and non-psychiatric controls (CTL) in the validation cohort. Levels of cytokines are reported as the log_{10} of plasma cytokine concentration (pg/mL).
Table 6: ANCOVA results exploring the effect of diagnosis on levels of peripheral IL-10 after controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use. There were no individual covariates that contributed significantly to the result. (Created with SPSS)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>0.286(^a)</td>
<td>7</td>
<td>0.041</td>
<td>1.143</td>
<td>0.383</td>
</tr>
<tr>
<td>Intercept</td>
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<td>1</td>
<td>0.221</td>
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<td>0.024</td>
</tr>
<tr>
<td>Cannabis Use</td>
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<td>1</td>
<td>0.006</td>
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<td>0.685</td>
</tr>
<tr>
<td>Tobacco Use</td>
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<td>1</td>
<td>0.112</td>
<td>3.127</td>
<td>0.095</td>
</tr>
<tr>
<td>Age</td>
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<td>1</td>
<td>0.024</td>
<td>0.658</td>
<td>0.428</td>
</tr>
<tr>
<td>Sex</td>
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<td>1</td>
<td>0.000</td>
<td>0.013</td>
<td>0.911</td>
</tr>
<tr>
<td>BMI</td>
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<td>0.044</td>
<td>1.227</td>
<td>0.283</td>
</tr>
<tr>
<td>Antipsychotic Use</td>
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<td>0.009</td>
<td>0.259</td>
<td>0.617</td>
</tr>
<tr>
<td>Diagnosis</td>
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<td>0.113</td>
<td>3.170</td>
<td>0.093</td>
</tr>
<tr>
<td>Error</td>
<td>0.608</td>
<td>17</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>0.894</td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) R Squared = .320 (Adjusted R Squared = .040)
Table 7: Partial MANCOVA results exploring the effect of diagnosis on levels of peripheral TNFα after controlling for covariate effects; BMI, age, sex, acute cannabis use, acute tobacco use, and antipsychotic use. There were no individual covariates that contributed significantly to the result. (Created with SPSS)

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.498</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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<td>2.253</td>
<td>1.912</td>
<td>0.183</td>
</tr>
<tr>
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<td>0.788</td>
<td>0.386</td>
</tr>
<tr>
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<td>0.140</td>
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<td>0.734</td>
</tr>
<tr>
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<td>0.056</td>
<td>0.047</td>
<td>0.830</td>
</tr>
<tr>
<td>Antipsychotic</td>
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<td>0.661</td>
<td>0.561</td>
<td>0.463</td>
</tr>
<tr>
<td>Diagnosis</td>
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<td>2.532</td>
<td>2.148</td>
<td>0.159</td>
</tr>
<tr>
<td>Error</td>
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</tr>
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<td>Total</td>
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<td>Corrected Total</td>
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</tr>
</tbody>
</table>

c. R Squared = .258 (Adjusted R Squared = -.016)
3.5.1. Inflammatory Cytokine Ratio

There were no differences in ratio of proinflammatory to anti-inflammatory cytokines between groups (CTL, CHR, early stage SCZ) when the first and validation cohorts were combined (F(2,69) = 1.40, p = 0.2534) (Figure 30).

Figure 30: Comparisons of the ratio of proinflammatory and anti-inflammatory cytokine levels between CTL, CHR and early stage SCZ groups.
4. DISCUSSION

The following section consists of portions adapted from Da Silva et al. 2018 and Wu et al. 2019 (submitted to Scientific Reports).

