MENTAL HEALTH, STRESS, AND METABOLOMICS IN INDIVIDUALS WITH PHYSICAL DISABILITIES: THE IMPACT OF A CARETAKER AND SPASTICITY

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Bachelor of Science, University of Lethbridge, 2023

A thesis submitted
in partial fulfillment of the requirements of the degree for

MASTERS OF SCIENCE
in
NEUROSCIENCE

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

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Date of Defense: August 13th, 2024

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Dedication

I want to dedicate this MSc. Thesis to my brother Parker, who has been my best friend throughout life, my motivation for everything and the strongest person I have ever met.

Abstract

Individuals who are limited to a wheelchair because of a physical disability (PDis) face unique daily stressors in their lives. It is not well understood how a PDis impacts an individual's stress vulnerability and mental/ metabolic health, as well as the social repercussions it may create. This thesis investigated (1) how living with a caretaker impacts stress vulnerability, mental health, perceived social support and metabolism of those with a PDis relative to individuals who have no access to a caretaker and (2) how spastic cerebral palsy differs from non-spastic PDis regarding stress vulnerability, mental health, and if metabolic changes reflect the psychological wellbeing. The analysis of questionnaire data and urine samples has been used to quantify and conceptualize the unique mental and biological differences within the PDis population. Through the comparison of questionnaire scores, it was determined if stress vulnerability, mental health, and perceived social support differ between the groups. Nuclear magnetic resonance spectra analysis identified specific metabolites, and then statistical inference techniques were used to identify potential biochemical pathway alterations. These metabolites were also correlated with specific questionnaire scores, showing unique correlations within each group. Through the questionnaire and metabolomic analyses, a better understanding of the quality of life among individuals living with a PDis population can guide future strategies. This could be directed towards effective treatments or interventions that could be used to ensure that those who live with PDis are getting the proper support, are becoming less vulnerable, and receiving the best possible health care to live better lives where their mental and biological differences are being understood and accounted for.

Ethics Statement

All data collection and recruitment methods were approved by the ethics committee from the University of Alberta, (Mental Health and Metabolomic Biomarkers in Individuals with Physical Disabilities, July 6th, 2022, Pro00120950) and Fanshawe College (Mental Health and Metabolomic Biomarkers in Individuals with Physical Disabilities, June 27th, 2023, Pro2306121).

Use of Generative AI

The generative AI software Grammarly was used to assist with spelling, grammar, and sentence rewriting. The AI search engine Consensus was also used to search for scholarly articles.

Contributions of Authors

The following authors' contributions and assistance with this manuscript-based thesis:

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The authors would also like to thank: Vince Weiler (MSc, Magnetic Resonance Technician, Faculty of Arts and Science) for his assistance with the Nuclear Magnetic Resonance machine and spectra acquisition; Michael Veltri (BSc, Student, Department of Neuroscience and Department of Biochemistry) for assistance with sample preparation and metabolomics analysis; Nasrin Soltanpour (BSc, Lab Technician, Department of Neuroscience) for assistance with sample preparation and transportation.

Grants from NSERC and CIJR supported this research. C.P. was supported by the Alberta Innovates Research award, SGS Fellowship award, Dr. Gary McPherson Leadership Scholarship, and CGS-M scholarship.

Acknowledgements

I want to acknowledge Dr. Gerlinde Metz for her willingness to take me into the lab during my undergraduate degree, for her infinite support, and for granting me the freedom and trust to begin a research project unique to my interests. I extend this to Dr. Jamshid Faraji for teaching me the foundation of academic writing and his assistance with data transportation across Alberta. I extend this to Tony Montina for taking me on within his metabolomics lab and for his effort, support and encouragement in this thesis's metabolomics data analysis portion. I extend this to those within the metabolomics lab, Michael Veltri, Carter Witbeck, and Thomas Kenny, for their camaraderie, assistance and support with any difficulties or questions I had throughout this thesis. Lastly, I am grateful for the neverending support and encouragement from my family and fiancé, Ryleigh.

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List of Abbreviations

AMC	Arthrogryposis multiplex congenita
AUC	Area under the curve
BCAA	Branched-chain amino acid
BRS	Brief Resilience Scale
CP	Cerebral palsy
CYP1A2	Cytochrome P450 enzyme
DMG	Dimethylglycine
GAD	Generalized Anxiety Disorder questionnaire
HMDB	Human Metabolome Database
ICU	Intensive Care Unit
MAO	Monoamine Oxidase
MDD	Major depressive disorder
MDI	Major Depression Inventory questionnaire
MW	Wilcoxon-Mann-Whitney U Test
NMR	Nuclear Magnetic resonance
OPLS-DA	Orthogonal Projection to Latent Structures—Discriminant Analysis
PDis	Physical disability
PHQ	Patient Health Questionnaire
PSS	Perceived Stress Scale
ROC	Receiver Operator Characteristic curve
SCI	Spinal cord injury
SMA	Spinal muscular atrophy
SSQ	Social Support Questionnaire
TSP	Trimethylsilyl propanoic acid
VIAVC	Variable Importance Analysis based on random Variable Combination

Chapter 1: Introduction

1.1 Introduction

There are 2.7 million individuals in the USA who have a physical disability (PDis) that limits them to a wheelchair (Koontz et al., 2015) and around 3.8 million individuals in Canada who have some form of mobility disability (StatCan, 2022). However, there is still uncertainty regarding the emotional, social and biological impact of being limited to a wheelchair. Therefore, individuals with a PDis may have more difficulty creating social bonds or being socially accepted, limiting their ability to create robust social support systems. This lack of proper social support can lead to adverse mental health outcomes and an increase in perceived stress (Blebu et al., 2022; Muñoz-Bermejo et al., 2020; Wickramaratne et al., 2022). Moreover, the sedentary lifestyle associated with wheelchair use may further exacerbate both biological and mental health concerns. Studies suggest that as physical activity levels decrease, adolescents tend to experience more mental health complications (Rodriguez-Ayllon et al., 2019). As a result, children and adolescents with PDis who engage in minimal physical activity are at heightened risk (Cribb et al., 2023). Additionally, research indicates that individuals who lead sedentary lifestyles despite being able to walk suffer the most. Given the highly sedentary lifestyle of PDis individuals, it stands to reason that they may experience these biological repercussions to an even greater extent. These biological repercussions can be measured via metabolomics, which is the study of the small molecules (or metabolites) that are involved in almost all biochemical functions of the body; however, very few metabolomics studies have been carried out on individuals with PDis. Before attempting to fill these gaps in the literature, we first need to consider the nature of physical impairments among individuals living with PDis.

1.2 Types of Physical Disabilities

PDis can be manifested through several different arrangements such as the degree of impairment, duration, temporal (progression/ regression/ permanent), congenital/ acquired and contextual factors such as age, sex or other conditions/ comorbidities. These different factors of a PDis may have implications on how an individual can navigate through daily life and how they perceive or adapt to their PDis. Through these manifestations of PDis, specific PDis can be classified, with the addition of analysis of physical symptoms, genetic factors, or physical damage to the brain or spine.

1.2.1 Cerebral Palsy

Cerebral palsy (CP) comprises a group of heterogeneous neurodevelopmental disorders functionally characterized by limitations in an individual's motor control capabilities. These limitations are reflected in postural difficulties, increased muscle tone, spastic/dyskinetic syndromes, and overall motor skill deficits (Gulati & Sondhi, 2018). The motor limitations are seen in limbs, joint contracture, speech, salivary glands, digestion and hearing/ sight issues, limiting these individuals' communication, movement and everyday tasks (Vitrikas et al., 2020). CP is the most significant cause of PDis in children, affecting around ~2/1000 children (Kolman, 2004; Van Naarden Braun et al., 2016), and has been estimated to cost 1 million USD per individual lifetime (Kancherla et al., 2012), costing the US 8.2 billion USD in 2002 (Koman et al., 2003). CP diagnosis is divided into three main types: ataxic, dyskinetic and spastic.

Spastic CP is characterized by stiff muscles and a lack of control when movements are induced. Spasticity is a product of antagonistic muscle incoordination. Spastic CP is then divided into subgroups based on the specific symptoms affecting the limbs. Diplegia (legs more affected than arms), hemiplegia (one arm and one leg affected, other leg and arm not as affected), double hemiplegia (arms more affected than legs), and quadriplegia (all limbs affected) (Kolman, 2004). Dyskinetic CP is characterized by uncontrollable movements and stiff limbs when voluntary movement is attempted. Ataxic CP is expressed via poor balance and coordination. The most significant indication of ataxic CP is the extensive delay of motor skill milestones. Mixed CP combines two or three of the previously mentioned types (Gulati & Sondhi, 2018).

1.2.2 Other Types of Physical Disabilities

Arthrogryposis multiplex congenita (AMC) is a birth disorder resulting in joint contractures, which limits an individual's ability to bend joints or may lock them in a singular position entirely. It is not primarily genetic but mainly due to a lack of movement within the womb (fetal akinesia) (Langston & Chu, 2020). Individuals with AMC rarely show spasticity, but joints may be contractured, affecting various joints simultaneously or just a single joint. Due to the nature of the joint contracture, the muscles adjacent to the impacted joint see limited hypertrophy dependent on the contracture level. This differs from CP joint contractures, where the contracture often occurs due to one spastic muscle being stronger than the antagonistic muscle (hypertonic spasticity), showing that muscle hypertrophy can still occur within these adjacent muscles (Hoffer et al., 1987).

Strokes are produced via damage caused to the brain due to lack of blood or an overexposure of blood. There are two forms of strokes: ischemic and hemorrhagic. Hemorrhagic strokes occur when a blood vessel within the brain bursts, resulting in an overexposure of blood to a brain region and cerebral hypoxia in another region (Hanley et al., 2013). The increased blood within the cranium results in increased pressure and compression, and while the blood breaks down, it creates toxic effects on the brain and induces an inflammatory response, triggering the immune system (Ohashi et al., 2023; Robinson et al., 2009). Ischemic strokes are characterized by cerebral arteries clotting due to emboli formation, causing localized cerebral hypoxia, leading to tissue damage and neuronal and glial cell death. Both of these types of strokes result in impairments from physical to cognitive loss, varying in severity from mild to extreme. The precise localization, the extent of clot accumulation/hemorrhage, the time before the emboli are removed, or the duration of the hemorrhage determine the degree of functional impairment in affected brain regions (Feske, 2021). Additionally, unlike the other PDis, there have been metabolites found that relate to both ischemic and hemorrhagic stroke and stroke implications (Petersson et al., 2024)

Spinal cord injury (SCI) is an acute injury to the spinal cord that may cause damage to the afferent and efferent pathways, resulting in a range of neurological deficits. Impairments can manifest as a loss of somatosensation, muscle weakness or paralysis, and various autonomic dysfunctions (Eli et al., 2021). The region of injury plays an immense role in the level of impairment. Damage to the cervical spine typically results in quadriplegia, thoracic usually impairs the lower back and legs, and lumbar and sacral usually result in partial loss of hip and leg function (Perrouin-Verbe et al., 2021). The severity and extent of the injury typically correlate with the degree of functional impairment and can have significant consequences for an individual's mobility, sensation, and overall quality of life (Karsy & Hawryluk, 2019).

Spinal muscular atrophy (SMA) is a genetic disease that induces neuronal loss within the spinal cord due to a gene mutation within the SMN1 gene that results in the lack of survival motor neuron protein, limiting muscle recruitment and leading to muscle weakness and atrophy across the entire body (Mercuri et al., 2022). SMA is characterized by various types that are differentiated by the age of onset, severity and genetic background. These are known as type I, II, III, and IV; the lower the number, the earlier the onset and the higher the symptom severity is (Wirth, 2000). Type I onset is during infancy, with life expectancy limited to two years, all the way to type IV, whose onset usually occurs after the age of thirty and has no impact on life expectancy (Lunn & Wang, 2008). This thesis will focus on type II SMA, characterized by muscle weakness onset between six to twelve months, which is the most common form of SMA that leads to wheelchair use (Coratti et al., 2024). These individuals often experience extreme motor limitations due to their restrictions in inducing muscle hypertrophy. Individuals with SMA, however, are unlikely to have speech difficulties or any form of spasticity. Instead, they are on the opposite spectrum compared to CP individuals regarding muscle movement on a day-to-day basis. CP has extreme involuntary muscle movement, and SMA has very little.

1.3 PDis Mental Health

Living with a PDis reduces the quality of life and can add increased difficulties and stressors to daily tasks. Reasons for a reduced quality of life can range from health or medication concerns to difficulty in everyday tasks (eating, hygiene, transportation) to social exclusion. The specific mental health complications (depression and anxiety levels) among individuals with PDis are not very well documented. There is also a lack of research regarding the social well-being of those with a PDis, where social well-being is characterized as perceived social support, social acceptance and social accessibility. The literature in this field is characterized by inconsistent results and a lack of follow-up publications to conceptualize how a PDis affects an individual's mental health. Recent literature documented that 18-33% of individuals with stroke experience depression, but it is still considered an under-researched area and an undertreated symptom among individuals who have experienced a stroke (Medeiros et al., 2020). Individuals with SCI may also have an increased risk of other adverse mental health outcomes; however, one study found that over time, they adapt to their situation and see a decrease in distress as time from injury increases (Schultz et al., 2022). There is also a critical difference between stroke/SCI individuals and CP, SMA and AMC individuals when it comes to their perception of their disability. In general, individuals with stroke or SCI were once entirely able to walk for a portion of their lives and then experienced an injury that led to a PDis. This relative change in physical ability may be one reason for changes in mental health outcomes, whereas individuals with CP, SMA, and AMC were born with their condition, and developmental trajectories may have enabled appropriate mental health adaptations.

Individuals with congenital PDis provide an opportunity to track their mental health and associated quality of life throughout their developmental trajectory. For example, adolescents with CP record higher rates of mental health disorders (75% vs 54% in controls) and were 2.6 times more likely to have anxiety and 1.8 times more likely to have depression than non-CP adolescents (Cribb et al., 2023). There is a lack of adolescent mental health research on other PDis, but these findings might be transferable to SMA and AMC adolescent mental health. However, recent studies have shown that children and adolescents with neuromuscular disease were not at increased risk of adverse mental health symptoms (Gosar et al., 2021). In regards to mental health in adults with PDis, individuals with CP show a higher risk of developing anxiety and depression (Smith et al., 2019), whereas individuals with SMA and AMC have not been found to have an increased risk of mental health symptoms (Sarveswaran et al., 2023; Wohnrade et al., 2023).

The impact of the COVID-19 pandemic on PDis individuals is also a unique situation that has impacted their ability to receive healthcare, increased isolation, and negatively impacted their mental and social well-being (Lebrasseur et al., 2021). Older PDis individuals experienced increased adverse mental health outcomes and loneliness relative to those who did not have a PDis during the early portions of the COVID-19 pandemic (Steptoe & Di Gessa, 2021). PDis individuals also recorded feeling invisible regarding healthcare, homecare and access to proper resources during the pandemic (Reber et al., 2021). There is also the possibility of long and short-term implications of the pandemic on those with a PDis, both with catching COVID-19 and its social isolation, furthermore identifying the need for more inclusive healthcare measurements and emergency preparedness while also attempting to measure these long-term impacts (Kendall et al., 2020).

Based on the previous findings, mental health symptoms in PDis are still under-investigated and poorly understood. By contrast, most PDis research focuses on the biological health of these individuals (National Guideline, 2019). While this dearth of data is understandable in areas of research such as SMA based on a curable component, for individuals who suffer from stroke, SCI, CP, SMA or AMC, where the majority of chronic symptoms are not curable, a better understanding of their mental health symptoms and risks is warranted.

1.3.1 Perceived Stress in PDis Conditions

Personal risk factors, such as stress, arguably represent significant determinants of mental health outcomes. There are unique stressors to individuals with a PDis, such as the availability of a caretaker, the caretaker's personality, medication, comorbidities, lifestyle restrictions, sedentary behaviour, surgeries, transportation, and accessibility. Not only may PDis individuals experience more stressors than a mobile person from day to day, but they may also be more vulnerable to these stressors (Bax, 1980). Given the limited existing data on the quality and quantity of perceived stress among individuals with PDis, it remains to be determined if stress can affect psychosomatic, biological and psychological functions. Of the limited PDis stress research, a previous study has shown that higher perceived stress in individuals with PDis significantly impacts physiological and cellular functions, including shortening the cells' telomere length, which is a robust marker of biological age (Lahav et al., 2022). Striking gaps in the literature about the psychological and biological consequences of PDis and perceived stress in this population highlights the urgent medical need for studies focusing on stress susceptibility and resilience to develop effective interventions, such as social support strategies.

1.4 The Caretaker-Patient Dynamic

The patient-caretaker dynamic may involve family and non-familial caretakers, who may experience significant stress and emotional burdens. Most research regarding the patient-caretaker relationship has been directed toward the caretaker's mental health, stress, social support and perspective (Stratton et al., 2014). On the other hand, very few studies have considered the patient's stress and emotional burden or confirmed that the availability of a caretaker has a positive psychological impact on patients. The family aspect, cognitive and physical capabilities and overall workload of the patient can dictate the quality of the relationship with their caretakers. The lack of data in this realm critically impacts how healthcare personnel and policymakers perceive this relationship. This thesis will address this gap by investigating a caretaker's impact on a patient's perceived stress and mental health outcomes.

1.4.1 Caretakers

Caretakers, also referenced as caregivers, are expected to provide primarily physical support to individuals with a PDis; however, human interaction arguably also provides emotional and social support. For individuals with PDis, caretaker support ranges from assistance with most activities to only a few. Assistance may involve using the bathroom, personal hygiene, transporting to/from a chair or bed, eating/preparing food, dressing and undressing, chores, household maintenance, day planning and more. Caretakers may be either family or non-family members. The emotional strain of being a caretaker has been widely recognized (Harrison, 2021), and it may negatively impact a caretaker's mental health (Malm-Buatsi et al., 2015). Previous research indicated that caretakers of children with PDis might experience increased perceived stress and adverse mental health symptoms (Malm-Buatsi et al., 2015). These observations are also made among unpaid caregivers, such as family members (Foster & Elntib, 2020). About 31% of caretakers additionally show signs of clinical depression (Scherer et al., 2019). In general, caretakers will require more robust social support systems (Namasaba et al., 2022) and are subject to compassion fatigue, becoming overworked and garnering empathy fatigue with their assigned patients (Harrison, 2021). Notably, the current literature highlights a striking gap in research on how the caretakers themselves and their mental health impact the individuals with PDis that they care for.

1.4.2 Individuals with a PDis Living with a Caretaker

While the caretakers' stresses have been well recognized, the client's side of this dynamic is poorly understood. Individuals who rely on a full-time caretaker may have more severe physical or cognitive limitations. Individuals with a PDis living with a caretaker do so because of physical limitations. Previous research described satisfaction in individuals with autism with their caretakers, yet this study did not consider potential mental health impacts (Song et al., 2022). Further research showed a positive correlation between caretaker mental health and adolescent mental health, which suggests that caretakers indeed have a mental health impact on their cared-for individuals (Stratton et al., 2014). To further understand the relationship between a person with a PDis and their caretaker, it is essential to consider the approach to how caretakers get assigned.