4.1. Mitochondrial OXPHOS Complex Expression

There were no significant differences in mitochondrial complex I–V function between CHR and CTL in the first cohort of participants. This result was successfully replicated in the validation cohort as well as when the cohorts were combined. These results differ from previously published literature that compared mitochondrial activity of individuals with SCZ to CTL (Gubert et al. 2013, Ben-Shachar et al. 1999, Dror et al. 2002). In the literature, mitochondrial complex I activity in platelets has been reported to be increased in patients in acute exacerbation of SCZ compared to CTL (Ben-Shachar et al. 1999, Dror et al. 2002) and decreased in patients in residual state SCZ (Dror et al. 2002). Additionally, a study which examined activity of mitochondrial complexes in the PBMCs of individuals with SCZ in stable chronic condition reported decreased mitochondrial complex I activity (Gubert et al. 2013). The alterations in mitochondrial electron transport chain activity seen in the literature differ from our results that show no alteration in mitochondrial electron transport chain expression in CHR or early stage SCZ groups. However, this is likely due to the difference in groups studied. The current literature related to psychosis and peripheral mitochondrial electron transport chain activity focuses on patients that have already developed psychosis or SCZ (Gubert et al. 2013, Ben-Shachar et al. 1999, Dror et al. 2002). However, the population examined in our study consists of individuals who are only at risk to develop psychosis or are only at the very early stages of disease. However, the previously mentioned studies also found no changes in mitochondrial complex IV
activity in platelets of individuals in acute exacerbation of SCZ (Ben-Shachar et al. 1999); and no changes in mitochondrial complex II and III in PBMCs of individuals with stable chronic SCZ (Gubert et al. 2013), which is consistent with our findings. Furthermore, our results are consistent with post-mortem studies that reported unaltered complex I function in the prefrontal cortex of schizophrenia patients (Andreazza et al. 2010) and no change in expression of nuclear mRNA coding for mitochondrial proteins in patients with schizophrenia (Konradi et al. 2004). As previously mentioned, CHR individuals are in the putative prodromal state of schizophrenia (Mizrahi et al. 2014) and have an estimated 30% transition risk to a full-blown psychotic episode in the first two years (Fusar-Poli et al. 2012). As a result of this, a longitudinal test following CHR individuals who progress to full psychosis and SCZ would be required to see how peripheral mitochondrial electron transport chain expression is altered during disease progression compared to CTL. This may explain the alteration in mitochondrial electron transport chain activity that is seen in the literature; and help determine if these peripheral alterations are present early on in the development of SCZ, or if the alterations are a consequence of long-term disease progression.

4.1.1. Prodromal Symptom Severity

In the first cohort, mitochondrial complex III expression in the CHR group was inversely associated with SOPS negative and SOPS total symptom severity score, suggesting that lower mitochondrial electron transport chain function is associated with greater prodromal symptom severity. In the validation cohort alone, this finding was not replicated; however, when the studies were combined, we were able to validate the negative correlation between mitochondrial complex III expression and negative prodromal symptom severity. Consistent with our results, a study reported a negative trend level association between complex I activity and negative
symptoms in patients with SCZ (Dror et al. 2002). Negative symptoms are characterized by anhedonia and avolition, which could be associated with lower energy levels caused by decreased complex expression. However, this is speculative, as we have not measured ATP levels in this study. In regards to the relationship between negative symptoms and oxidative phosphorylation, the literature is sparse; with one study reporting that coenzyme Q10, an antioxidant, is capable of reversing chronic restrain stress-induced anhedonia in rats, and another study that reported an alleviation of depression-like symptoms in behavioural tests of chronically stressed rats with the administration of coenzyme Q10 (Aboul-Fotouh 2013a, Aboul-Fotouh 2013b).

In addition to this correlation, a positive correlation between mitochondrial complex V expression and the SOPS disorganization symptoms score was found in CHR individuals when the first and validation cohorts were combined. Independently, each cohort had non-significant positive correlations, suggesting that increased mitochondrial electron transport chain expression is associated with greater disorganization symptoms in CHR. Complex V is an ATP synthase in the electron transport chain, and is responsible for creating ATP via the phosphorylation of ADP to ATP using the energy created by the proton gradient between the mitochondrial matrix and the intermembrane space (Jonckheere, Smeitink and Rodenburg 2012). Deficient complex V has been demonstrated to result in many disease symptoms including lactic acidosis, dysmorphic features, and methyl glutaconic aciduria which are present early in development (Jonckheere et al. 2012, De Meirleir et al. 2004). The literature related to mitochondrial electron transport chain function and disorganization symptoms in SCZ is scarce. To our knowledge there are no studies examining the effect of complex V dysfunction to disorganization symptoms. However, a study conducted in 2014 examined mRNA levels of mitochondrial complex I genes in different
subtypes of SCZ and found that expression of NDUFV1, NDUFV2, and NDUFS1 to be significantly increased in individuals of the disorganized schizophrenia subtype (Akarsu et al. 2014), indicating that changes in the mitochondrial electron transport chain may be associated with disorganization symptoms.

Additionally, studies have also reported positive associations between increase in complex I activity and positive symptom severity in patients with SCZ, which was not seen in our results (Dror et al. 2002, Ben-Shachar et al. 2007). However, this difference of results may be the result of differences in disease stage.

### 4.1.2. Neurocognition

In the first cohort, mitochondrial complex V expression was inversely related with RBANS attention subscale in the sample as a whole, suggesting that higher mitochondrial function is associated with poorer cognitive performance. However, we were unable to replicate the inverse relationship between complex V expression and RBANS attention subscale in either the validation cohort or when the cohorts were combined. “Mitochondria have been shown to regulate synaptic transmission, brain function and cognition (Clay et al. 2011, Picard and McEwen 2014). Thus, changes in mitochondrial [electron transport chain] expression and activity may alter ATP synthesis and consequently brain energy metabolism, which can disrupt normal brain development leading to cognitive impairments often seen in schizophrenia (Rajasekaran et al. 2015, Picard and McEwen 2014)” (Da Silva et al. 2018).

### 4.2. Levels of Peripheral Lactate and Pyruvate

There were no significant differences in peripheral lactate and pyruvate levels between CHR and CTL in the first cohort. This result was successfully replicated in the validation cohort and when
the cohorts were combined. This differs from the literature with studies reporting increased levels of lactate in serum, cerebral spinal fluid, and post mortem samples of medicated individuals with SCZ (Fukushima et al. 2014, Regenold et al. 2009, Dean et al. 2016). However other studies have suggested that the increase in lactate is associated with antipsychotic treatment rather than SCZ pathology or etiology (Halim et al. 2008, Elmorsy et al. 2016). Elmorsy et al. previously reported significant increases in blood lactate levels from baseline in individuals with SCZ 90 days after the start of antipsychotic treatment (2016). Contrary to this study, a 2019 study reported increased lactate in the dorsolateral prefrontal cortex of individuals with SCZ and abnormal lactate levels in the frontal cortex of schizophrenia mice models, both of which were not attributed to pH, age, post-mortem interval (PMI) effects, or the use of antipsychotics (Sullivan et al.).

In the first cohort, levels of peripheral lactate were positively associated with SOPS total symptom severity score and SOPS negative severity score. In the validation cohort, there were no associations between lactate or pyruvate levels and SOPS or RBANS scores. However, when the cohorts were combined, we replicated a positive association between levels of peripheral lactate and SOPS total symptom severity. This suggests that higher lactate levels are associated with greater prodromal symptom severity. This result is supported by a previous study in the literature that reported a trend level positive association between increased levels of brain \(^1\text{H}\)-MRS lactate and greater negative symptom severity in SCZ (Rowland et al. 2016). Additionally, in the validation cohort, levels of peripheral lactate were significantly associated with the RBANS total scale and RBANS immediate memory subscale in the group as a whole. These correlations were both present in the CHR group while only the correlation between lactate and RBANS immediate memory subscale was present in the CTL group. This suggests that higher
levels of peripheral lactate are associated with poorer cognitive performance. In the literature, it has been reported that increased levels of brain $^1$H-MRS lactate are associated with lower general cognitive function and lower functional capacity in SCZ and CTL groups (Rowland et al. 2016, Rowland et al. 2018), consistent with our results. Furthermore, a study examining the relationship between cerebral lactic acidosis and mitochondrial diseases reported a positive correlation between elevated lactate levels and neuropsychological symptoms in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) patients (Kaufmann et al. 2004), which is in line with our results.