In some cases, a caretaker is a family member, while in other cases, within Canada, a caretaker can be assigned via a government program (AHS, 2024), a government caretaker agency (Canada, 2024) or a third party caretaker agency (Options, 2024). These live-in care workers allocate and alternate jobs between other caretakers, such as overnight care, community support, personal care, and respite care. This system does not give the client much of a voice when choosing their caretaker. Furthermore, caretakers are not assigned by best fit but instead by availability. In most cases, clients cannot request a new caretaker if they do not get along. The caretaker can request a new assignment, and when a client's family is involved, they can try to request a new caretaker for this individual. Another option is to live at a care home if the individual with a PDis requires full-time care. Here, they will live with other individuals with disabilities and, once again, are non-specifically assigned caretakers. In addition, there are systemic issues with many current caretaker homecare systems, such as the lack of available caretakers and often a lack of specialization. These situations may have repercussions on both the caretaker and the client's mental health and overall wellbeing.

1.4.3 Individuals with PDis who Live Alone

Individuals with PDis may also live independently, depending on the availability of accessible housing and greater upper body movement abilities. In Canada, these individuals may still receive government grants for cleaning assistance and acquiring groceries if necessary. Very little research has focused on these individuals and how living alone impacts their mental and physical health. A few of the following studies considered the situation of independent living in non-disabled individuals; it can be assumed that those findings may be generalizable to individuals with PDis. For example, elderly individuals who live alone may establish their social support networks through alternative ways relative to elderly individuals who live with caretakers, such as via local eateries (Torres, 2019). Individuals with PDis who live alone may not be as successful in creating social networks at places like local eateries due to accessibility issues or social barriers and prejudices. Additionally, individuals who live alone are at a higher suicide risk compared to individuals who live with others (Olfson et al., 2022). Moreover, living alone may be associated with a higher risk of muscular disorders, such as sarcopenia (Yang et al., 2023), which may present further disadvantages to individuals with PDis who live by themselves.

1.5 Biomarkers of Mental Health and Stress

Biological markers provide the opportunity to objectively quantify aspects of research and diagnoses that were previously subjective and produced diagnoses with variability factors that create a lack of standardization between diagnosed individuals. Mental health biomarkers can help the individual, their family, and healthcare workers better objectively conceptualize an individual's mental health and perceived stress. Previous studies on non-physically disabled individuals have looked at specific genomic factors that increase susceptibility to depression (Tenenbaum et al., 2019) and also looked at inflammatory cytokines that have been used as biomarkers for depression (Harsanyi et al., 2022). Within anxiety research, heart rate variability, neuroimaging and genetics have been used as biological markers, showing the possibility of a heart-brain connection that may help anxiety treatment and diagnosis while relating to the physiological symptoms of stress (Tomasi et al., 2024). The physiological responses of anxiety and depression are complex and highly variable among patients (Chesnut et al., 2021), whereas stress is the response of a well-understood biochemical process known as the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a central part of the neuroendocrine system that controls reactions to stress and regulates many body processes, including digestion, the immune system, mood and emotions, and energy storage and expenditure (Herman et al., 2016). It involves three components: the hypothalamus, the pituitary gland, and the adrenal glands. Its direct role in the body's stress response makes stress biomarkers more reliable and with less variability than anxiety and depression biomarkers, while the biological mechanisms are better understood and researched (Mello et al., 2003). Of these stress biomarkers, there are hormonal ones, such as cortisol (Blair et al., 2017) and leptin (Bouillon-Minois et al., 2021), as well as neurological ones, such as glucocorticoid and mineral corticoid receptor densities in the brain (McEwen et al., 2016). The small molecule components of cellular metabolism, known as metabolites, also provide an avenue for potential biomarkers. Variations in biological activity occur in response to both internal and external factors, such as diet, activity levels, and environment, and lead to distinct metabolomic profiles among differing populations. For example, metabolomic profiles have been used to differentiate between anxiety and depression in depressed individuals (de Kluiver et al., 2021). Metabolomics has also been used better to understand the biological response to posttraumatic stress disorders (Mellon et al., 2018). As biomarker discovery develops, the data from subjective subjects can be combined with objective biomarker data and streamline and improve the process of detecting or predicting if an individual is at risk of adverse mental health outcomes and stress susceptibility.

1.6 NMR-Based Metabolomics

Metabolomics is the study of metabolism and refers to the comprehensive analysis of an organism's biological functions and dynamic regulations (Gardner et al., 2020). Through the quantitative and statistical analysis of variations in metabolite levels in biological fluids and tissues, metabolomics provides novel perspectives on the impacts of dietary constituents, pharmaceutical interventions, environmental factors and pathological conditions (Nicholson & Lindon, 2008). Nuclear Magnetic Resonance (NMR) spectroscopy is a valuable tool for identifying and quantifying metabolites within a tissue or bio-fluid, as it is a semi-automated process that involves comparing the spectra from the target sample with a comprehensive library of reference spectra derived from pure compounds with known concentrations. This methodology, known as quantitative metabolomics or targeted metabolic profiling, is increasingly applied to characterize the metabolomes of diverse biological samples (Wishart, 2008).

NMR spectra are detected via the interaction of nonzero magnetic moment (spin) of nuclei within molecules and a strong static magnetic field. In the case of 1H NMR-based metabolomics, the various hydrogen nuclei in each molecule are investigated. Since hydrogen nuclei only contain one proton, the nuclei will be referred to as "protons" from this point forward. The magnetic moment of each proton in a molecule aligns with the static magnetic field of the NMR instrument and rotates at an angular frequency that is unique to the chemical environment of each proton. A radiofrequency pulse (RF) is then utilized to move these magnetic moments into the transverse plane and out of alignment with the static field. The RF pulse is then turned off, and the protons return to alignment with the magnetic field while continuing to rotate at an angular frequency unique to the chemical environment of each proton. As a result, a current is induced in a coil (or antenna) that is detected as the signal by the NMR instrument. The detected current is encoded with the rotational information of each proton from each molecule, which results in a frequency spectrum. The observed spectral peaks provide information on the local chemical environment (PPM or frequency on the x-axis), neighbouring protons (multiplicity or J-Coupling), and concentration (area of the peak), resulting in an excellent chemical fingerprint for each unique molecule (Gronenborn & Polenova, 2022; Koutcher & Burt, 1984). Within NMR metabolomics, these spectral peaks are binned based on multiplicity (singlets, triplets, doublets) and chemical shift and the area of each bin is first compared between groups and then compared to a database to identify the metabolites. The metabolite concentrations and perturbations can reflect the impact of environmental and biopsychosocial factors on a living system (Emwas et al., 2016). Thus, metabolic changes and metabolite profiles may be robust markers of specific environmental or pathological impacts. (Steckl & Ray, 2018).

Previous research by our team has identified potential urine metabolite biomarkers for prenatal stress in children whose mothers were affected by significant stressors, such as a natural disaster (Heynen et al., 2023; Paxman et al., 2018). Moreover, urine metabolite biomarkers may identify the severity and recovery from a stroke while supplying insights into post-stroke cellular functions (Petersson et al., 2024). Additionally, our lab has identified potential blood and urinary biomarker metabolites to measure the severity and recovery following traumatic brain injury and spinal cord injury (Bykowski et al., 2021a, 2021b, 2023; Bykowski et al., 2024). Metabolism and the outliers we can discover, particularly via NMR metabolomics studies of urine, hold excellent value in biomarker discovery and understanding of biology and pathology of biopsychosocial factors (DeBerardinis & Thompson, 2012).

1.6.1 Urine Metabolomics

Urine is an optimal biofluid due to its non-invasive collection and ability to be valuable in biological information due to being a waste product. Urine has historically been a favoured biofluid in metabolomics research for several reasons. It boasts sterility, ease of acquisition in large quantities, and minimal interference from proteins or lipids. Nevertheless, its chemical intricacy poses significant challenges to complete bio-fluid analysis. As a biological waste product, urine commonly harbours metabolic remnants from diverse sources such as dietary intake, pharmaceuticals, environmental pollutants, endogenous metabolites, and microbial byproducts. Many of these constituents remain inadequately defined and comprehended. The human urine metabolome database (HMDB), along with previously published urine metabolomics research, can be utilized to mitigate these issues when interpreting our results (Bouatra et al., 2013; HMDB). Previous research on colorectal cancer has utilized a comprehensive assessment of urine using NMR and results in the identification of specific metabolites associated with diseases, such as creatinine, 4-hydroxybenzoic acid, acetone, and carnitine, among others, indicating potential pathways like pyruvate metabolism and glycolysis/gluconeogenesis being affected in conditions like colorectal cancer (Brezmes et al., 2022). Similarly, urine metabolomics has been applied to possibly diagnose diseases such as ovarian and breast cancers (Slupsky et al., 2010). These approaches to urine metabolomics could be adapted to identify biomarkers for psychiatric conditions, offering a non-invasive tool for early diagnosis and monitoring.

1.7 Objectives and Hypotheses

The primary objective of this thesis is to gain deeper insights into the PDis community by exploring facets of their mental health, perceived social support, and metabolic profiles. Understanding the heterogeneity within the PDis population is crucial, beginning with examining the impact of caretaker involvement on individuals with PDis and discerning differences between CP and other PDis subtypes.

The first study aims to elucidate emotional, social, and biological distinctions between PDis individuals living with caretakers and those living independently. It is hypothesized that PDis individuals living with a caretaker will exhibit decreased levels of adverse mental health outcomes, decreased susceptibility to stress, and increased social support compared to their counterparts who are living alone. Furthermore, PDis living arrangements are anticipated to manifest in discernible differences in metabolomic signatures, with specific metabolites being identified and some metabolites correlating with mental health symptoms and social support scores.

In the second study, the focus shifts to comparing CP individuals with other PDis subtypes, particularly emphasizing the aspect of spasticity within CP. It is hypothesized that spastic CP individuals will demonstrate similar levels of adverse mental health outcomes and stress vulnerability compared to other non-spastic PDis individuals. Additionally, compared to other PDis, CP individuals are expected to exhibit distinctive metabolomic signatures, with identified metabolites associated with CP and its characteristic spasticity relative to other PDis.

Through this thesis, we will better conceptualize the PDis population and their dynamic with caretakers vs living alone while also increasing the understanding of the difference between those with CP and those with other forms of PDis. This research will increase understanding of the mental and physical health of individuals with PDis. It will identify specific areas where support is insufficient, providing insights into how to address these gaps better. Additionally, this study will outline future directions for the treatment and support mechanisms for the PDis community, aiming to improve their overall wellbeing. The metabolomics findings would result in a better biological understanding of the PDis pollution based on objective metabolite and pathway differences between the compared group while having biomarkers that may correlate with questionnaire data to help further conceptualize treatments and lifestyle or biological differences between those with a PDis.

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Chapter 2: The Mental Health, Stress and Metabolomic Impact of Caretakers on Physically Disabled Individuals

2.1 Introduction

The caretaker-patient dynamic is a complex relationship. Those who require a caretaker, also known as a caregiver, need help with daily tasks such as cleaning, dressing, cooking, and transportation. Individuals who need full-time support rely on homecare caretakers who often live with these individuals. These caretakers may be family members, supportive roommates, or several assigned formal paid homecare caretakers who rotate shifts. Those who require full-time homecare caretakers may be elders or individuals with a disability. It has been found that 49.5% of individuals with a disability of any kind rely on either formal or informal family homecare caretakers (Del Duca et al., 2011). In North America, homecare caretakers are a significant medical expense amounting on average to about $ 12,000 USD per caretaker per year per patient (Coast et al., 1998; Curioni et al., 2023; Goossens et al., 2013). To better understand the complexity of the patient-caretaker relationship, the perspective of and benefits to the patient warrants further consideration.

In the case where an individual with a physical disability (PDis) requires a caretaker for extended periods or even full-time, it is reasonable to expect that the relationship between the patient and the caretaker has a direct emotional, social, and physical impact on both individuals. The patient-caretaker dynamic may involve family or non-familial caretakers, who may experience significant stress and emotional burdens. Most research regarding caretaker relationships has been directed toward the caretaker's mental health, stress, and social support systems (Pinquart & Sörensen, 2003). Previous studies have shown that the emotional weight that comes with being a caretaker often negatively impacts their mental health. Caretakers of individuals with PDis may perceive higher levels of distress and adverse mental health symptoms (Malm-Buatsi et al., 2015) (Foster & Elntib, 2020). Very few studies have considered the patient's stress and emotional burden or have attempted to confirm that the availability of a caretaker has a positive psychological impact on the perceived stress of the patient. Of the limited research in this area, one study showed that mental health among caretakers and their adolescent patients is positively correlated (Stratton et al., 2014). The family aspect, cognitive and physical capabilities, and overall workload relating to the patient's care can dictate the quality of the relationship between the caretakers and the patient (Del-Pino-Casado et al., 2019). The lack of data in this realm critically impacts how healthcare personnel and policymakers perceive this relationship. Additionally, a limited understanding is known of how living alone impacts individuals with PDis. Based on previous research that relates to living alone, non-disabled individuals who live alone are at a higher suicide risk compared to individuals who live with others (Olfson et al., 2022). In addition, the risk of muscular disorder (sarcopenia) increased while living alone (Yang et al., 2023). Based on the lack of literature regarding the living situation of those with PDis, understanding potential social and emotional impacts is limited.

Living with a PDis can significantly impact the quality of life, adding challenges to daily tasks and potentially leading to social exclusion (Morris, 2001). Mental health complications among individuals with PDis, such as depression and anxiety, are not extensively researched and have inconsistent findings. Recent studies indicate that 18-33% of stroke survivors experience depression, yet there is little information about its intensity and treatment (Medeiros et al., 2020). Similarly, individuals with spinal cord injury (SCI) are at risk of adverse mental health outcomes, although symptoms often improve over time (Schultz et al., 2022). The perception of an individual with PDis may differ between individuals with acquired PDis like stroke or SCI, who once had full mobility, and those with congenital conditions like cerebral palsy (CP), spinal muscular atrophy (SMA), and arthrogryposis multiplex congenita (AMC). Adolescents with CP, for instance, exhibit higher rates of adverse mental health outcomes compared to controls (Cribb et al., 2023), while research on mental health in SMA and AMC adolescents is limited. In adults, individuals with CP are at higher risk of anxiety and depression (Smith et al., 2019), whereas those with SMA and AMC do not show an increased risk of adverse mental health symptoms (Sarveswaran et al., 2023; Wohnrade et al., 2023).

Another factor within PDis mental health is how the COVID-19 pandemic impacted their ability to receive healthcare, increased isolation, and negatively impacted their mental and social well-being while also possibly increasing vulnerability to stress (Faraji & Metz, 2021; Lebrasseur et al., 2021). Older PDis individuals recorded higher adverse mental health outcomes than controls, and another study found that those with a PDis recorded feeling ignored by healthcare and homecare systems during the pandemic (Reber et al., 2021; Steptoe & Di Gessa, 2021). Among caretakers, symptoms of depression and anxiety often increased proportionally to their workload during COVID-19 (Karni-Efrati et al., 2022). It is also known that social support may play a foundational role in the mental health of individuals; however, within the disabled community, results are inconsistent (Thoits, 2011; Tough et al., 2017). Adverse mental health outcomes in PDis are underinvestigated, while the majority of research is focused on physical health (National Guideline, 2019). Understanding mental health risks is crucial due to the inherent reciprocal relationship between mental and physical health.

Personal risk factors, such as stress, arguably represent significant determinants of mental health outcomes. There are unique stressors to individuals with a PDis, such as the availability of a caretaker and caretaker's personalities, medication, comorbidities, lifestyle restrictions, sedentary behaviour, surgeries, transportation, and accessibility. Not only may PDis individuals experience more stressors than those without a PDis from day to day, but they may also be more vulnerable to these stressors (Bax, 1980). Given the limited existing data on the quality and quantity of perceived stress among individuals with PDis, the psychological, physical and psychosomatic consequences are not well researched. One study has shown that higher perceived stress in the PDis population significantly impacts physiological and cellular functions, including shortening the cells' telomere length, a robust marker of biological age (Lahav et al., 2022). Striking gaps in the literature about the psychological and biological consequences of PDis and perceived stress in this population highlights the urgent need for studies focusing on stress susceptibility to develop effective interventions, such as social support strategies and the role of the caretaker. One approach to better understanding psychosomatic impacts is studying biological data, such as metabolomics.

Metabolomics is the study of metabolism and can comprehensively analyze an organism's biological functions and regulations (Gardner et al., 2020). Metabonomics provides novel perspectives on the impacts of dietary constituents, pharmaceutical interventions, and pathological conditions through metabolite concentrations (Nicholson & Lindon, 2008). Metabolites can be measured and detected via mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy in both biofluid and tissue samples. A semi-automated process involves comparing the metabolic profile of a sample with a comprehensive library of reference spectra derived from pure compounds with known concentrations. This methodology, known as chemometrics, is increasingly applied to characterize the metabolomes of diverse biological samples and disease states (Wishart, 2008). Previous research by our team has utilized NMR metabolomics to identify unique urine metabolite profiles in children exposed to prenatal stress *in utero* due to a natural disaster (Heynen et al., 2023; Paxman et al., 2018). Urine metabolite biomarkers may also identify the recovery from a stroke while supplying insights into post-stroke cellular functions (Petersson et al., 2024). Additionally, our lab has identified potential blood and urinary biomarker metabolites to measure the severity and recovery following traumatic brain injury and SCI (Bykowski et al., 2021a, 2021b, 2023; Bykowski et al., 2024; Wanner et al., 2021). Other literature showcases how metabolomic profiles have been used to differentiate between anxiety and depression (de Kluiver et al., 2021). Thus, NMR metabolomics holds excellent value in biomarker discovery and understanding biopsychosocial factors, including adverse mental health outcomes (DeBerardinis & Thompson, 2012). Animal models of stress have also utilized metabolomics to show linkages between metabolite levels and adverse mental health outcomes (McCreary et al., 2019; Zaytsoff et al., 2019).

The purpose of the present study is to consider the emotional and biopsychosocial well-being of individuals with PDis, emphasizing the patient aspect of the patient-caretaker relationship. The study considers patients with PDis limited to a wheelchair as their primary mode of transportation. This includes individuals with spastic quadriplegia CP, AMC, type 2 SMA, ischemic stroke, and SCI. This study will utilize questionnaires and urinary metabolomics to investigate the mental state of the patient and any associated biochemical changes, respectively. We expected to find higher perceived social support and lower adverse mental health symptoms among those with PDis who live with a full-time caretaker relative to those with PDis who live alone. We also expected an inverse relationship between perceived social support and adverse mental health outcomes and that these changes are reflected in metabolomic changes.

2.2 Methods

All data collection and recruitment methods were approved by the ethics committee from the University of Alberta (July 6th, 2022, Pro00120950) and Fanshawe College (June 27th, 2023, Pro2306121).