The following is an excerpt from Da Silva et al. 2018 explaining the effect of tobacco use on peripheral levels of lactate and pyruvate in the first cohort. The validation cohort did not have enough tobacco users for analysis:

“In addition, we observed a significant effect of tobacco use on pyruvate and lactate levels in CHR individuals, such that CHR tobacco smokers (n = 6) had lower lactate and pyruvate levels than CHR non-smokers (n = 14). The interpretation of these results is limited by the small sample size; however, a study reported that during acute abstinence (3–6 hours), as was the case for the CHR individuals in this study, smokers had lower lactate levels compared to non-smokers (Laudon Meyer, Waldenlind and Marcus 2005).”

4.3. Levels of Peripheral Cytokines

In the first cohort, there were no differences in levels of peripheral cytokines between CHR and CTL. We were able to replicate this finding in the validation cohort as well as when the cohorts were combined after controlling for covariate effects in the analysis. In the validation cohort, there were also no differences in cytokine levels in the early stage SCZ group compared CTL
after controlling for covariate effects in the analysis. Additionally, there were no differences in inflammatory cytokine ratios between groups indicating that there are no differences in relative proinflammatory and anti-inflammatory levels between groups. This suggests that there is no increase in inflammatory activity in the early stage SCZ group or CHR group compared to CTL; however, this is exploratory as the sample size for early stage SCZ is small. This is contrary to multiple reported evidence of altered cytokine levels in SCZ. The cytokines that we studied included IFNγ, IL-10, IL-1β, IL-2, IL-6, IL-8 and TNFα. All of these cytokines have been investigated in the context of SCZ in the literature (Rodrigues-Amorim et al. 2018). INFγ, IL-6, and TNFα are cytokines that display both pro- and anti-inflammatory activity, and a 2018 systematic review found that multiple studies reported that increased levels of these cytokines play a role in SCZ and psychosis (Rodrigues-Amorim et al.). Increased levels of INFγ were associated with acute exacerbations of SCZ, increased levels of IL-6 is seen to be related to SCZ pathophysiology (Khandaker and Dantzer 2016, Mahadevan et al. 2017, Al-Asmari and Khan 2014, Kaminska et al. 2001), and increased TNFα and TNFα receptors sTNFR1 and sTNFR2 is a marker of inflammation in severe mental disorders (Mørch et al. 2016, Al-Asmari and Khan 2014). This review also reported that multiple studies examined IL-10, an anti-inflammatory cytokine, and found that levels of IL-10 have been shown to be both increased (Maes et al. 2002), and decreased in patients with chronic SCZ (Maes et al. 2002, Kaminska et al. 2001). Furthermore, IL-1β and IL-8, pro-inflammatory cytokines, have been reported to be increased in SCZ (Khandaker and Dantzer 2016, Al-Asmari and Khan 2014, Maes et al. 2002).

Further contrary to our results, a meta-analysis conducted in 2011 compiled 14 studies on blood cytokine levels in FEP individuals and reported a significant increase in IL-1β, IL-6, IL-12, IFN-γ, TNF-α, TGF-β, and sIL-2R levels compared to controls, suggesting increased peripheral
inflammation as early as FEP (Miller et al. 2011). Furthermore, a 2015 study reported elevated levels of IL-2 and IL-6 in serum of antipsychotic-naïve FEP individuals, but no change in levels of IL-10, IL-17, TGF-β2 (Petrikis et al. 2015). This indicates that altered inflammatory blood cytokine levels are present even in early stages of the disease, which we do not see in our early stage SCZ results. Furthermore, differences in serum IL-6 levels in CHR has been reported in literature, and the same study reported a trending p-value for individuals with psychotic disorders, indicating that changes in peripheral inflammation can be detected even in a putative prodromal stage (Stojanovic et al. 2014). This is contrary to our CHR results, but there were differences in the tests used for this study compared to ours that could account for the differing results. This group determined that individuals were at risk to develop psychosis using the Comprehensive Assessment of At-Risk Mental States instead of the COPS, and detected cytokine levels in serum instead of plasma, which may account for our differing results (Stojanovic et al. 2014). Furthermore, another study reported that differences in cytokine levels in combination with other analytes have been detected in CHR individuals compared to CTL.

The North American Prodrome Longitudinal Study (NAPLS), a multisite study researching the SCZ prodrome, measured 185 analytes in plasma samples from individuals at CHR in a multiplex immunoassay; and followed these individuals for two years to determine whether they converted to psychosis in that time (Perkins et al. 2014). The study aimed to create a profile of analytes that could be predictive of the risk of transition to psychosis, and this group reported that an index of 15 analytes including pro-inflammatory cytokines IL-1 β, IL-7, and IL-8 was promising in potentially discriminating subjects to either CTL, CHR with transition, or CHR without transition to psychosis (Perkins et al. 2014). This indicates that not only are there detectable changes in inflammatory cytokine levels in CHR, but that they are different enough to
be used to create a potential predictive profile to discriminate between those who will and will not convert to psychosis. However, these changes may be subtle alone and may need to be seen in the context of other blood analytes. Overall, there is data in the literature suggesting changes in inflammatory cytokines of early stage SCZ and CHR populations compared to CTL. However, we were unable to replicate this finding in our study, potentially due to differences in samples used or differences in methods of study. Additionally, in our study we were unable to make the distinction between CHR individuals that will and will not convert to psychosis; which would require a longitudinal study.

4.3.1. Relationship with Mitochondrial OXPHOS Complex Expression

In our study, we found negative correlations between expression of mitochondrial complex I and levels of IL-10, IL-8, and TNFα in the sample as a whole. These associations seemed to be driven by the CHR group, which also had correlations with IFNγ and IL-1β, suggesting that decreased complex I expression is associated with increased levels of these cytokines in CHR individuals. We also found negative correlations between complex IV and IFNγ, IL-10, IL-1β, IL-6, IL-8 and TNFα in the sample as a whole; with both CTL and CHR contributing to these correlations. All correlations were present in the CHR group aside from IL-6 and all correlations were present in the CTL group aside from IFNγ and IL-6, suggesting that decreased complex IV expression is associated with increased levels of these cytokines in both CTL and CHR individuals.

This is in line with the current literature on SCZ that indicate decreased mitochondrial electron transport chain activity and increased inflammation are related to disease pathology (Gubert et al. 2013, Ben-Shachar et al. 1999, Dror et al. 2002, Rodrigues-Amorim et al. 2018). A study on murine microglial cells reported that release of IL-1β, IL-6, IL-12 and TNFα occurred through
the increased production of mitochondrial ROS caused by the inhibition of complex I via rotenone and complex III via antimycin A (Ye et al. 2016). This suggests that the changes in inflammation seen in SCZ could be the result of changes in mitochondrial complex function, causing the release of inflammatory cytokines. However, this will need to be explored further in the context of SCZ pathology.

As previously stated, multiple reports show changes in inflammatory cytokines in SCZ that are already evident in the early stages of the disease. Our study was not able to show differences in OXPHOS complexes or inflammatory cytokines between CTL and CHR, but we were able to demonstrate that a correlation between increased inflammation and decreased mitochondrial electron transport chain function is present in CHR individuals.