2.2.1 Study Participants

Participant Recruitment: For this study, 21 individuals with PDis were recruited from various community organizations, including the Calgary Power Hockey League, Calgary Cerebral Palsy Association, Alberta Cerebral Palsy Sports Association, Spinal Muscular Atrophy Canada, Lethbridge Powerchair Soccer Program, Canadian Electric Wheelchair Hockey Association and via word of mouth. It is worth noting that these individuals did not regularly participate in a powerchair sport for at least six months when data was initially collected. The inclusion criterion was that individuals were limited to a wheelchair as their primary mode of transportation for at least the previous ten years.

Study Participants: PDis participants were differentiated by either living with a full-time caretaker (caretaker group) or no caretaker or roommates (alone group). The caretaker and alone groups involved twelve (female n=2, male n=10, overall mean age=34.5) and nine (female n=1, male n=8, overall mean age=32.4) individuals, respectively. PDis participants in the caretaker group had relied on full-time caretakers, whether familial or non-familial, for a minimum duration of one year. However, participants in this group showed variation in how much assistance they needed. Some individuals needed help with a few tasks such as transportation (both to and from locations, including assistance in and out of wheelchairs/ powerchairs) and food preparation or consumption, to instances where caretaker support was necessary for all activities of daily living, including eating, sleeping, transportation, and communication. Individuals in the alone group were included in this study if they had lived independently without the presence of caretakers, family members, or roommates for a minimum duration of one year.

2.2.2 Data Collection

Questionnaire data and urine samples were collected during a home visit. Data were collected between July and September in the summer of 2022 and 2023. Fasting urine was collected using a sterilized collection cup between 7 am and 10:30 am before the participants had breakfast or used the bathroom, and individuals fasted for at least 8 hours prior to urine collection. Subsequently, the cup was capped, the collection date and time were recorded, and the sample was promptly frozen at -18°C and transported within four days to the University of Lethbridge for storage in a -80°C freezer. The assessment of psychological distress and mental health utilized self-administered questionnaires. Assistance was provided as needed to complete the questionnaires by reading them out and filling out the form based on the participants' answers. Questionnaire completion took approximately 20-40 minutes, with breaks offered as needed.

2.2.3 Questionnaire Assessment

Questionnaires covered a range of domains, including demographics, analog scales, perceived stress, anxiety, depression, exercise, independent mobility, resilience and social support. The sociodemographic questionnaire captured information on age, sex, housing situation, caretaker status, education, employment, religion, the nature of PDis symptoms, and age at diagnosis. Analog scales assessed current stress levels, mental well-being, and screen time. Standardized questionnaires of stress and well-being included the following: the Perceived Stress

Scale (PSS-10) was used to measure the individual's relative recorded stress (Camargo et al., 2023) and was broken down into three sections: before, during COVID-19 lockdowns, and within the last month; the Generalized Anxiety Disorder questionnaire (GAD-7) assessed anxiety (Simon, 2021) the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2007) depression levels; the Major Depression Inventory questionnaire (MDI), which provided a more detailed exploration of depressive symptoms (Nielsen et al., 2017) the Godin Leisure-Time Exercise Questionnaire (Godin) evaluated exercise habits over a typical one-week span (Sikes et al., 2019); the Intensive Care Unit (ICU) Mobility Scale measured participants' mobility levels (Tipping et al., 2016). In addition, after the first round of data collection, the Brief Resilience Scale (BRS) for resilience (Julian et al., 2022) and the Social Support Questionnaire (SSQ-12) for social support (İnan-Budak et al., 2023) was added (n=14). SSQ scoring was then broken down into appraisal, belonging and tangible social support.

Table 1: An overview of questionnaires used for the assessment of psychological distress and mental health.

Questionnaires	Screens for	Measurement	References for Validity and Reliability
General demographics	Age, sex, housing situation, caretakers, education, work, income, religion, PDis, diagnosis, age	Collects basic information about the participants to understand the individual's situations while ensuring certain factors were controlled.	N/A
Analog scales	Stress, well-being, screen time	Measures stress levels, well-being, and screen time habits, respectively.	N/A
Perceived Stress Scale (PSS-10)	Relative stress	Assesses perceived stress levels during different periods (before COVID-19, during COVID-19, and during the previous month).	(Camargo et al., 2023)
Generalized Anxiety Disorder	Anxiety symptoms	Identifies patients potentially suffering from generalized anxiety disorder and records the severity of anxiety symptoms.	(Simon, 2021)

Questionnaire (GAD-7)			
Major Depression Inventory (MDI)	Depression symptoms	Assesses the severity of depression symptoms and provides more detailed answer choices compared to PHQ-9.	(Nielsen et al., 2017)
Patient Health Questionnaire (PHQ-9)	Depression symptoms	Screens, diagnoses, and monitors the severity of depression symptoms, including suicide ideation and functional impairment.	(Kroenke et al., 2007)
Godin Leisure-Time Exercise Questionnaire	Leisure time and exercise habits	Assesses leisure time and exercise habits based on strenuous, moderate, and light activities.	(Sikes et al., 2019)
ICU mobility scale	Mobility scale	Identifies the individual's level of mobility, chosen over other mobility scales due to increased specificity	(Tipping et al., 2016)
Brief Resilience Scale (BRS)	Resilience	Assesses individual resilience by measuring the ability to recover from stress and adversity.	(Julian et al., 2022)
Social Support Scale (SSQ-12)	Social support	It measures the perceived availability of social support, broken down into three main components: appraisal, belonging, and tangible.	(İnan-Budak et al., 2023)

2.2.4 Urine Metabolomics

Sample Preparation of Urine: A standardized procedure was followed for all 20 urine samples (Heynen et al., 2023). Each microcentrifuge tube received a mixture comprising 160 µL of buffer, 40 µL of 0.02709% w/v D_2O containing trimethylsilyl propanoic acid (TSP), and 500 µL of urine. The buffer (pH 7.4 ± 0.05) consisted of a 4:1 ratio of dibasic potassium phosphate (K_2HPO_4) to monobasic potassium phosphate (KH_2PO4) with a concentration of 0.625 M, 3.75 mM of NaN_3 as an antimicrobial agent, and 3.75 mM of potassium fluoride (KF). Following vortexing and centrifugation at 12,000 rpm at 4°C for 5 min, 550 µL of the resulting supernatant was transferred to an NMR tube.

NMR Spectroscopy: NMR spectra were acquired using a 700 MHz Bruker Avance III HD spectrometer (Bruker Ltd., Milton, ON, Canada) employing a 1-D NOESY gradient water suppression pulse sequence. Each urine sample underwent 128 scans, resulting in a total acquisition size of 128 k, a spectral window of 20.51 ppm, which was zero-filled to 256 k. Spectra were automatically phased, baseline corrected, and line-broadened by 0.3 Hz before being exported to MATLAB (MathWorks, Natick, MA, USA) for recursive segment-wise peak alignment (Veselkov et al., 2009) and subsequent binning using Dynamic Adaptive Binning followed by manual correction (Anderson et al., 2011). Data were normalized, log-transformed, and Pareto-scaled prior to modelling (Craig et al., 2006), with all spectra referenced to the TSP peak (0.00δ).

2.2.5 Data Analysis

In metabolomics analyses, the normality of data was assessed using the Shapiro-Wilk test, with the result that all bins were non-parametric. The Wilcoxon-Mann-Whitney U test (MW) was then used for non-parametric data analysis (Goodpaster et al., 2010), with Bonferroni-Holm correction applied to correct for multiple comparisons. Variable Importance Analysis based on random Variable Combination (VIAVC) identified significantly altered bins for metabolite identification (Yun et al., 2015). Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA), a supervised multivariate test (Worley & Powers, 2013), was conducted for group comparisons. Multivariate models were validated by double ten-fold cross-validation and permutation testing using 2000 permutations (Szymanska et al., 2012; Westerhuis et al., 2008). Metabolite identification utilized Chenomx 8.2 NMR suite software (Chenomx Inc., Edmonton, AB, Canada) with the Human Metabolome Database (HMDB), followed by pathway topology analysis using Metaboanalyst with human pathway library and hypergeometric test (Wishart et al., 2012; Wishart et al., 2009; Wishart et al., 2007). Subsequent analyses used SPSS, encompassing parametric and non-parametric tests for hypothesis testing, with correlations analyzed using Pearson's and Spearman's methods.

Questionnaire analysis was performed using SPSS (IBM, version 27, Armok, NY, USA), where independent t-tests combined with Levene's Test for Equality of Variances were used to find p-values when comparing means between the two groups. Correlations were analyzed using Pearson or Spearman tests based on homogeneity of variance tests. These tests were conducted across the questionnaire and metabolite data to determine correlations between metabolite levels and questionnaire scores. The relative differences between the three PSS time frames, the difference between before COVID-19 and during COVID-19 was labelled as ΔPSS1, and the difference between during COVID-19 and the last month was labelled as ΔPSS2. Bonferroni correction was conducted for the questionnaire data based on total comparisons conducted.

2.3 Results

2.3.1 PDis Patient Characteristics

The PDis patient characteristics between those who live with a caretaker (caretaker group) and those who live alone (alone group) can be seen in Table 2. This table highlights the patient information within each group, including sample size, age, sex, PDis, mean ICU mobility score, the highest level of education attained the highest level of education attained, employment, and religiousness. One individual from the alone group did not fill out the questionnaire aside from PDis, age and caretaker situation, and a different individual within the caretaker group did not supply urine.

Table 2: Patient characteristics table (n=21), displaying information for those with a PDis who live with a caretaker (caretaker group) and those with a PDis who live alone (alone group).

	Caretaker	**Alone**
n=	12	9
Age	34.5 ± 15.2	32.4 ± 6.3
Sex		
Male	10	8
Female	2	1
PDis		
AMC	0	2
CP	8	4
Stroke	1	1
SCI	1	1
SMA	2	1
ICU Mobility Score	5.2 ± 0.85	5.5 ± 0.95
Highest Level of Education		
Partial High School	1	0
Highschool Diploma	7	1
Partial Post Secondary	2	4
Post Secondary Degree	2	3
Employment Status		
Employed	4	4
Gov. Subsidy	6	3
In School	2	1
Religious	7	6

2.3.2 The Presence of a Caretaker and its Psychological Impact on the patient

Figure 1 shows the questionnaire results concerning mental health, stress, resilience, and physical activity levels. None of the questionnaire scores showed a statistically significant difference, but slight differences were observed in some areas. In the GAD questionnaire for anxiety symptoms, the caretaker group recorded a higher score than the alone group. In the PHQ and MDI questionnaires for depressive symptoms, the PHQ screened better for suicidal ideation, and MDI had more in-depth answer choices. The caretaker group recorded a higher MDI score but a lower PHQ score than the alone group. The caretaker group scored higher than the alone group regarding the PSS over the last month. The BRS for resilience was slightly higher for the alone group. The analog stress scale was an analog scale from 1-5 where the participant could indicate their recorded current perceived stress at the time of assessment, which revealed similar results for each group. The Godin score used to measure physical activity was lower for the caretaker group. The higher the score, the higher the recorded symptoms for the MDI, PHQ, GAD, PSS, and analog stress scale. The higher the BRS and Godin score, the higher the recorded resilience or physical activity.

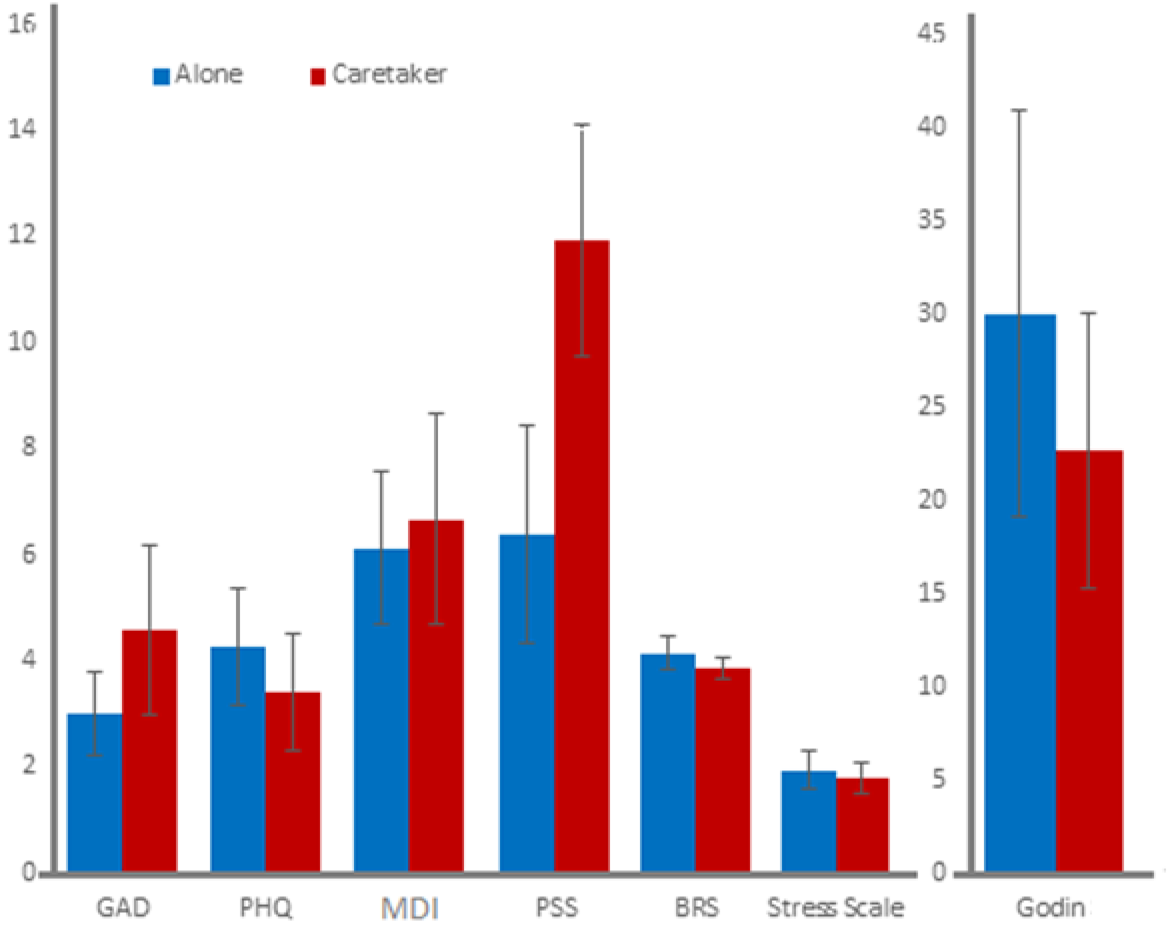

Figure 1: Mental health, stress, activity levels (n=20) and resilience scores (n=14) from indicated questionnaires. Blue bars represent the alone group, and red bars represent the caretaker group. The x-axis represents the questionnaire types, and the y-axis represents the questionnaire score. Depression symptoms were assessed through the Major Depression Inventory (MDI)/ Patient Health Questionnaire (PHQ), anxiety through the Generalized Anxiety Disorder Questionnaire (GAD), and stress-related emotions through the Perceived Stress Scale (PSS) (p=0.09)/ analog stress scale. The resilience questionnaire is the Brief Resilience Scale (BRS), and the physical activity level questionnaire is the Godin. Error bars represent the standard error of the mean.

Figure 2 showcases the SSQ scores, broken down into SSQa for appraisal, SSQb for belonging and SSQt for tangible social support. Appraisal refers to social support involving confidence building, positive feedback, and affirmation. Belonging refers to a sense of social inclusion, the perception of being part of a group or community. Tangible, or instrumental support, involves receiving concrete assistance through financial aid, services, or physical assistance. None of the results regarding SSQ scores showed a statistically significant difference between the two groups. The SSQ scores were similar across all portions of the SSQ, where SSQ total, SSQa, and SSQb all showed lower scores for the caretaker group, and SSQt showed a higher score for the caretaker group relative to the alone group. The higher the score, the greater the perceived social support.

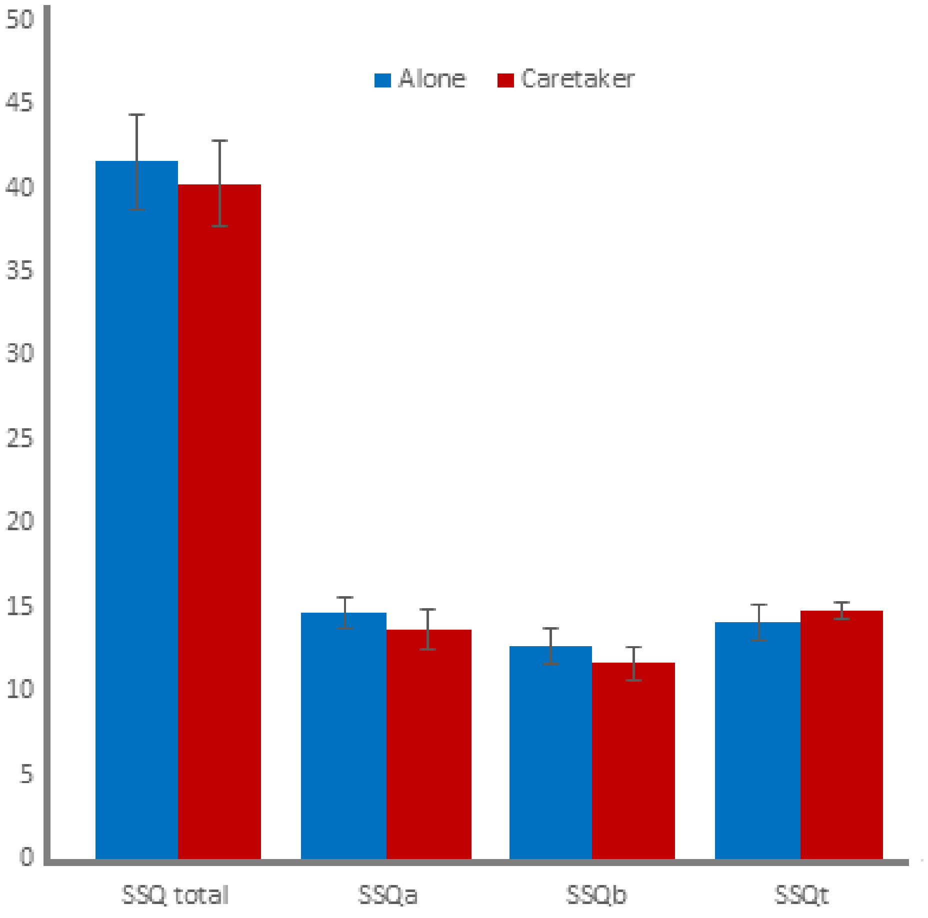

Figure 2: The questionnaire results for the SSQ section (n=14). The x-axis represents the Social Support Questionnaire (SSQ) sections, and the y-axis represents the SSQ score. The error bars represent standard error. The red represents the caretaker group, and the blue represents the alone group. SSQ total for the accumulated score, SSQa is for appraisal, SSQb is for belonging, and SSQt is for tangible.