4.4. Limitations

There are several limitations to this study that need to be considered when interpreting data. First, is that this study focuses on finding changes in the periphery as an indicator of a disease of the brain. Changes in the periphery may not be representative of changes in the brain because alterations in the periphery are a result of total biochemical processes in the entire body. In addition to this, mitochondrial complexes were measured in the PBMCs of patients, which may significantly differ in metabolic environment from cells of the brain. Despite this being a limitation, it is also a strength of the study; as we are able to determine that changes in mitochondrial electron transport chain expression cannot be detected in the PBMCs of CHR individuals and individuals in the early stages of SCZ, indicating that this is not a peripheral biomarker of SCZ development. A second limitation to this study is that there was a significant difference in tobacco use between CHR and healthy controls (p = 0.03). Although there was no
effect of tobacco use on mitochondrial electron transport chain function, CHR tobacco smokers (n = 6) had significantly lower peripheral lactate and pyruvate levels than CHR non-smokers (n = 14); however, this was only studied in the first cohort, and the small sample size precludes any meaningful interpretation at this stage. Third, participants were not required to fast before blood sample collection, and the effect of this on mitochondrial markers remains unknown. Fourth, the sample size of the early stage SCZ group may have been too small to detect differences between groups. Using data from the second cohort, an exploratory power calculation indicated that with the average observed effect size of 0.2; 394 individuals would need to be recruited for both CTL and early stage SCZ groups in order to detect differences in mitochondrial electron transport chain expression between the groups with a power of 0.8 and a two-tailed alpha value of 0.05. Contrarily, in a similar study looking for differences in peripheral mitochondrial function between SCZ and CTL individuals, a difference in PBMC mitochondrial function was reported with sample sizes of 30 and 18 respectively (Gubert et al. 2013). However, the participants in the Gubert et al. study were significantly older than the participants of this study; with an average length of disease of 19.9 years (2013). The participants in our study are still in the early phases of the disease, and as such, may not have reached the same levels of detectable mitochondrial dysfunction. Lastly, the clinical high risk prodromal group consists of individuals who will not experience psychosis or develop SCZ in their lifetime, and therefore are not truly in the prodromal stage of SCZ. The subset of individuals that will not go on to develop psychosis may differ biologically from those that will progress to psychosis. Speculatively, this group may even present closer to CTL individuals, which could alter results. If this were the case, it would be inappropriate to have all CHR individuals in the same group during analysis. In the literature, there is evidence of a difference in blood analyte profile between CHR individuals
who will not develop psychosis and CHR individuals that will develop psychosis (Perkins et al. 2014). At this point, we are unable to separate the CHR group into individuals who will and will not convert to psychosis and develop SCZ. A longitudinal study that follows these CHR individuals would be required to see if there is a conversion to psychosis and SCZ, and if there is a difference between those in the prodromal state and those that only present with symptoms, but do not develop psychosis.

5. FUTURE DIRECTIONS AND CONCLUSION

5.1. Future Directions

In order to better understand our results, future studies are warranted. Firstly, a new cohort of early SCZ patients should be recruited to validate the results of this study. There were 11 early stage SCZ participants in this study, which may not be sufficient in detecting differences in mitochondrial function and inflammation. Exploratory power analyses indicate that a sample size of 394 individuals per group would be required to detect differences between early stage SCZ and CTL groups with a small effect size of 0.2, which is not consistent with logistics and sample accessibility.

A longitudinal study following our CHR participants would be needed in order to determine if they convert to psychosis, and to follow their progression from CHR to FEP to progressed SCZ. This will allow for the characterization of peripheral mitochondrial function and inflammation as the disease progresses in these individuals; and help to determine when these peripheral changes begin to appear, and if the systemic manifestations are a consequence of disease progression.

Lastly, we could investigate these changes in the brain of these participants. This could be
achieved with the use of induced pluripotent stem cell (iPSC)-derived neurons created from the PBMCs of participants. Alongside the longitudinal study, iPSC-derived neurons could be created at each stage of disease that participants experience; starting from CHR to FEP to more progressed SCZ. This would allow for the investigation of mitochondrial function and inflammation that may be present in the brain of individuals at the early stages of disease development that have not yet manifested systemically.

5.2. Study Conclusions and Significance of Study

In conclusion, we were unable to detect alterations in peripheral mitochondrial function in PBMCs and plasma samples of clinical high risk or early stage schizophrenia individuals, but peripheral mitochondrial function may be associated with prodromal symptom severity; particularly negative symptoms. We were able to validate this in an independent sample set with our validation cohort, as well as in a larger sample set when the first and validation cohorts were combined.