Figure 3 showcases the retrospective PSS data regarding before, during COVID-19 and within the last month to evaluate participants' perceived stress. The thick black dotted line in Figure 3 represents the global average score of 13 for the PSS scale at a singular point in time (n=2387) (Cohen et al., 1983). None of the differences within the COVID-19 results showed statistical significance, but some trends were observed. Before COVID-19, perceived stress levels among the caretaker group were slightly below the alone group and the global average. During COVID-19, both groups saw a sharp increase in perceived stress levels. The caretaker group recorded a slightly higher score than the alone group. This increase in perceived stress from before to during COVID-19 is conceptualized in the ΔPSS1 bar graph on the right side of the figure labelled b. Perceived stress levels dropped during the period from during COVID-19 to within the last month (i.e., post-COVID-19) in both groups. The results obtained within the last month show that the caretaker group was close to the global average and similar to the levels observed before COVID-19. By contrast, the alone group dropped lower than the global average and the levels before COVID-19. This change from during COVID-19 to the levels reported last month (i.e., post-COVID-19) is conceptualized via ΔPSS2 viewed in graph 3b.

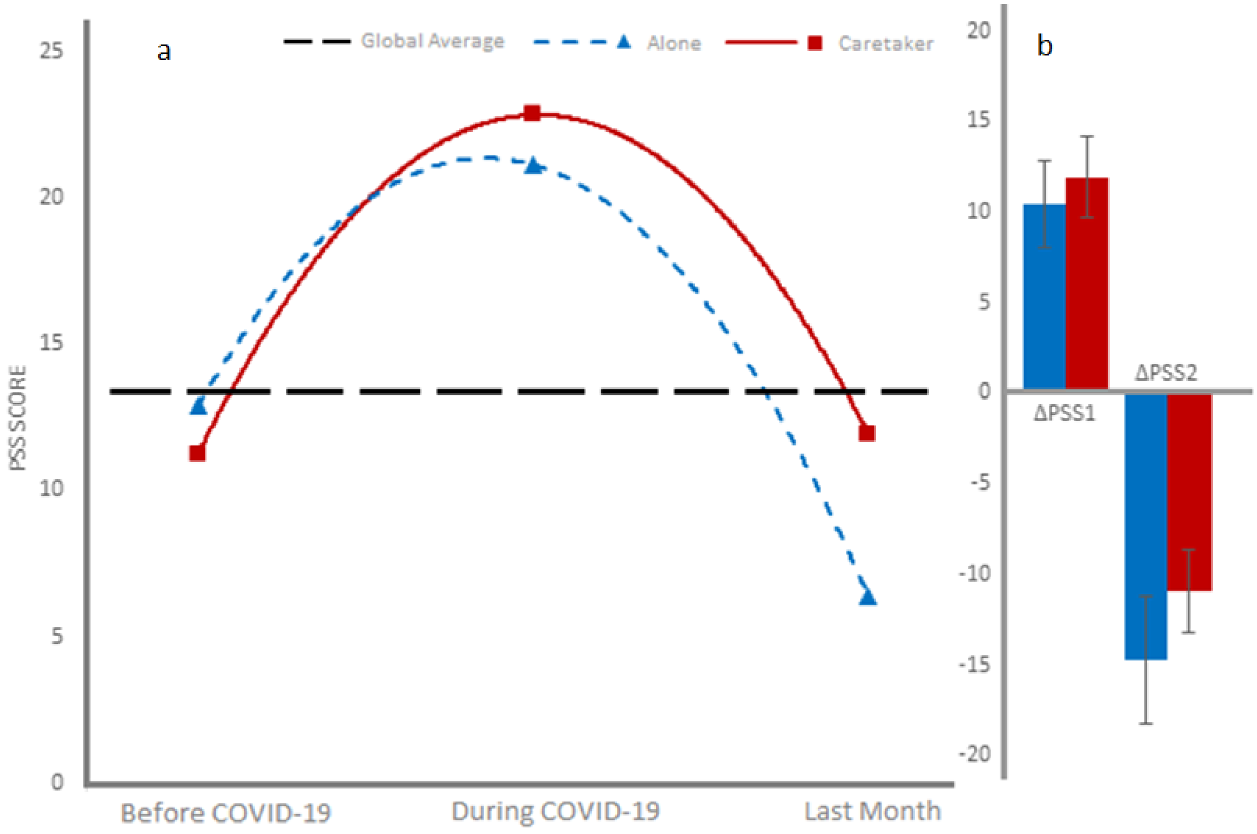

Figure 3: Retrospective Perceived Stress Scale (PSS) scores over three time points (n=20). The red solid line and squares represent the caretaker group, and the blue dotted line and triangles represent the alone group. The x-axis indicated the PSS time point inquired about in the questionnaire, and the y-axis indicated the PSS score. The error bars represent standard error. The thick black dotted line represents the global average of the PSS at a one-time point (n=2397). The mean difference between the PSS score before and during COVID-19 is represented on the right bar graph labelled ΔPSS1, and the difference between the PSS score during COVID-19 and the last month is represented by the rightmost bar graph labelled ΔPSS2.

Figure 4 illustrates a correlational analysis between different questionnaire scores in each group. Data were initially tested for homogeneity of variance to determine which correlation test to apply. All three correlations were non-parametric; therefore, Spearman's correlation was calculated. Figure 4A shows a strong negative correlation between the caretaker group's analog stress scale score and their SSQt score (Rho= -.932 and p=.002). Thus, perceived stress relating to the analog stress scale decreased as tangible social support increased. Figure 4B shows another strong negative correlation between the pre-COVID-19 PSS score and the BRS score for the caretaker group (Rho= -.973 and a p=.001). Showing that as reliance increased before COVID-19, perceived stress decreased. These correlations did not exist for the alone group. Finally, in graph C, the alone group showed a strong negative correlation between the SSQ total and PHQ scores (Rho= -.927 and p=0.003). Illustrating that as total social support levels increased, adverse depression symptoms decreased.

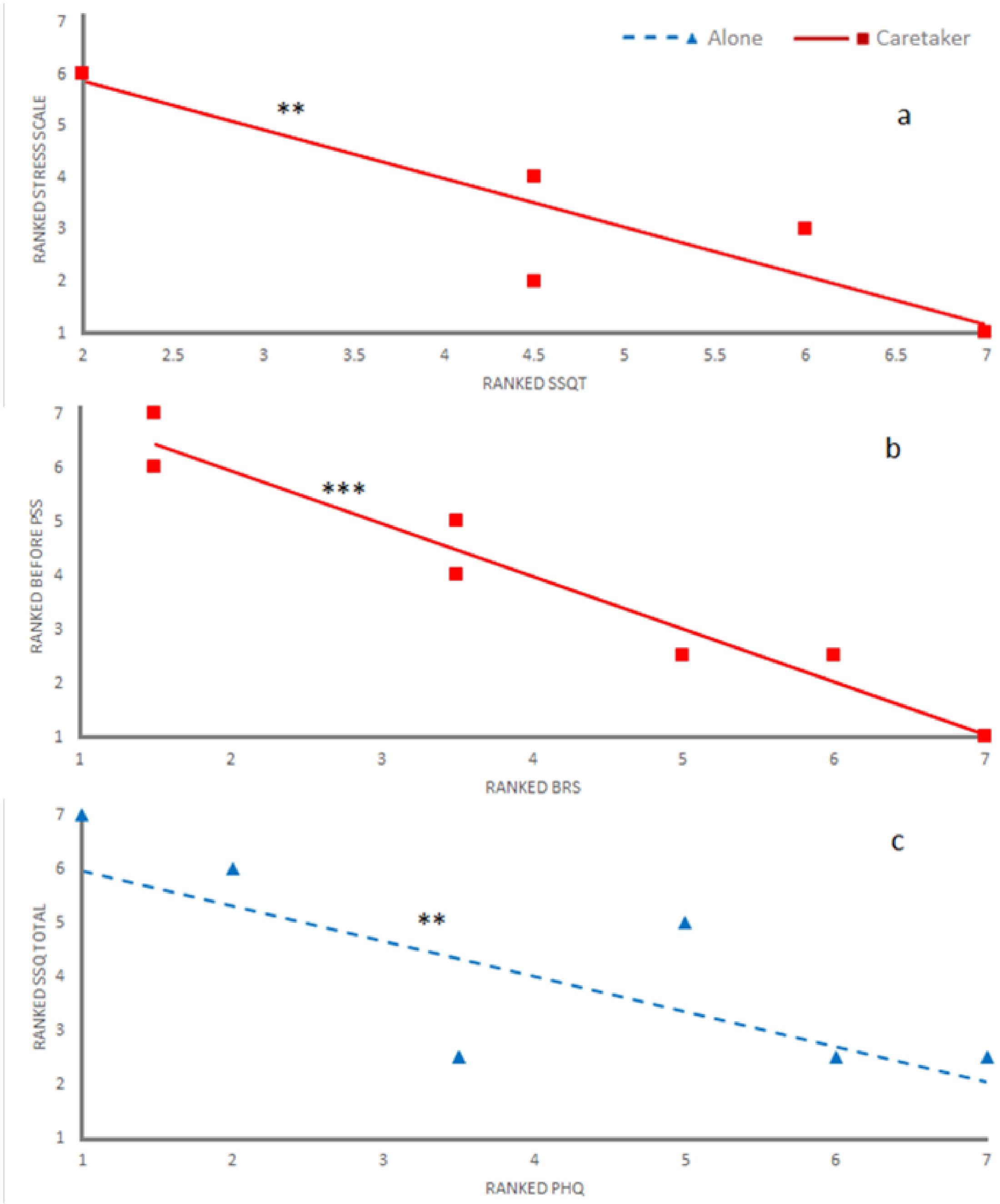

Figure 4: Correlational analysis relating scores between different questionnaires. The red solid line and squares represent the caretaker group, and the blue dotted line and triangles represent the alone group. Both axes are ranked. Graph A represents the correlation between the analog stress scale and Tangible Social Support (SSQt) results. The caretaker group recorded a Spearman Rho= -.932 and p=.002 (n=7). Graph B represents a correlation between Perceived Stress Scale (PSS) scores before COVID-19 and Brief Resilience Scale (BRS) scores. The caretaker group recorded a spearman Rho= -.973 and a p=.0002 (n=7). Graph C represents the correlation between the SSQ total and the Patient Health Questionnaire (PHQ). The alone group recorded a Rho= -.927 and p=0.003 (n=7). Both axes are ranked in descending order. Results with one asterisk (*) represent a p-value of less than 0.05, and results with two asterisks (**) represent a p-value of less than 0.005 and (***) represents the Bonferroni corrected 0.0009 p-values based on total compairisons conducted.

2.3.3 Urine Metabolome Differences Between the Caretaker and the Alone Groups

Table 3 displays the most significantly altered metabolites, their chemical shift, p-values and their regulation when comparing the caretaker group to the alone group. These 16 unique metabolites were identified out of 385 bins that were deemed significant via VIAVC or MW tests. Of these metabolites, six were found to be upregulated, and ten were found to be downregulated in the caretaker group relative to the alone group.

Table 3: Metabolites with statistically significant difference between the caretaker (n=11) and the alone group (n=9) according to the MW tests and VIAVC analysis. Metabolites are displayed in order of chemical shift. Each metabolite's corresponding chemical shift, VAIVC/ MW p-value and regulation are displayed in each respective row. The up-regulation arrow indicates positive regulation, and the down-regulation arrow indicates negative regulation when comparing the caretaker group to the alone group.

Metabolite	Chemical Shift (PPM)	VAIVC p- Value	Mann-Whitney p-Value	Regulation
Hippuric acid, Histamine	7.792	4.91E-68	6.18E-03	↑ 30.366
N-Acetyl-L-aspartic acid	4.412	5.35E-13	Not Sig.	↑ 16.588
Pseudouridine	4.297	9.28E-17	5.02E-03	↓ -17.451
Thiamine	3.873	1.30E-16	Not Sig.	↑ 23.405
Inosine	3.844	3.34E-41	5.69E-03	↓ -23.211
Guanidoacetic acid	3.758	2.57E-36	Not Sig.	↑ 14.306
Trimethylamine N-oxide, Betaine	3.258	8.13E-14	5.15E-03	↓ -34.516
L-Histidine	3.150	1.77E-04	6.44E-03	↓ -19.284
Deoxyadenosine	2.835	3.20E-15	5.06E-03	↓ -20.594
Citramalic acid	2.755	4.18E-11	4.70E-03	↓ -23.901
Aspartic acid	2.723	Not Sig.	5.67E-03	↓ -22.363
3-Hydroxymethylglutaric acid	2.467	7.09E-13	5.85E-03	↓ -11.913
4-Methylcatechol	2.193	1.40E-14	Not Sig.	↑ 18.245
L-Isoleucine	1.988	1.20E-21	Not Sig.	↓ -10.053
L-methionine	1.926	Not Sig.	5.58E-03	↑ 48.373
3-Hydroxyisovalerate	1.263	2.32E-01	6.05E-03	↓ -17.513

Figure 5 shows multivariate modelling techniques to analyze the metabolomic data across the two groups. Initially, unsupervised principal component analysis (PCA) was created via all bins, and then a supervised Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA), which also utilized all bins, failed to meet the criteria for validation during permutation and cross-validation tests. Following these findings, VIAVC bins, identified as significantly altered through multivariate analyses, were used for further modelling. Using the VIAVC bins, the supervised OPLS-DA modelling showed clear and significant separation between the groups, as illustrated in the OPLS-DA scores plot in Figure 5. Figure 6 represents the Receiver Operator Characteristic (ROC) curve that was produced to measure the model's classification and predictive accuracy, with the area under the curve (AUC), 95% confidence interval, and predictive accuracy displayed in the figure.

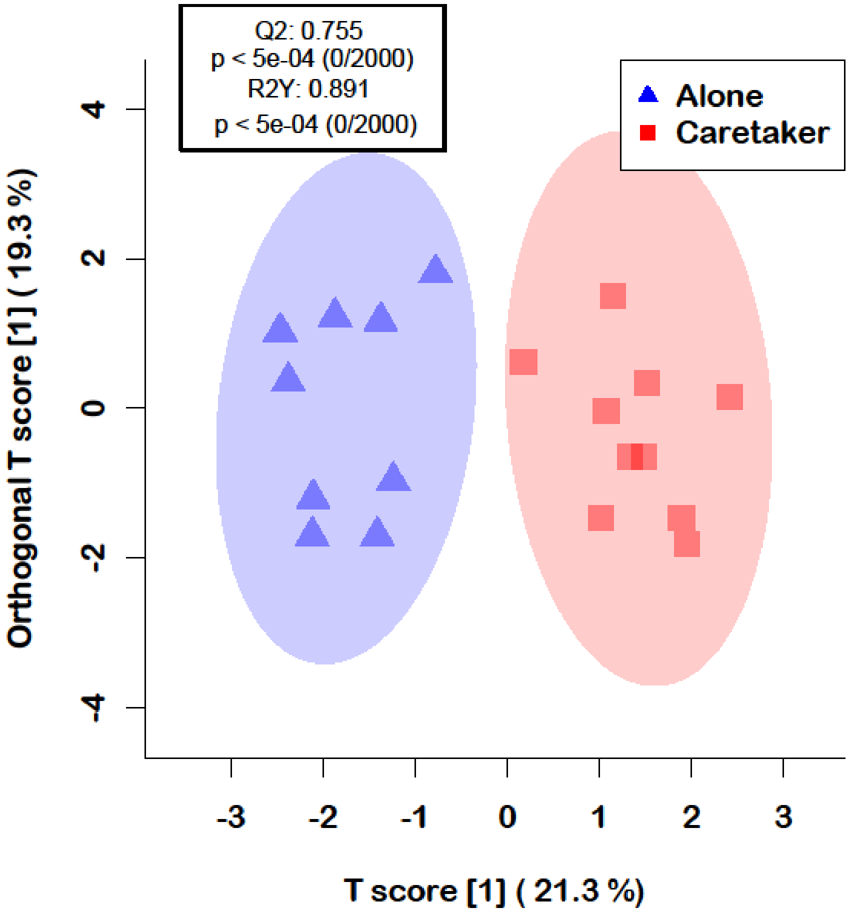

Figure 5: OPLS-DA scores plot for urine metabolites found to be statistically significant via VIAVC best subset testing. Red and squares represent the caretaker group (n=11), and blue and triangles represent the alone group (n=9). Each square/ triangle represents one participant. The shaded oval regions show the 95% confidence interval for each respective group. The x-axis represents predictive variation (between-group differences), while the y-axis indicates orthogonal variation (within-group variation). The plot also includes measures from cross-validation (Q2) and permutation (R2) testing for the OPLS-DA model.

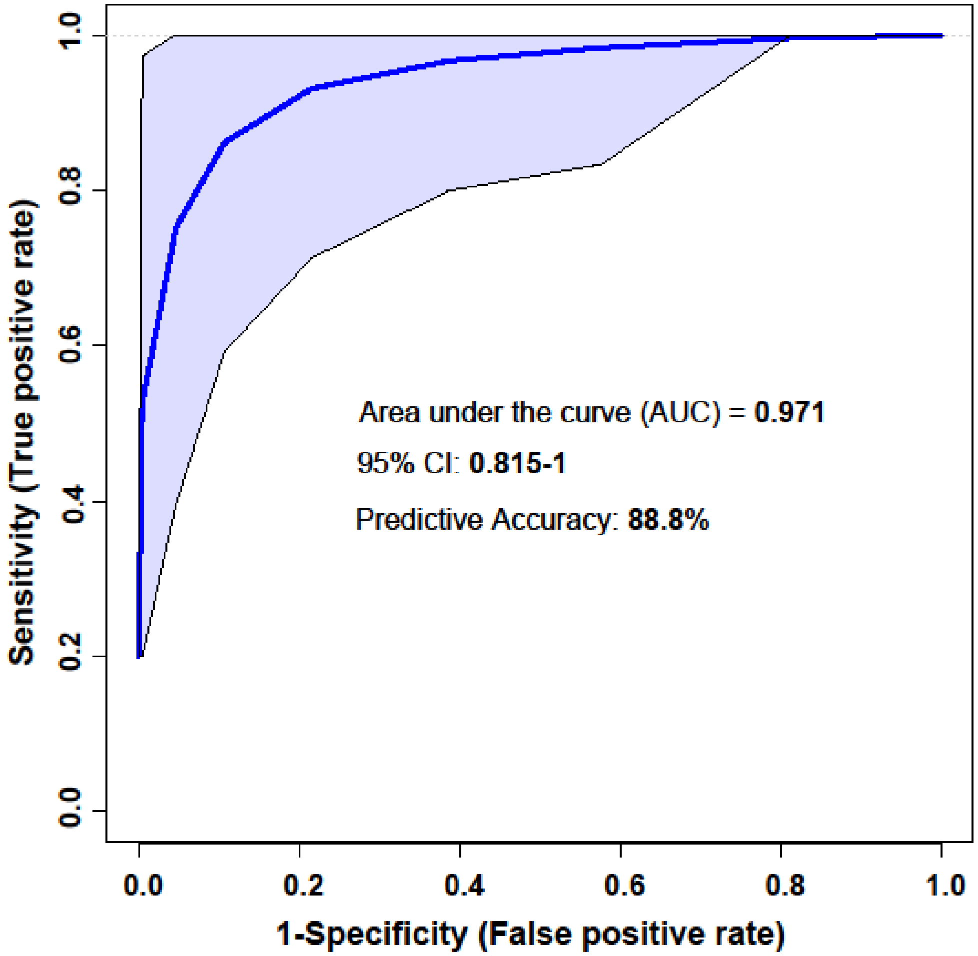

Figure 6: Reciever Operator Characteristic (ROC) curved based on the Variable Importance Analysis based on random Variable Combination (VIAVC) best subset bins. The related area under the curve (AUC), 95% confidence interval and the predictive accuracy are specified in the figure. The predictive accuracy is based on 14 VIAVC bins.

Figure 7 shows the pathway topology analysis results used to investigate potential underlying pathway disruptions that may be present based on the metabolites identified as significantly altered by either the MW or VIAVC tests. The metabolism pathways displayed in order of p-value are histidine (1), beta-alanine (2), alanine aspartate and glutamate (3), glycine, serine, threonine (4), and thiamine (5) metabolism. The metabolites identified via the VIAVC and the MW tests were used for the pathway analysis.

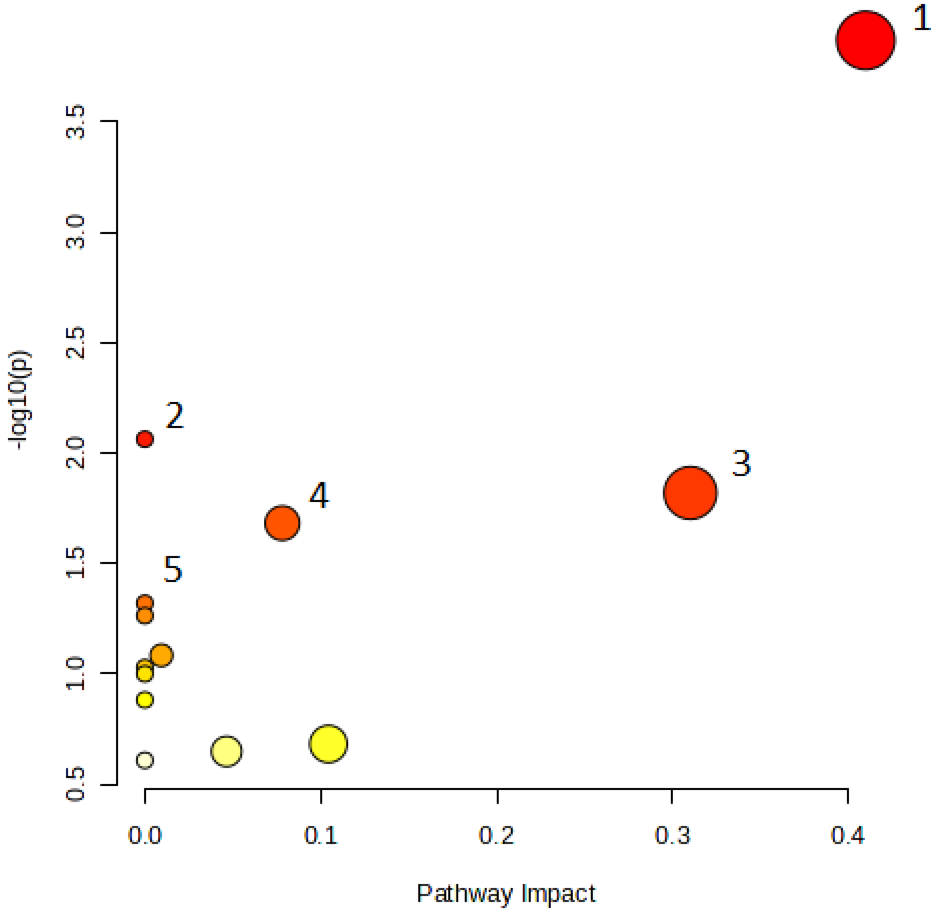

Figure 7: Metabolic Pathway Topology Analysis. The x-axis and the size of the circle represent the impact factor, which measures how the identified metabolites influence the given pathway. The y-axis and the colour represent the p-value in -log10; the higher the value, the greater the statistical significance and the darker the colour. The pathways that are less than p=.05 are labelled. 1. Histidine metabolism, p=0.0001, impact = 0.410. 2. beta-Alanine metabolism, p=0.009, impact=0. 3. Alanine, aspartate and glutamate metabolism, p=0.01, impact=0.310. 4. Glycine, serine and threonine metabolism, p=0.02, impact=0.078. 5. Thiamine metabolism, p=0.05, impact=0.

2.3.4 Metabolite Levels Reflect Perceived Stress, Mental Health and Physical Exercise Habits

Correlations between metabolite levels and the questionnaire data are presented in Figure 8, and all correlates were initially tested for homogeneity variance to determine the correlation test to apply. All data used was found to be non-parametric; therefore, the Spearman correlation was calculated for each graph. For the caretaker group, there were positive correlations between aspartic acid/ PSS (Rho=.882, p=.001) and aspartic acid/ PHQ (Rho =.812, p=0.002) and negative correlations between l-isoleucine/ Godin (Rho=-.927, p=0.002) and 3-hydroxy isovalerate/ Godin group (Rho=-.817, p=.002). These significant correlations were not found for the alone group. Thus, higher levels of aspartic acid reflected higher levels of perceived stress and depression, and lower levels of l-isoleucine and isovalerate reflected participation in fewer physical activities. A positive correlation between inosine and the analog stress scale was found for the alone group (Rho=0.933, p=0.001). This correlation was not found for the caretaker group. Thus, in the alone group, higher levels of inosine reflected higher levels of perceived stress.

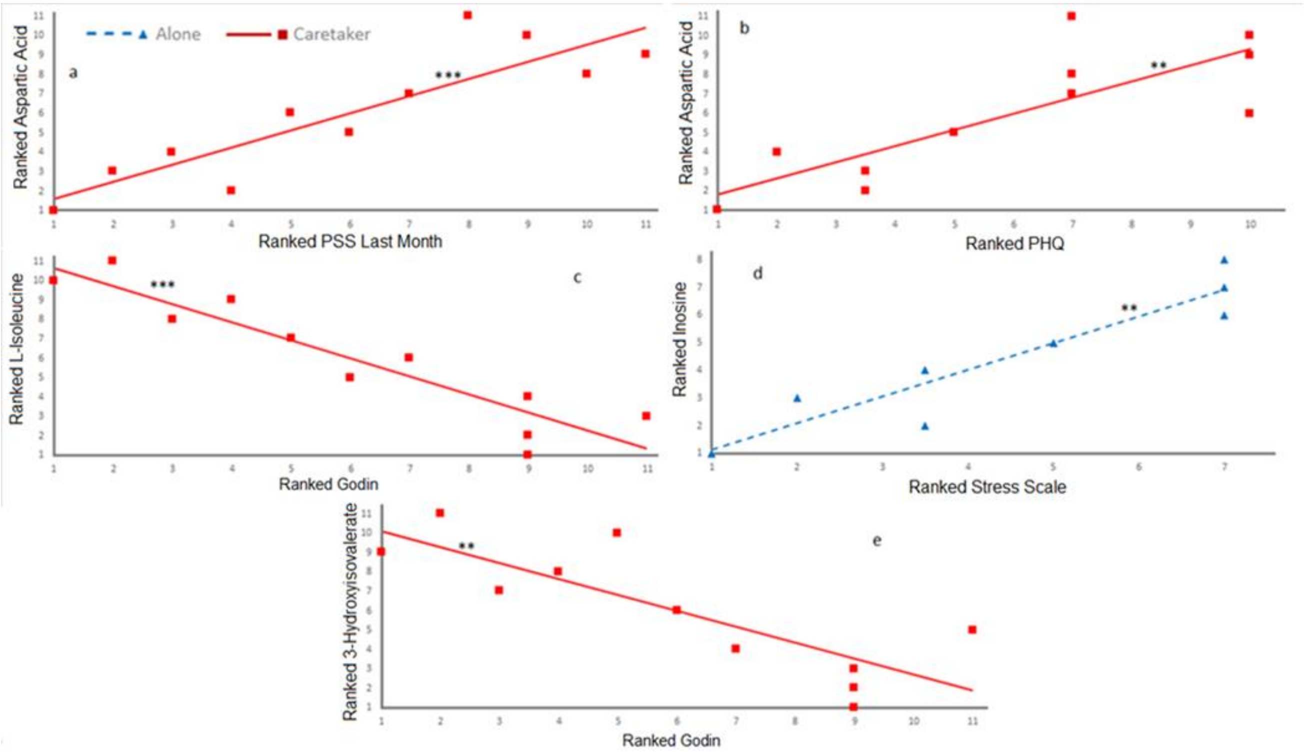

Figure 8: Correlations between metabolites and questionnaire data. The blue dotted line and triangles represent the alone group, and the red solid line and squares represent the caretaker group. All correlations were found via Spearmans due to non-parametric data. Both axes are ranked, questionnaire data on the x-axis and metabolite data on the y-axis. For graph A, aspartic acid and Perceived Stress Scale (PSS) scores were found to correlate within the caretaker group Rho=.882, p=0.0003. For graph B, aspartic acid was again found to correlate with Patient Health Questionnaire (PHQ) scores within the caretaker group Rho =.812, p=0.002. For graph C, l-isoleucine negatively correlated with Godin Leisure-Time Exercise (Godin) scores within the caretaker group Rho=-.927, p=0.00004. For graph D, inosine levels correlated with the analog stress scale core within the alone

*group Rho=0.933, p=0.0007. For graph E, 3-hydroxy isovalerate correlated with Godin scores within the caretaker group Rho=-.817, p=.002. For graphs A, B, C, and E, no significant correlations were found for the alone group; for graph D, no significant correlation was found for the caretaker group. Both axes are ranked in descending order. Asterisks represent significances: * 0.005, ** represent a p-value of less than 0.005 and *** represents the Bonferroni corrected 0.0004 p-values based on the total number of comparisons.*

2.4 Discussion

This pilot study found that those with PDis who live with a caretaker full time do not show a difference in adverse mental health outcomes, physical activity, resilience, perceived stress levels or perceived social support when compared to those with a PDis who live alone. Additionally, there were specific correlations within the psychosocial scores for each group. A urine metabolic difference was discovered between the two groups in terms of metabolite regulation differences and biochemical pathway differences. Specific correlations regarding metabolite levels and questionnaire scores were also found for each group.

2.4.1 Overview of Patient Characteristics

The demographic data indicate that the living alone and the caretaker groups were comparable in age, ICU mobility scores, and religiosity. There were, however, notable differences in the distribution of several characteristics, such as PDis among each group, sex ratio, employment status, and education levels. These demographic differences could impact the psychological and metabolomic results between the two groups, which may explain some of the differences.

Differences in PDis between the two groups were mainly due to an unequal distribution of individuals with CP: 44% of the alone group had CP versus 66% of the caretaker group. All CP individuals within this cohort had spastic quadriplegia CP, which may explain why more individuals with CP relied on caretakers. Previous studies have found a higher rate of adverse mental health outcomes in individuals with CP when compared to non-CP individuals or other types of PDis, such as SMA or AMC (Sarveswaran et al., 2023; Smith et al., 2019; Wohnrade et al., 2023). Differences in spasticity between groups may potentially affect the questionnaire and metabolomic results. These differences between spastic CP and non-spastic PDis within this cohort will be a focus of Chapter 3.

In the present participants, 11% of the living alone group and 16% of the caretaker group were female, with the study only including 14% females. The distribution of sex within PDis is dependent on the specific PDis; CP, SCI, and stroke <65 years old are more prominent within males, whereas SMA, AMC and stroke >65 years old are equal in their sex distribution (Chan et al., 2013; Gall et al., 2012; Mawaddah Ar et al., 2017; Priya et al., 2019; Tatavarti et al., 2018). This difference may originate from recruitment difficulties and the male predominance in CP, SCI and early to middle-aged stroke victims.

The differences in employment status were seen most notably in the number of individuals whose only income relied on government subsidies, which affected 38% in the alone group and 50% in the caretaker group. The amount of government subsidies varied between individuals, depending on need and place of residence. Literature is limited regarding the potential mental health impacts of reliance on government subsidy versus a regular job in individuals living with PDis. Non-PDis-based studies, however, reported that longer times of unemployment gradually diminish an individual's mental health (McKee-Ryan et al., 2005). It is unknown if this correlation also applies to those with PDis and their employment status. There is the possibility that different employment statuses may affect the psychological and metabolomic findings of the present study.

Education levels differed between the two groups, where 13% of the alone group reported reaching a high school diploma, compared to 58% of the caretaker group. In turn, the alone group included more individuals with a post-secondary degree (38%) versus only 17% of the caretaker group. Previous literature has shown that higher education levels are associated with a decrease in adverse mental health outcomes (Bjelland et al., 2008). The alone group recording higher levels of education may have implications on the metabolomic and questionnaire results relating to adverse mental health symptoms.

2.4.2 Mental Health, Stress and COVID-19 Impact

The data showed a trend of caretaker individuals recording higher scores across the PSS, GAD, and MDI and individuals who lived alone also recorded higher scores across the PHQ, Godin, and BRS. The difference between the two questionnaire screening for depressive symptoms of the MDI and PHQ highlights the complexity of living conditions' impact on mental health. The MDI is slightly broader and designed for diagnostic purposes aligned with DSM-IV and ICD-10 criteria, whereas the PHQ is more commonly used for screening and monitoring depression severity in clinical practice (Bech et al., 2001; Kroenke et al., 2001). Though these results are similar within the questionnaires, those who live with a caretaker overall appear to experience slightly higher levels of anxiety, depression, and stress compared to those who live alone. Those living alone appear to have slightly higher levels of resilience and engage in more physical activity. Demographic factors, such as higher employment rates and higher education levels among the alone group, may also account for variance in these results. Despite these observed trends, the results are not statistically significant, and these observations should be interpreted cautiously and warrant further investigation in a follow-up study with a larger patient population.

The retrospective perceived stress concerning COVID-19 also showed a similar trend as the other questionnaire results, where the caretaker group experienced a greater change in perceived stress from before to during COVID-19 and the alone group experienced a larger decrease in perceived stress from during COVID-19 to the last month timepoint. Though not significant, this trend may indicate resilience within the alone group as their recovery from COVID-19-related stress decreased more substantially relative to the caretaker group. There was also a similarity in the before-COVID-19 PSS scores between the two groups, suggesting that a change occurred during the pandemic to create this separation in the PSS score of the last month. It is possible that the caretaker group's caretakers experienced heightened stress, similar to other public health workers, and that those with a PDis who were being cared for indirectly increased their stress levels via bystander or vicarious stress effect (Bryant-Genevier et al., 2021; Guitar & Molinaro, 2017). Again, these results were not statistically significant, and the observed trends should be interpreted cautiously and warrant further investigation in a larger population of patients. Of note is the retrospective nature of these data, and it is important to consider that the results may have differed if data collection had occurred longitudinally. Further research could explore the specific factors contributing to these stress changes, such as the role of social support, coping mechanisms, and the impact of living arrangements during COVID-19.

A strong negative correlation between resilience and perceived stress before COVID-19 is expected; however, it is notable that this correlation was only found in the caretaker group. This correlation was expected due to previous literature showing that resilience can mitigate the impacts of stress (Lara-Cabrera et al., 2021). Considering the alone group's more substantial decrease in perceived stress from the pandemic and their slightly higher resilience score, the findings highlight the unique circumstances of each group's resilience and coping mechanisms.

Individuals with caretakers might develop different coping strategies closely tied to their caretakers' presence. Previous studies have shown that caretakers for stroke patients use positive reappraisal support on themselves; this may be applicable for other PDis caretakers and may be indirectly transferred to the individual with a PDis (Monteiro et al., 2018). This would also explain why the caretaker group experienced a more drastic increase in their PSS scores from before to during COVID-19 relative to the alone group. As the caretakers arguably also experienced increased perceived stress during the pandemic, it is possible that they could not provide their patients with the same level of support as before COVID-19. Accordingly, it was reported that caretakers experienced significant empathy fatigue or burnout during the COVID-19 pandemic (Ruiz-Fernández et al., 2020). Those living alone may have developed independent coping mechanisms and placed a higher importance on an alternative social network, which may have been more stable during the COVID-19 pandemic. Further research should investigate the factors influencing resilience and perceived stress regarding living situations for individuals with PDis, especially concerning high-impact situations such as global pandemics.

2.4.3 Social Support Dynamics

The social support results from this cohort suggest that individuals with PDis who live alone may perceive slightly better social support appraisal and a stronger sense of belonging than those with a caretaker; however, the perception of tangible support is slightly higher for those living with a caretaker. Despite these trends, the results were not statistically significant, as the social support differences between groups were minimal. This lack of significant difference indicates that the presence of a caretaker does not substantially alter the perception of overall social support for individuals with PDis. There is also the possibility that the caretaker does supply a substantial form of perceived social support, but the alone group's ability to create their unique social support network or other forms of social support strategies appears comparable to the social support by a caretaker. This has been observed in elders who live alone but were able to create and seek their unique social networks at local eateries as an effective buffer against loneliness (Torres, 2019). It is also possible that the questionnaire did not cover the relevant areas of social support provided by caretakers versus other social support networks.

Correlations between questionnaires also showed the differences between the caretaker and the alone groups' perceived social support. There was a strong negative correlation between tangible social support and analog stress scale scores for individuals with caretakers. This correlation, which did not exist for the alone group, shows that the individuals living with caretakers may rely on tangible support, which may reduce their stress levels. This group might value and benefit more from practical support, explaining the strong negative correlation between SSQt and the analog stress scale scores in this group. The practical support provided by caretakers might be pivotal in managing daily stressors, making tangible support a more significant factor for stress reduction in this group. The presence of this correlation shows the tangible impact of social support on those with PDis who live with a caretaker.

There was a strong negative correlation between depression scores and total social support for individuals living alone but not for the caretaker group. For individuals living alone, the sense of belonging and overall social support might be more critical in combating depression. Social support in this group may come from a broader network rather than a single caretaker, making the total social support more relevant for mitigating depressive symptoms. For example, research on elderly populations has shown that the size of a social network can help mitigate depressive symptoms (Oxman et al., 1992). Supportive relationships may strongly influence stress and resilience, while negative or less supportive relationships may not show the same beneficial effects. For those living alone, the quality and quantity of broader social interactions can be more varied. The total social support score might capture the cumulative effect of various relationships, which could be more effective in mitigating depression.

2.4.4 Metabolomic Pathway Analysis

Pathway analysis reveals specific metabolic pathways that may be differentially regulated in individuals living with caretakers from those living alone, including the histidine metabolism pathway. The upregulation of l-histidine and histamine and the downregulation of aspartic acid in the caretaker group suggest substantial metabolic adaptations. Histidine and its derivatives play critical roles in numerous physiological processes, including neurotransmission, immune response, and gastric secretion (Brosnan & Brosnan, 2020). The elevated histamine levels may indicate increased stress or inflammatory responses in the caretaker group, potentially reflecting greater psychological and physical demands on these individuals. Previous studies have linked upregulated histidine metabolism to stress and inflammatory pathways (Hirasawa, 2019; Joseph et al., 1990). Additional research has shown that dysregulation of histidine metabolism can lead to conditions like hyperhistaminemia, which is observed due to increased decarboxylation of histidine to histamine (Tanabe & Sakura, 1989). There has also been a recorded disruption of histidine within individuals with chronic obstructive pulmonary disease (Diao et al., 2019). Ten of the sixteen metabolites in the histidine pathway can be detected via NMR (Du et al., 2017); within the comparison of the caretaker group and the alone group, 30% of the detectable NMR urine metabolites within this pathway were found to have a regulation difference amongst the groups, reinforcing the impact living with a PDis has on histidine metabolism.

Pathway analysis also indicated that the alanine, aspartate and glutamate metabolism pathway might have also been affected by the presence of a caretaker. The downregulation of aspartic acid and upregulation of l-histidine in the caretaker group highlights critical differences in amino acid metabolism. Aspartic acid and glutamate are pivotal in energy production and neurotransmitter synthesis (Reitzer, 2004). The observed changes could impact the physiological stress responses and cognitive functions of individuals in the caretaker group, potentially leading to increased vulnerability to stress-related disorders (Engskog et al., 2017; Popoli et al., 2011). This pathway was also disrupted in serum analysis following SCI and traumatic brain injury patients, suggesting that this pathway may have direct, measurable effects on the nervous system and its regulatory responses to neuronal injuries (Bykowski et al., 2023; Bykowski et al., 2024). Of the twenty-eight metabolites in the pathway, fifteen of them are detectable via NMR, and 13% of these were found to be statistically different between these groups, showing that this pathway may not be as impacted but still may hold some implications regarding the living situation with a PDis (Botosoa et al., 2012; Jingjing et al., 2009; Monika et al., 2000).

Glycine, serine, and threonine metabolism showed a moderate pathway impact and statistical significance. The upregulation of betaine and guanidoacetic acid in the caretaker group suggests alterations in methylation processes and creatine synthesis (Marie Joncquel-Chevalier et al., 2015; Tachikawa et al., 2004). These metabolites are crucial for cellular energy homeostasis and neurotransmission (Marques et al., 2019). Changes in this pathway could reflect adaptations to the increased physical and mental demands experienced by individuals living with caretakers (Oudman et al., 2013). Of the thirty-three metabolites within this pathway, twenty of them are detectable via NMR, and 10% of the detectable urine NMR metabolites were found with regulation differences between the two groups, indicating that this pathway may be impacted by those with a PDis and their living conditions but much less than the other two pathways indicated thus far (Cecilia et al., 1997; Emmanuel et al., 2009).

The two other pathways of beta-alanine metabolism and thiamine metabolism were statistically significant, but they did not reveal any level of impact on their pathways. The lack of impact indicates that although these pathways have metabolite regulation differences, they do not translate into substantial biochemical effects with functional significance.

2.4.5 Correlation of Metabolites with Questionnaire Data

The relationship between metabolite levels and questionnaire scores within each group provided insights into how metabolic profiles might be associated with psychological well-being and physical activity levels. The correlation between aspartic acid and both PSS and PHQ scores within the caretaker group indicates that aspartic acid may play a critical role in the stress and depressive states of individuals with PDis who rely on caretakers. Aspartic acid is an important neurotransmitter and is involved in the urea cycle and energy production, potentially linking it to stress and mood regulation (Birken & Oldendorf, 1989). Previous research has shown that aspartic acid levels were found to be significantly lower in patients with major depressive disorder (MDD) compared to healthy controls. This reduction in aspartic acid may serve as a clinical biomarker for MDD, indicating a potential link between aspartic acid levels and depressive symptoms (Fu et al., 2012). Abnormalities in neurotransmission involving amino acids like aspartic acid have been implicated in the pathophysiology of depression. Studies suggest neurotransmitter disruptions affect mood and stress responses (Salvadore et al., 2012). The alone group did not display this correlation yet had similar PHQ/MDI scores, but this difference could be based on lower PSS scores and the complexity of metabolic responses to different living conditions. The specific nature of stressors, coping mechanisms, and metabolic pathways likely varies between those living with caretakers and those living alone; furthermore, this could diminish the direct influence of aspartic acid on stress and depressive symptoms.

Understanding these nuanced differences is crucial for developing tailored interventions that address the unique needs of individuals with PDis in varying living arrangements.

The negative correlation between the Godin score and both l-isoleucine and 3-hydroxyisovalerate within the caretaker group indicates that these metabolites may be related to physical activity levels. L-isoleucine is a branched-chain amino acid (BCAA) involved in muscle metabolism, and its reduction may reflect increased muscle utilization during physical activity (Kota et al., 2016). 3-Hydroxyisovalerate, a metabolite in the leucine degradation pathway, also showed a similar correlation and suggests that higher physical activity is linked to increased catabolism of BCAAs, possibly as an adaptive mechanism to meet energy demands (Sureda et al., 2010). Previous studies have shown that higher physical activity levels are associated with lower serum levels of isoleucine and leucine (Brodan et al., 1976; Xiaobo et al., 2017). These authors reported that these two amino acids increased significantly in individuals with low physical activity, suggesting that higher physical activity is linked to lower levels of these amino acids, potentially due to increased muscle utilization during exercise. The presence of a caretaker might facilitate higher-intensity physical activities, leading to increased muscle metabolism and energy demands. Higher overall physical activity levels recorded by the alone group might reflect a broader range of activities, some of which may not be as metabolically demanding. This variation in activity intensity could result in less pronounced metabolic changes, thus the absence of strong correlations between physical activity and BCAA levels in the alone group.

The correlation between the analog stress scale score and inosine observed for the alone group may reflect the metabolomic impacts of stress on those with PDis who live alone. Inosine is a purine nucleoside that can have neuroprotective effects and is involved in energy metabolism (Srinivasan et al., 2021). The elevated levels of inosine in response to stress might reflect a compensatory mechanism to counteract stress-induced neuronal damage. The neuroprotective effects of inosine have been shown in multiple sclerosis research, where raising serum uric acid levels via inosine administration reduced oxidative stress markers and improved clinical outcomes (Spitsin et al., 2001). In patients with chronic obstructive pulmonary disease, elevated inosine monophosphate levels were linked to disturbances in energy metabolism in muscle tissue (Pouw et al., 1998). This finding underscores the potential relationship between increased inosine levels and chronic metabolic stress. In addition, studies on ischemic conditions in both animal models and human subjects revealed that inosine levels increased significantly during reperfusion following ischemia, reflecting the body's response to acute cellular stress and damage (Fox et al., 1979). The alone group showed this correlation but not the caretaker group, even with both groups showing similar analog stress scale scores, which may be due to distinct metabolic adaptations required by their living conditions. Inosine's role in neuroprotection and energy metabolism may be particularly relevant for individuals who manage stress independently without the immediate support of a caretaker. Elevated inosine levels in response to stress reflect a compensatory mechanism that counteracts stress-induced neuronal damage and maintains metabolic balance.

2.4.6 Future Directions and Limitations

Limitations within this study include small sample size, retrospective data, data collected over two different time points, PDis distribution, and sub-optimal alignment of the two groups. This includes sub-optimal matching of participants by sex, PDis, clinical characteristics, education levels, employment, and socioeconomic status. These group differences, combined with the smaller sample size, increase variability factors within each group, expanding the possibility of confounding variables within this study. The data collection occurring over two different summers also increases possibly confounding variables within the metabolomics outcomes and makes the retrospective PSS data further from their designated time points. Another limitation of this study is the lack of direct measurement of physical activity levels, such as VO2 max, accelerometer data, or heart rate monitoring. Additionally, ICU mobility scores could have been objectively measured rather than relying solely on self-reported assessments.

The findings from this study highlight several areas for future research that could enhance the understanding of the metabolic and psychological differences in individuals with PDis based on their living arrangements. The following are several areas that future studies should consider to understand better how lived experiences with PDis affect mental and physical health trajectories. It would be necessary to better align control groups by ensuring comparable demographic and clinical characteristics. Investigating the differences between varying levels of care needed within the caretaker group could provide insights into how the intensity and type of care impact metabolic and psychological outcomes. Stratifying the caretaker group based on the level of dependency and type of support could reveal more detail about the complex relationship between care dynamics and health markers. Integrating cognitive assessments into the study design would evaluate the relationship between living situations, cognitive function, stress, and metabolic profiles. Identifying how cognitive performance correlates with metabolic changes and psychological well-being in caretaker and alone groups could further help conceptualize group differences. A larger patient population would increase the study's statistical power, allowing for more vigorous conclusions and the ability to detect smaller effect sizes. Conducting sample collections at different time points, particularly avoiding times of widespread stress, such as the end of COVID-19 lockdowns, can provide more representative baseline data. Expanding the analysis to include other biofluids, such as saliva, blood and cerebrospinal fluid, could provide a more holistic view of the metabolic changes associated with various types of PDis and the associated living situation. Investigating how different types of PDis are associated with distinct mental health profiles and metabolomic fingerprints could uncover specific biomarkers and therapeutic targets. Understanding these variations may advance personalized interventions tailored to individual needs depending on their specific PDis type and stress level.

2.5 Conclusion

This pilot study explores the complex relationships between metabolomic profiles, psychological well-being, perceived stress, perceived social support and living arrangements among individuals with PDis. Key findings indicate significant metabolic differences between individuals living with caretakers and those living alone, particularly in histidine and aspartic acid pathways. These differences suggest that the metabolic adaptations associated with different living conditions may influence stress and depressive states, especially in individuals reliant on caretakers. Despite these metabolic insights, questionnaire assessments revealed no statistically significant differences in social support, mental health and stress levels between the two groups, although trends suggest that those who live with caretakers might experience higher levels of anxiety and stress. The impact of COVID-19 on mental and physical well-being further underlines the complex interplay between living arrangements, stress susceptibility and resilience. The pathway analysis revealed possible significant impacts on several pathways, and these dysregulations can be the focus of potential targeted interventions to address the specific metabolic differences associated with those who have PDis across living situations. Correlation analyses between metabolite levels and questionnaire data provide further insights into the biochemical foundations of stress and depressive states in PDis individuals while also indicating the potential for possible biomarkers. This proof-of-principle study highlights the importance of a holistic approach to understanding the complex interactions between those with PDis who live with a caretaker versus those who live alone, as well as their psychological well-being and the metabolic impact of these experiences. If a more extensive study reinforces these findings, developing tailored interventions may address specific needs based on living situations to improve quality of life and overall health.

2.6 Supplemental Materials

Table 4: Correlations between questionnaire scores

Group	Sample Size	Questionnaire	Questionnaire	Spearman Rho	P value	Assigned Letter
Caretaker	11	SSQt	Analog Stress Scale	-0.932	0.002	a
Caretaker	11	BRS	Before COVID-19 PSS	-0.973	0.0002	b
Alone	9	PHQ	SSQ Total	-0.927	0.003	c

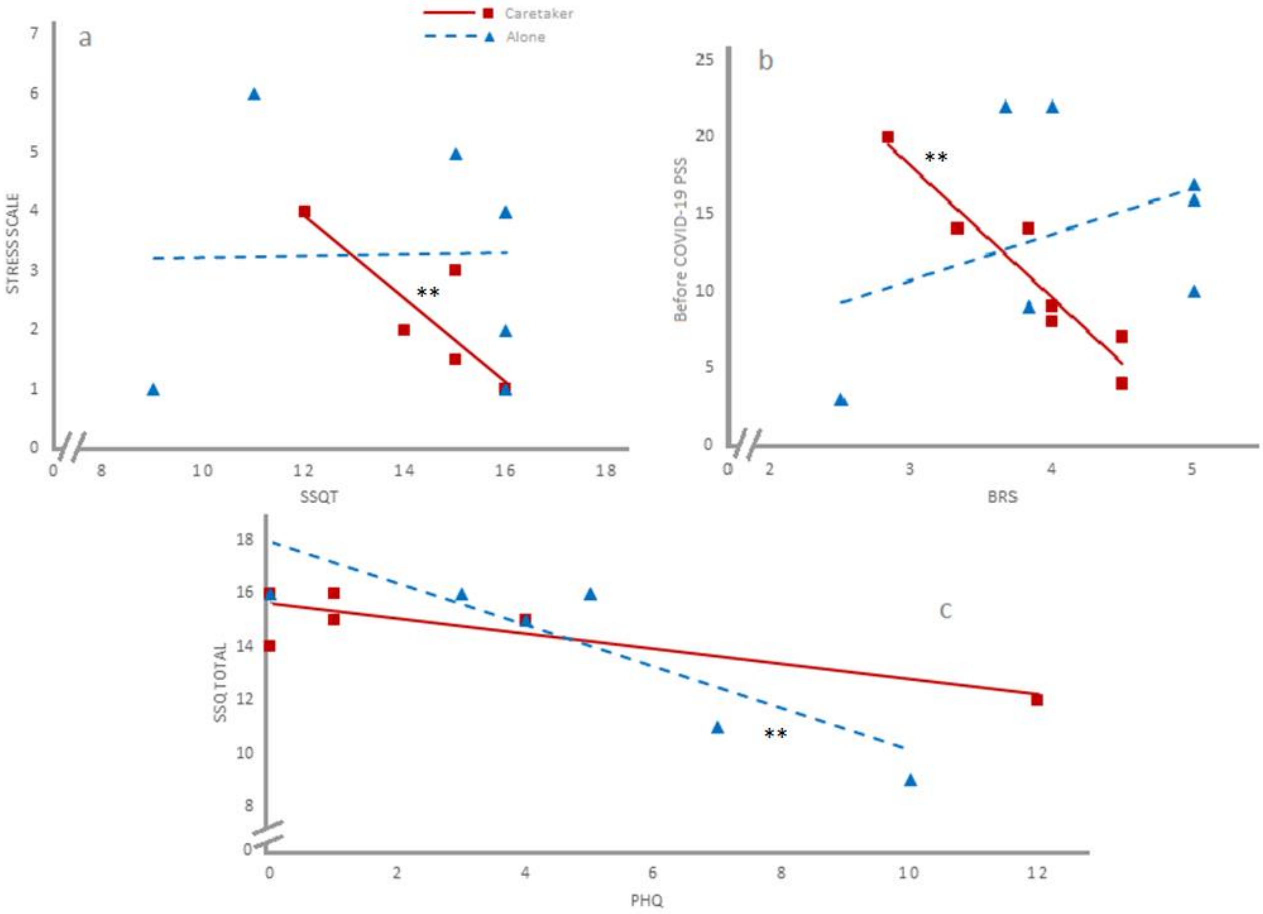

Figure 9: Correlation graphs relating scores between different questionnaires. The red solid line and squares represent the caretaker group, and the blue dotted line and triangles represent the alone group. Graph A represents the correlation between the stress scale and SSQt results for both the caretakers and the alone group. The caretaker group recorded a spearman Rho= -.932

and p=.002 (n=7). Graph B represents a correlation between PSS scores before COVID-19 and BRS scores for the alone and the caretaker groups. The caretaker group recorded a spearman Rho= -.973 and a p=.001 (n=7). Graph C represents the correlation between SSQ total and PHQ for both the caretaker and the alone group. The alone group recorded an Rho=-.927 and p=0.003 (n=7).

Table 5: Topology Pathway Analysis

Pathway	Total	Hits	P-Value	Impact	Metabolite(s)
Histidine metabolism (1)	16	3	0.000135	0.410	L-Histidine, Histamine, Aspartic acid
beta-Alanine metabolism (2)	21	2	0.00866	0.000	N-Acetyl-L-aspartic acid, Aspartic Acid
Alanine, aspartate and glutamate metabolism (3)	28	2	0.0151	0.310	Aspartate, L-Histidine
Glycine, serine and threonine metabolism (4)	33	2	0.0208	0.078	Betaine, Guanidoacetic acid
Thiamine metabolism (5)	7	1	0.0479	0.000	Thiamine
Valine, leucine and isoleucine biosynthesis (6)	8	1	0.0546	0.000	L-Isoleucine

Table 6: Correlations between questionnaire scores and metabolite levels.

Group	**Sample Size**	**Questionnaire**	**Metabolite**	**Spearman Rho**	**P value**	**Assigned Letter**
Caretaker	11	PSS	Aspartic Acid	0.882	0.0003	a
Caretaker	11	PHQ	Aspartic Acid	0.812	0.002	b
Caretaker	11	Godin	L-Isoleucine	-0.927	0.00004	c
Alone	8	Stress Scale	Inosine	0.933	0.001	d
Caretaker	11	Godin	3-Hydroxyisovalerate	-0.817	0.002	e

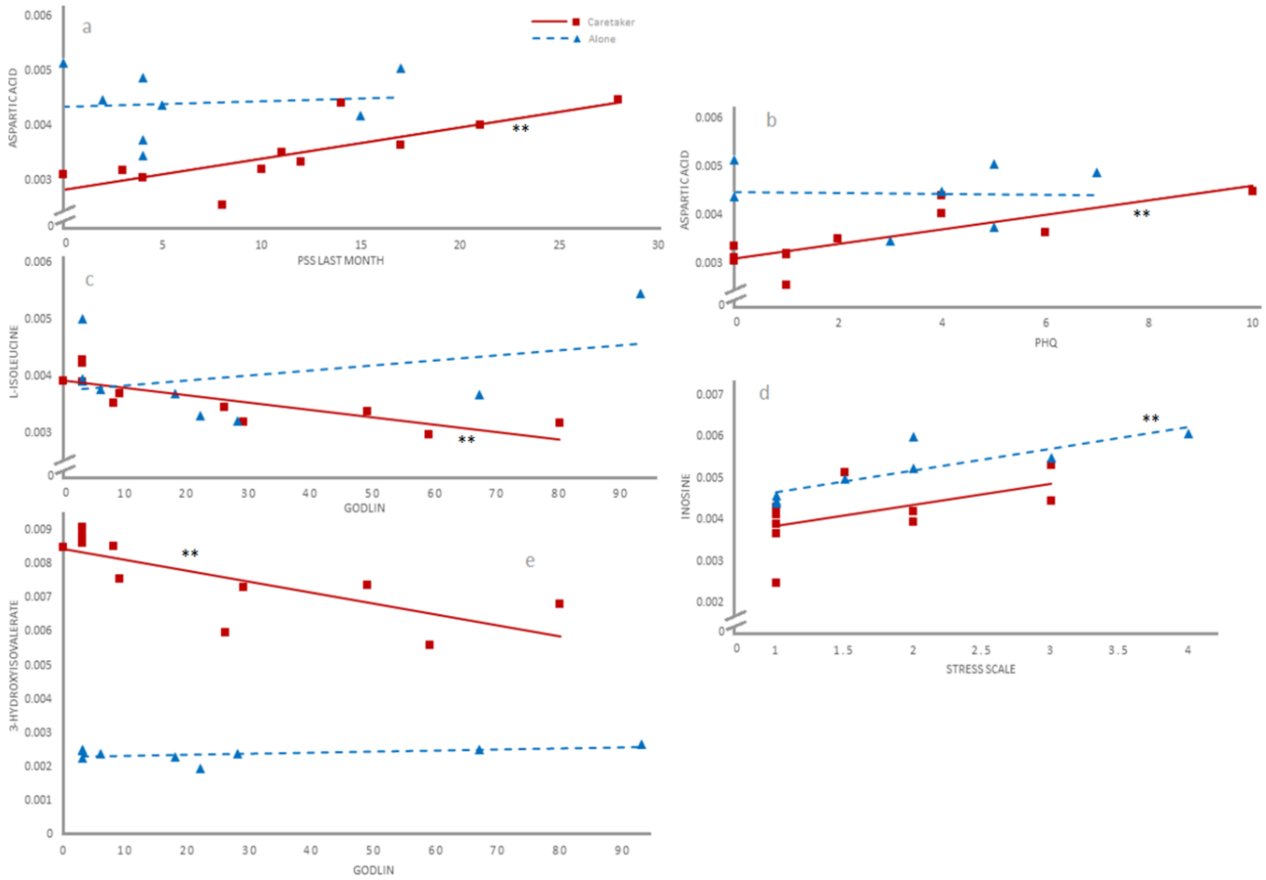

Figure 10: Correlations between metabolites and questionnaire data. The red solid line and squares represent the caretaker group, and the blue dotted line and triangles represent the alone group. All correlations were found via Spearmans due to non-parametric data. For graph A, aspartic acid and PSS scores was found to correlate within the caretaker group Rho=.882, p=0.001. For graph B, aspartic acid was again found to correlatewith PHQ scores within the caretaker group Rho=.812, p=0.002. For graph C l-isoleucine negatively correlated with godin scores within the caretaker group Rho=-.927, p=0.002. For graph d, inosine levels correlated with the stress scale core within the alone group Rho=0.933, p=0.001. For graph E, 3 hydroxy isovalerate was found to correlate with Godin scores within the caretaker group. For graphs A, B, C, E, there was no significant correlations found for the alone group and for graph D there was no significant correlatin found for the caretaker group.

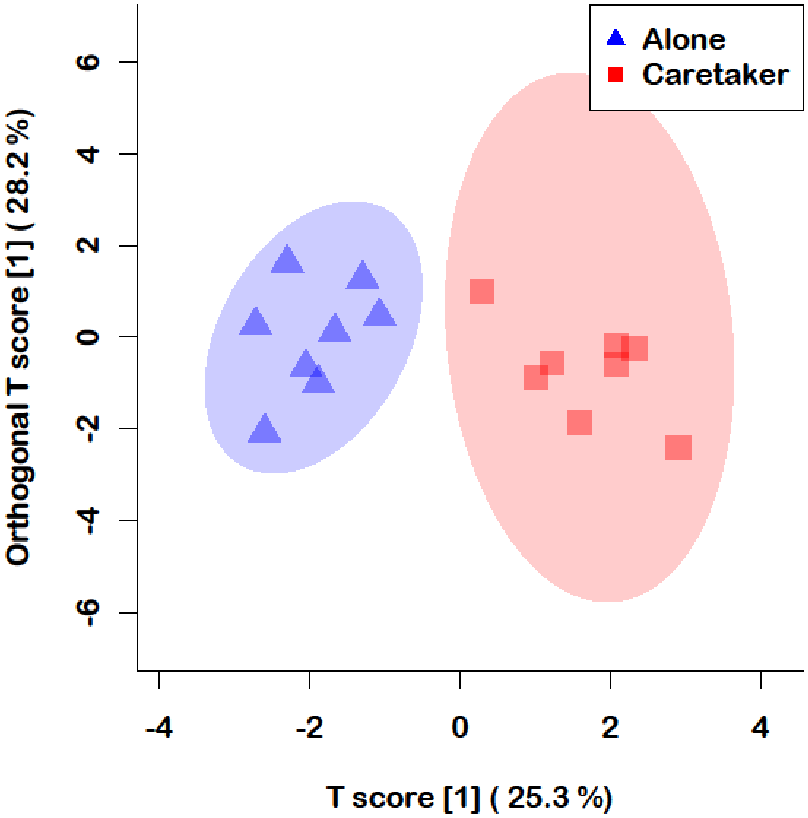

Figure 11: OPLS-DA scores plot for urine metabolites found to be statistically significant via VIAVC best subset testing with female participants removed. Red and squares represent the caretaker group (n=9), and blue and triangles represent the alone group (n=8). Each square/ triangle represents one participant. The shaded oval regions show the 95% confidence interval for each respective group. The x-axis represents predictive variation (between-group differences), while the y-axis indicates orthogonal variation (within-group variation). The plot also includes measures from cross-validation and permutation testing for the OPLS-DA model. Q2= .738, R2= .886

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Chapter 3: Mental Health and Metabolomic Differences Between Those With Spastic Quadriplegic Cerebral Palsy and Those With Non-Spastic Physical Disabilities

3.1 Introduction

Cerebral palsy (CP) is estimated to cost the USA around 1 million USD per individual per lifetime (Kancherla et al., 2012), costing the USA 8.2 billion USD in 2002 (Koman et al., 2003). CP is also the most significant cause of physical disability (PDis) in children, affecting around ~1/500 children (Kolman, 2004; Van Naarden Braun et al., 2016). Even considering these substantial costs and the frequency of CP, research on these individuals regarding their mental and resting biological health is scarce. Additionally, within the literature, CP is often grouped with other physical disabilities (PDis). The biological and adverse mental health repercussions linked to CP, however, may be very different from other PDis due to their neurological differences, particularly considering spastic quadriplegia CP which is associated with impaired upper and lower limb function. These severe functional limitations may be linked to biological and mental health repercussions.

CP comprises a group of heterogeneous neurodevelopmental disorders functionally characterized by limitations in an individual’s motor control capabilities. These limitations are reflected in postural difficulties, increased muscle tone, spastic/dyskinetic syndromes, and overall motor skill deficits (Gulati & Sondhi, 2018). The motor limitations may affect upper and lower limbs, joint contracture, speech, salivary glands, digestion and hearing/vision problems, limiting these individuals’ communication, movement and everyday tasks (Vitrikas et al., 2020). The most severe CP diagnosis involves spastic symptoms. Spastic CP is characterized by stiff muscles and a lack of control when movements are induced. Spasticity is a product of antagonistic muscle incoordination. Spastic CP is then divided into specific subgroups based on the symptoms affecting the limbs. Diplegia (legs more affected than arms), hemiplegia (one arm and one leg affected, other leg and arm not as affected), double hemiplegia (arms more affected than legs), and quadriplegia (all limbs affected) (Kolman, 2004). Some individuals with spastic CP also have dyskinetic CP, which is characterized by uncontrollable movements and stiff limbs when voluntary movement is attempted; these individuals with both spastic and dyskinetic CP are categorized as mixed CP. All other types of PDis differ from spastic CP due to these neurological motor recruitment symptoms.

Non-spastic PDis such as arthrogryposis multiplex congenita (AMC), flaccid paralysis caused by ischemic stroke or spinal cord injury (SCI), and spinal muscular atrophy (SMA), are associated with reduced muscle tone as opposed to increased muscle tone in spastic CP. AMC is a birth disorder resulting in joint contractures, which limits an individual's ability to bend joints or may lock them in a singular position entirely. It is mainly due to a lack of movement within the womb (fetal akinesia) (Langston & Chu, 2020). Ischemic strokes are characterized by cerebral arteries clotting due to emboli formation, causing localized cerebral hypoxia, leading to tissue damage and neuronal and glial cell death. The precise localization, the extent of clot accumulation, and the time before the emboli are removed determine the degree of functional impairment in affected brain regions (Feske, 2021). SCI is an acute injury to the spinal cord that can cause damage to the afferent and efferent pathways, resulting in a range of neurological deficits. Impairments can manifest as a loss of somatosensation, muscle weakness or paralysis, spasticity and various autonomic dysfunctions dependent upon the SCI (Karsy & Hawryluk, 2019). SMA is a genetic disease that induces neuronal loss within the spinal cord due to the lack of survival motor neuron protein, limiting muscle recruitment and leading to muscle weakness and atrophy across the entire body (Mercuri et al., 2022). How these PDis may impact mental health is an area that academia has yet to conceptualize with regular, consistent findings.

Living with a PDis reduces the quality of life and can add significant stress to daily life. Reasons for a reduced quality of life can range from health or medication concerns to difficulty in everyday tasks (eating, hygiene, transportation) to social exclusion. The increased difficulties in daily living could cause adverse mental health outcomes, such as symptoms of depression and anxiety and increased susceptibility to stress. Indeed, adolescents with CP have recorded higher rates of mental health disorders (75% vs 54% in controls) and were 2.6 times more likely to have anxiety and 1.8 times more likely to have depression than non-CP adolescents (Cribb et al., 2023), and this continues in adults with CP showing a higher risk of developing anxiety and depression (Smith et al., 2019). However, other congenital PDis, such as adolescents with muscular dystrophy (Gosar et al., 2021) and adults with AMC or SMA, do not report increased risks of adverse mental health symptoms (Sarveswaran et al., 2023; Wohnrade et al., 2023). For non-congenital PDis, recent literature documented that 18-33% of individuals with stroke experience depression, and individuals with SCI are also at an increased risk of adverse mental health outcomes; however, over time, they have been found to adapt to their situation and experience a decrease in adverse mental health symptoms as time from injury increases (Medeiros et al., 2020; Schultz et al., 2022).

In addition to the regularly experienced daily stress-related challenges that the PDis populations encounter, the recent COVID-19 pandemic has potentially added further psychological burden to these vulnerable individuals. Studies have shown that COVID-19 impacted the access to healthcare among individuals with PDis, increased their social isolation and loneliness, raised their risk of adverse mental health outcomes, and they recorded feeling invisible regarding healthcare and homecare during the pandemic (Lebrasseur et al., 2021; Reber et al., 2021; Steptoe & Di Gessa, 2021). There are still unanswered questions about how different groups within the PDis population were impacted relative to other PDis groups. Understanding the relationship between mental health and perceived stress in individuals with PDis represents an urgent medical need that has yet to be adequately answered.

There are unique stressors to individuals with a PDis, including the availability of a caretaker and the caretaker's personality, access to health care, receiving treatments and their financial burdens, dealing with comorbidities, lifestyle restrictions, transportation and infrastructure accessibility. PDis individuals may experience additional stressors compared to more mobile individuals; many of these stressors may occur over extended periods (Bax, 1980). It is not yet known if individuals with CP show different outcomes than other PDis types. Previous studies have shown that higher perceived stress in individuals with PDis significantly impacts physiological and cellular functions, including shortening the cells' telomere length, a robust marker of biological age (Lahav et al., 2022). Markers such as telomere length may reflect altered cellular function and metabolism (Denham et al., 2016). To better understand how the stresses of PDis, and CP in particular, affect mental health, the study of cellular metabolism and other biological markers may provide relevant objective data.

Biological markers may facilitate objective diagnosis and monitoring of PDis symptoms over time. Previous studies have considered specific biomarkers for depression, anxiety and stress (Blair et al., 2017; Bouillon-Minois et al., 2021; Harsanyi et al., 2022; McEwen et al., 2016; Tenenbaum et al., 2019; Tomasi et al., 2024). Notably, metabolomic profiling has been proven particularly valuable in the differentiation between anxiety and depression (de Kluiver et al., 2021). As biomarker discovery develops, subjective data can be combined with objective data and streamline the process of detecting or predicting if an individual is at risk of adverse mental health outcomes and stress susceptibility. Metabolomics based on nuclear magnetic resonance (NMR) spectroscopy can help discover these markers.

Metabolomics is the comprehensive analysis of an organism's biological functions based on dynamic metabolic regulations (Gardner et al., 2020) by identifying and quantifying metabolites detected via NMR spectroscopy. A semi-automated process involves comparing the target sample with a comprehensive library of reference spectra derived from pure compounds with known concentrations. This methodology, known as chemometrics, is increasingly applied to characterize the metabolomes of diverse biological samples (Wishart, 2008). A distinctive metabolite profile from biological samples (biofluids, extracts and tissues) can be obtained within the NMR spectra. The specific metabolite concentrations and perturbations reflect the impact of environmental and biopsychosocial factors (Emwas et al., 2016). Thus, metabolic changes and metabolite profiles may be robust markers of specific environmental or pathological impacts, such as stress (Steckl & Ray, 2018).

Previous research by our lab has identified potential urine metabolite biomarkers for prenatal stress through maternal stress analysis from children affected by stress, such as a natural disaster (Heynen et al., 2023; Paxman et al., 2018). Moreover, urine metabolite biomarkers may provide a prognostic tool for stroke and monitor post-stroke recovery through the lens of cellular functions (Petersson et al., 2024). Blood and urine biomarker metabolites also enable the assessment of severity and recovery following traumatic brain injury and SCI (Bykowski et al., 2021a, 2021b, 2023; Bykowski et al., 2024). Thus, NMR metabolomics and the study of characteristic metabolic pathways hold excellent value in biomarker discovery and understanding the biology and pathology of biopsychosocial factors (DeBerardinis & Thompson, 2012). These metabolomics findings concerning adverse mental health outcomes and metabolites have also been measured in animal models (McCreary et al., 2019; Zaytsoff et al., 2019).

Existing literature so far has not considered the specific lived experiences of individuals with CP compared to Other PDis. The present study aims to identify the impact of lived experiences in individuals with PDis and identify the specific perceived stress burden among individuals living with CP. Using NMR spectroscopy, the aim was to link the psychosocial factors reported by individuals with spastic quadriplegia CP to objective urine metabolomic profiles. Based on urine and questionnaire data, we expected to find a metabolomic signature that reflects adverse mental health outcomes in those with a spastic quadriplegia CP which is clearly different from individuals living with a non-spastic PDis. The findings may aid in the efforts of personalized health care and policymaking in support of specific needs for individuals living with spastic CP.

3.2 Methods

All data collection and recruitment methods were approved by the Alberta Research Information Services (ARISE) ethics committee from the University of Alberta (July 6th, 2022, Pro00120950) and Fanshawe College (June 27th, 2023, Pro2306121).

3.2.1 Recruitment and Participant Selection Criteria

Participant Recruitment: For this study, 21 individuals with extensive experience with PDis were recruited from various community organizations, including the Calgary Power Hockey League, Calgary Cerebral Palsy Association, Alberta Cerebral Palsy Sports Association, Spinal Muscular Atrophy Canada, Lethbridge Powerchair Soccer Program, Canadian Electric Wheelchair Hockey Association and via word of mouth. It is worth noting that these individuals were not participating in a powerchair sport for at least six months when data were initially collected. The inclusion criterion was that all individuals were limited to a wheelchair as their primary mode of transportation for at least the previous ten years. *Study Participants*: Participants in the “CP group” had spastic quadriplegia CP or mixed CP diagnosis; this involved twelve individuals (male n=9, female n=3, were also dyskinetic n=2, mean age=32). The non-spastic “Other PDis” group included individuals with a non-spastic PDis diagnosis; this involved nine individuals (males n=9, females n=0, AMC n=2, stroke n=2, SCI n=2, SMA n=3, mean age=35.7). The SCI participants included in this study did not show spasticity symptoms.

3.2.2 Data Collection

Data collection, including questionnaire data and urine samples, was conducted during home visits in the summer months between July 2022 and September 2023. Fasting urine samples were collected using sterilized collection cups between 7 am and 10:30 am before participants had breakfast or used the bathroom, ensuring a fasting period of at least 8 hours. The collection cups were capped and labelled with the date and time, and samples were promptly frozen at -18°C. Within four days, samples were transported to the University of Lethbridge for storage in a -80°C freezer. Psychological distress and mental health assessments were performed using self-administered questionnaires. Assistance was provided as needed, with questionnaires being read out loud and filled in based on the participants’ responses. Questionnaires were completed in approximately 20-40 minutes, with breaks offered as needed.

3.2.3 Questionnaire Assessment

The questionnaires covered various domains: demographics, analog scales, perceived stress, anxiety, depression, exercise, and independent mobility. The sociodemographic questionnaire collected information on age, sex, housing situation, caretaker status, education, employment, religion, nature of PDis symptoms, and age at diagnosis. Analog scales assessed current stress levels, mental wellbeing, and screen time. Standardized questionnaires for stress and wellbeing included the Perceived Stress Scale (PSS-10) to measure recorded stress (Camargo et al., 2023), segmented into three sections: before the COVID-29 pandemic, during COVID-19 lockdowns, and within the last month; the Generalized Anxiety Disorder questionnaire (GAD-7) for anxiety (Simon, 2021); the Patient Health Questionnaire (PHQ-9) for depression levels (Kroenke et al., 2007); and the Major Depression Inventory (MDI) for detailed depressive symptoms (Nielsen et al., 2017). The Godin Leisure-Time Exercise Questionnaire (Godin) evaluated exercise habits over a typical one-week span, while the Intensive Care Unit (ICU) Mobility Scale measured participants' mobility levels (Sikes et al., 2019; Tipping et al., 2016).

Table 7: Summary of questionnaires used to assess demographics, adverse mental health symptoms, stress, exercise and mobility.

Questionnaires	Screens for	Measurement	References for Validity and Reliability
General demographics	Age, sex, housing situation, caretakers, education, work, income, religion, PDis, diagnosis, age	Basic information about the participants was needed to understand the individual's conditions while confirming certain factors were monitored.	N/A
Analog scales	Stress, wellbeing, screen time	Stress levels, wellbeing, and screen time habits, respectively.	N/A
Perceived Stress Scale (PSS-10)	Relative stress	Measures perceived stress levels during different periods (before COVID-19, during COVID-19, and during the previous month).	(Camargo et al., 2023)
Generalized Anxiety Disorder Questionnaire (GAD-7)	Anxiety symptoms	Finds patients potentially suffering from generalized anxiety disorder and records the severity of anxiety symptoms.	(Simon, 2021)

Major Depression Inventory (MDI)	Depression symptoms	Evaluate the severity of depression symptoms and provide more detailed answer choices compared to PHQ-9.	(Nielsen et al., 2017)
Patient Health Questionnaire (PHQ-9)	Depression symptoms	Screens, diagnoses, and monitors the severity of depression symptoms, including suicide ideation and functional impairment.	(Kroenke et al., 2007)
Godin Leisure-Time Exercise Questionnaire	Leisure time and exercise habits	Measures leisure time and exercise habits based on strenuous, moderate, and light activities.	(Sikes et al., 2019)
ICU mobility scale	Mobility scale	Measures the individual's level of mobility, chosen over other mobility scales due to increased specificity	(Tipping et al., 2016)

3.2.4 Urine Metabolomics

Sample Preparation of Urine: Urine samples were prepared following a standardized procedure (Heynen et al., 2023). All microcentrifuge tubes received 160 µL of buffer, 40 µL of 0.02709% w/v D_2O containing trimethylsilyl propanoic acid (TSP), and 500 µL of urine. The buffer (pH 7.4 ± 0.05) consisted of a 4:1 ratio of dibasic potassium phosphate (K_2HPO_4) to monobasic potassium phosphate (KH_2PO_4) with a concentration of 0.625 M, 3.75 mM of NaN_3 as an antimicrobial agent, and 3.75 mM of potassium fluoride (KF). Following vortexing and centrifugation at 12,000 rpm at 4°C for 5 minutes, 550 µL of the supernatant was moved to an NMR tube.

NMR Spectroscopy: NMR spectra were obtained using a 700 MHz Bruker Avance III HD spectrometer (Bruker Ltd., Milton, ON, Canada) with a 1-D NOESY gradient water suppression pulse sequence. All urine samples underwent 128 scans and were acquired with 128 k points, a spectral window of 20.51 ppm, and a recycle delay of one second. Spectra were zero-filled to 256 k, automatically phased, baseline corrected, and line-broadened by 0.3 Hz prior to being exported to MATLAB (MathWorks, Natick, MA, USA) for recursive segment-wise peak alignment (Veselkov et al., 2009) and binning using Dynamic Adaptive Binning followed by manual correction (Anderson et al., 2011). Data were normalized, log-transformed, and Pareto-scaled prior to modelling (Craig et al., 2006), with all spectra referenced to the TSP peak (0.00δ).

3.2.5 Data Analysis

Questionnaire data analysis was performed using SPSS (IBM, version 27, Armok, NY, USA), where independent t-tests shared with Levene's Test for Equality of Variances were used to find p-values when assessing means between two groups. Relative differences between the three PSS time frames were labelled ΔPSS1 (before COVID-19 vs. during COVID-19) and ΔPSS2 (during COVID-19 vs. the last month). Bonferroni correction was applied to each test based on the number of comparisons.

In metabolomics analyses, the normality of each bin was assessed using the Shapiro-Wilk test, revealing a non-parametric distribution for all bins; subsequently, the Wilcoxon-Mann-Whitney U test (MW) was utilized (Goodpaster et al., 2010), with Bonferroni-Holm correction applied based on the number of comparisons. Variable Importance Analysis based on random Variable Combination (VIAVC) discovered significantly altered bins for metabolite identification (Yun et al., 2015). Orthogonal Projection to Latent Structures-Discriminant Analysis (OPLS-DA), a supervised multivariate test (Worley & Powers, 2013), was performed for group comparisons. Multivariate models were validated by double ten-fold cross-validation and permutation testing using 2000 permutations (Wishart et al., 2012; Wishart et al., 2009; Wishart et al., 2007). Metabolite identification was carried out using Chenomx 8.2 NMR suite software (Chenomx Inc., Edmonton, AB, Canada) with the Human Metabolome Database (HMDB), followed by pathway topology analysis using Metaboanalyst with the human pathway library and hypergeometric test (Wishart et al., 2012; Wishart et al., 2009; Wishart et al., 2007). Lastly, Pearson or Spearman correlation tests were completed, based on homogeneity of variance tests conducted across questionnaire and metabolite data, to investigate correlations relating metabolite levels and questionnaire scores.

3.3 Results

3.3.1 PDis Patient Characteristics

The demographics and patient characteristics of the individuals with CP (the CP group) and those with non-spastic PDis (the Other PDis group) can be seen in Table 2. Sample size, mean age, mean ICU mobility score, religiosity, living with a full-time caretaker, sex, PDis information, employment and education are shown for each group. One individual within the CP group did not fill out the questionnaire outside of PDis, age and caretaker situation, and a different individual within the CP group did not supply urine.

Table 2: Patient characteristics table (n=21) shows the demographic difference between the CP and Other PDis groups. The information displayed includes sample size, age, mobility, religion, caretaker situation, sex, PDis, employment, and education.

	CP	**Other PDis**
n=	12	9
Age	32 ± 8.28	35.67 ± 15.832
ICU Mobility	6.19 ± 0.78	4.22 ± 0.93
Sex		
Male	9	9
Female	3	0
PDis		
AMC	NA	2
Stroke	NA	2
SCI	NA	2
SMA	NA	3
Highest Level of Education		
Partial High School	0	1
Highschool Diploma	6	2
Partial Post Secondary	2	3

Post Secondary Degree	2	3
Employment Status		
Employed	5	3
Gov. Subsidy	5	4
In School	2	2
Caretaker	8	4
Religious	9	3

3.3.2 Questionnaire Results

The results of the questionnaires regarding anxiety, depression, and stress activity levels are shown in Figure 1. The PHQ, MDI, GAD, PSS and stress scale scores all relate to symptoms; the higher the score, the higher the symptoms. The Godin score relates to physical activity levels; the higher the score, the higher the activity levels. The CP group recorded higher scores for the GAD, PSS, analog stress scale, and Godin. The Other PDis group recorded higher scores for the MDI and slightly higher scores for the PHQ. The Godin Leisure-Time Exercise Questionnaire score was the only measure that exhibited a statistically significant difference between the CP and Other PDis groups (p=.05).

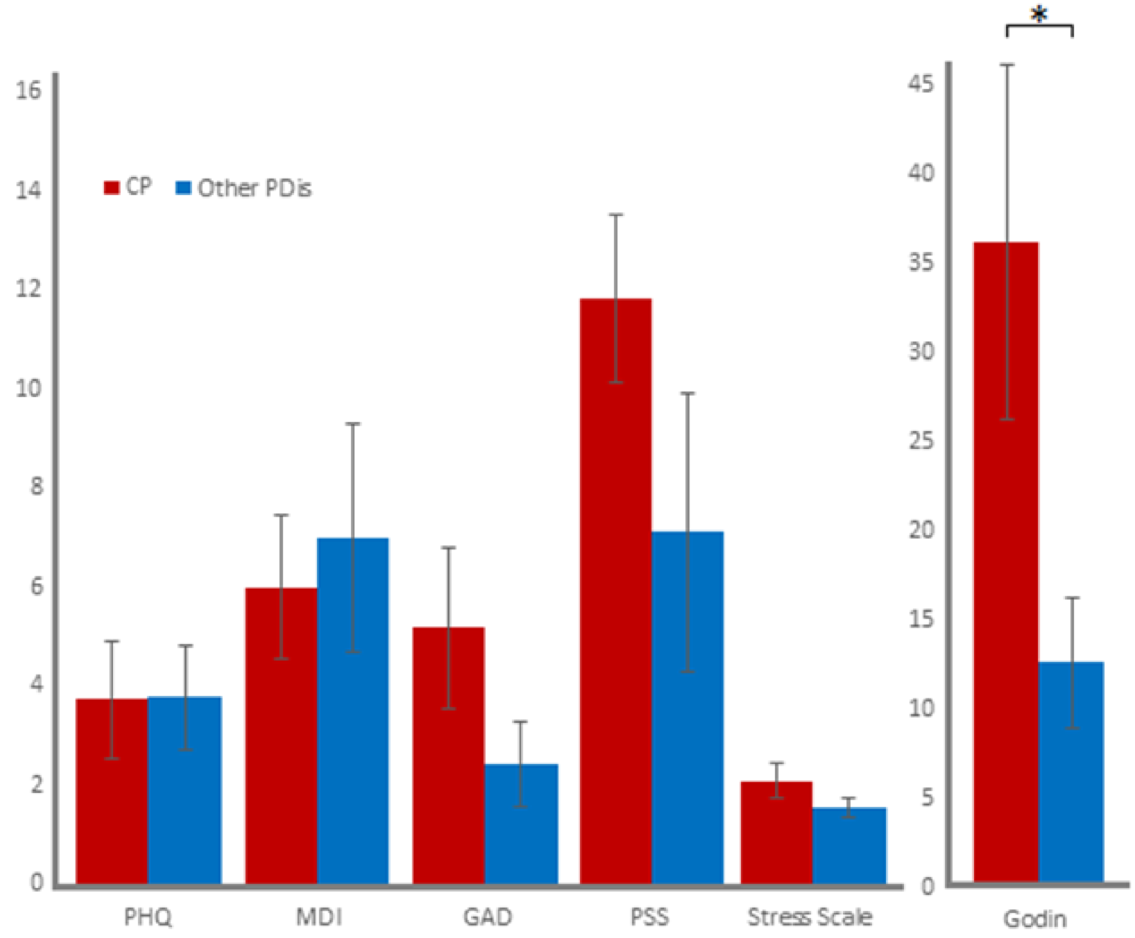

*Figure 5: Questionnaire results from both the CP group (red) (n=11) and the Other PDis group (blue) (n=9). The x-axis shows the given questionnaire, and the y-axis shows the questionnaire score. The error bars represent standard error. The depression questionnaires are the Major Depression Inventory (MDI)/ Patient Health Questionnaire (PHQ-9). The anxiety questionnaire is the Generalized Anxiety Disorder Questionnaire (GAD-7). The stress-related questionnaires are the Perceived Stress Scale (PSS-10)/ stress scale. The physical activity questionnaire is the Godin Leisure-Time Exercise Questionnaire (Godin) (p=.05). Results indicated with asterisks * and ** represent p<0.05 and 0.0083, the Bonferroni corrected value of corrected for the number of questionnaires assessed, respectively.*

Retrospective perceived stress in the PSS scale is illustrated in Figure 2 concerning before, during COVID-19 and within the last month. The thick black dotted line shows the global average score of 13 for PSS at a singular point in time (sample size n=2397) (Cohen et al., 1983). PSS scores for CP and Other PDis groups before COVID-19 were comparable and close to the global average. The CP group experienced increased scores during COVID-19, whereas the Other PDis group only showed a minor increase. This relative change in PSS score can be seen on the graph section labelled as B as ΔPSS1. The difference between the PSS scores recorded for each group during COVID-19 was statistically significant; this was the only difference that showed statistical significance (p=.05). The CP group then reduced PSS scores to the global average levels in post-COVID-19 assessments at the "last month" timepoint, to levels comparable to before COVID-19 PSS scores. The Other PDis group also experienced a decrease in PSS score, but post-COVID-19, last month's PSS score dropped below their before COVID-19 score and was lower than the global average. The relative change in PSS scores from during COVID-19 to the last month is shown on the bar graph labelled B as ΔPSS2.

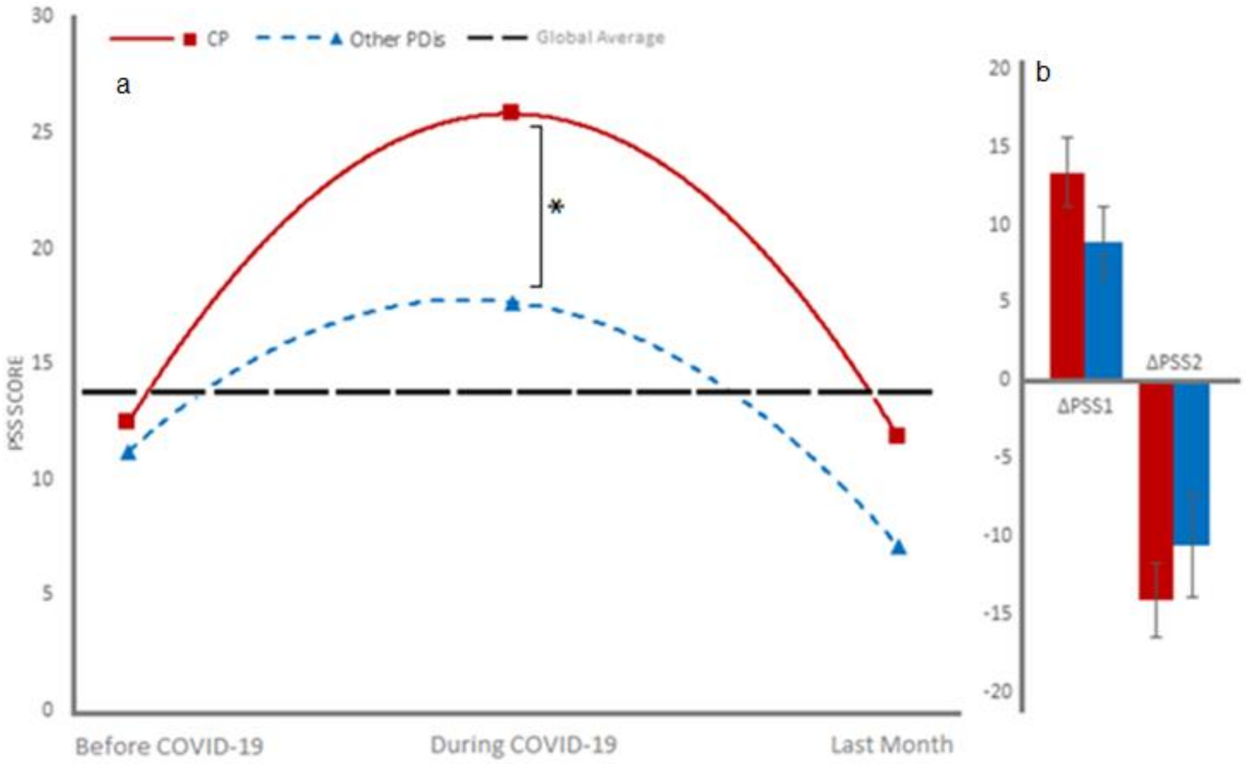

*Figure 6: Retrospective Perceived Stress Scale (PSS-10) scores over three-time points for the CP group (red, square, solid line) (n=11) and the Other PDis group (blue, dotted line, triangle) (n=9). The x-axis shows the time points before COVID-19, during COVID-19 (p=.05), and the last month. The y-axis shows the PSS scores for each time point. The black dotted line represents the global average in 1983 (n=2397). The mean difference between the time points before vs during COVID-19 is represented on the ΔPSS1 bar graph. The mean difference between the time points during COVID-19 and the last month is represented on the ΔPSS2 bar graph. The error bars represent +/- standard error. Results indicated with asterisks * and ** represent p<.05 and .0083, the Bonferroni corrected value of corrected for the number of questionnaires assessed, respectively.*

The correlation between questionnaire scores can be seen in Figure 3. Within the CP group, it is seen that as Godin scores increase, GAD levels decrease (p=0.023, Rho=-0.673); this correlation does not pass the p-value threshold for the Other PDis group. For the Other PDis group, it is seen that as ICU mobility levels increase, the recorded stress scale score decreases (p=0.033, Rho= -0.709); this correlation does not exist for the CP group.

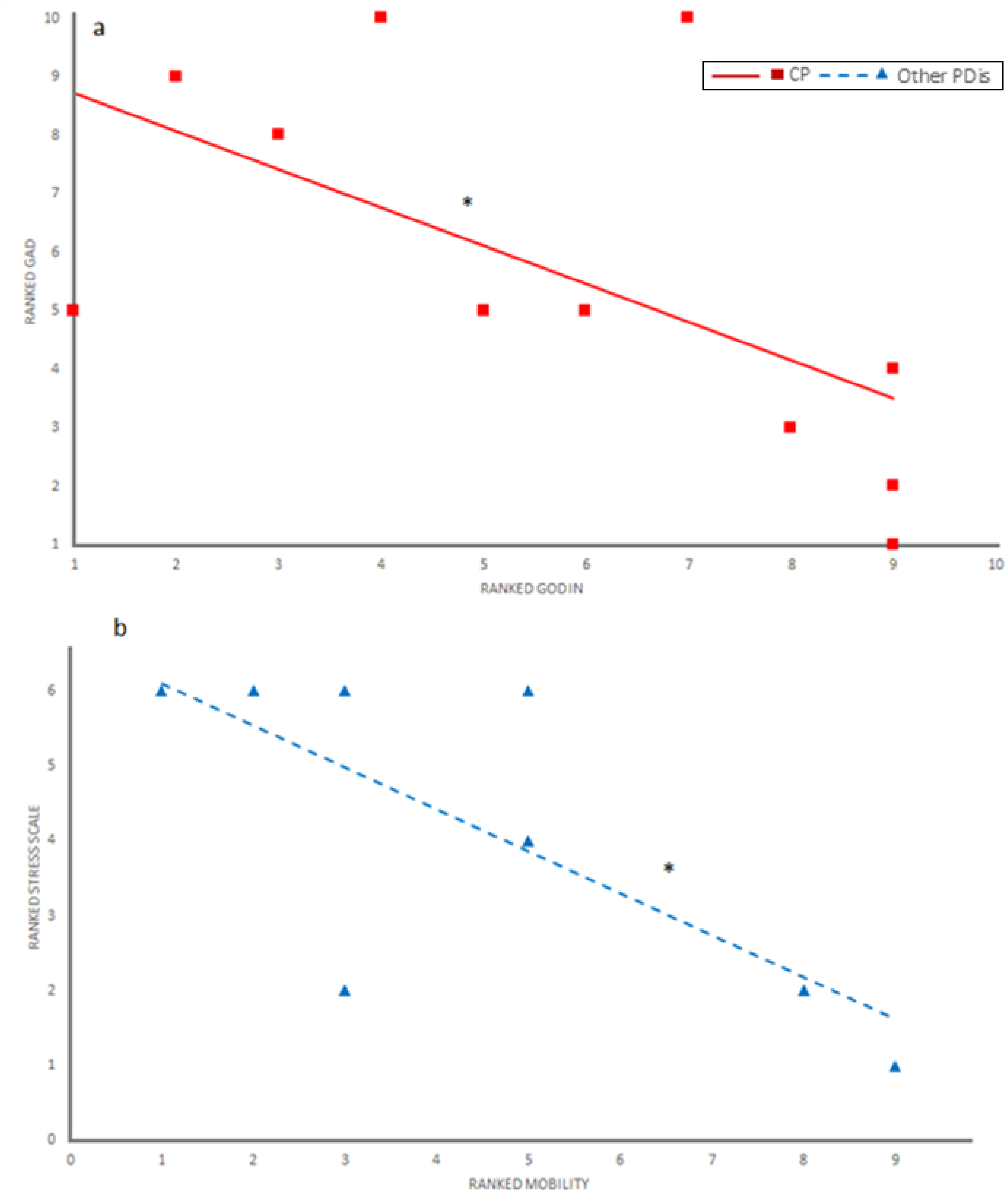

Figure 7: Correlations between questionnaire scores. The red solid line with squares represents the CP group (n=11), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and

*direction of the relationships between questionnaire responses in each group. Each axis is ranked, and questionnaire scores are on the x-axis and y-axis. Graph A shows that within the CP group, as Godin Leisure-Time Exercise (Godin) scores increase, Generalized Anxiety Disorder Questionnaire (GAD) scores decrease (p=0.023, Rho=-0.673). Graph B shows that the analog stress scale score decreases within the Other PDis group as the ICU mobility score increases (p=0.033, Rho= -0.709). Both axes are ranked in descending order. Results indicated with asterisks * represent p<.05 and ** represent a p-value of less than 0.005 and *** represents the Bonferroni corrected 0.0016 p-values based on the total number of compairisons.*

3.3.3 Urine Metabolome Differences between CP and