There were no alterations detected in levels of inflammatory cytokines in the clinical high risk or early stage schizophrenia groups. However, past studies indicate that there are changes in the profile of blood analytes, including cytokines, in the clinical high risk and first episode of psychosis populations. However, we report that decreased mitochondrial electron transport chain expression is correlated to increased levels of cytokines in the clinical high risk group, indicating that greater mitochondrial dysfunction is associated with increased inflammation in disease development; supporting the hypothesis that mitochondrial dysfunction and inflammation play a role in disease development. The lack of differences in peripheral mitochondrial function and inflammation that we report between our early stage schizophrenic patients, clinical high risk
participants, and non-psychiatric controls may indicate that changes in these pathways are not yet systemic, or may only be present in the brain at these stages. Additional studies are warranted to further elucidate changes in peripheral mitochondrial systems during the prodromal phase and the start of disease development.

6. OTHER PROJECTS

During my thesis I also have been involved in other studies in the laboratory that are complementary but not directly linked to this thesis. These studies provided me with the opportunity to increase my knowledge and laboratory skills.

6.1. Bipolar Disorder and the Regulation of Mitochondrial Function by Fxr1

In addition to the study above, we explored the role of fragile X mental retardation syndrome-related protein 1 (Fxr1) on mitochondrial function in the context of bipolar disorder (BP). It has previously been reported that, the gene encoding Fxr1, FXRI, is associated with SCZ and bipolar disorder (Liu, Kelsoe and Greenwood 2016, Hauberg et al. 2016). Gene ontology overrepresentation analyses showed that the most overrepresented cellular component was the mitochondrial inner membrane, which may indicate a role of Fxr1 in mitochondrial function.

With the use of CRISPR-Cas9 Fxr1 knockout mouse Neuro2a (N2a) cells, we compared mitochondrial changes in knockout and wildtype cell lines. This is an ongoing study, and preliminary results have indicated no change in levels of mitochondrial DNA (mtDNA) content (Figure 31), levels of mtDNA oxidation (Figure 32), levels of nitric oxide (Figure 33), and levels of ROS (Figure 34) between Fxr1 knockout and wildtype cell lines (p >0.05). Our preliminary
results suggest no phenotypic alterations of the mitochondria in the Fxr1 knockout N2a cell line, however additional studies are required to draw conclusions.

**Figure 31:** Mitochondrial DNA (mtDNA) content of Fxr1 knockout (KO) Neuro2a (N2a) cell line compared to wildtype (WT) N2a cell line analyzed using real-time quantitative polymerase chain reaction (qPCR) in NADH-ubiquinone oxidoreductase chain 1 (ND1) and ND4 genes. mtDNA content was calculated with the formula $2 \times 2^{\Delta Ct}$, where $\Delta Ct = (nDNA \ average \ Ct) - (mtDNA \ average \ Ct)$. Beta-2-microglobulin (B2M) was used as the nuclear DNA control. No significant differences were reported ($p>0.05$).
**Figure 32:** Mitochondrial DNA oxidation in Fxr1 knockout (KO) Neuro2a (N2a) cell line compared to WT N2a cell line, quantified using real-time quantitative polymerase chain reaction (qPCR) in ND1 and ND4 genes. No significant differences were reported (p>0.05).
Figure 33: Relative levels of nitric oxide present in cell media supernatant of Fxr1 knockout (KO) Neuro2a (N2a) cell line compared to WT N2a cell line, quantified with Griess reagent absorbance assay (540 nm). No significant differences were reported (p>0.05).
Figure 34: Concentration of reactive oxygen species present in cell cultures of Fxr1 knockout (KO) Neuro2a (N2a) cell line compared to WT N2a cell line, quantified with 2’,7’-dichlorofluorescin diacetate (DCFH-DA) absorbance assay. No significant differences were reported (p>0.05).
REFERENCES

--- (2013b) Coenzyme Q10 displays antidepressant-like activity with reduction of hippocampal oxidative/nitrosative DNA damage in chronically stressed rats. *Pharmacol Biochem Behav, 104*, 105-12.


Sokolov, B. P. (1998) Expression of NMDAR1, GluR1, GluR7, and KA1 glutamate receptor mRNAs is decreased in frontal cortex of "neuroleptic-free" schizophrenics: evidence on reversible up-regulation by typical neuroleptics. J Neurochem, 71, 2454-64.


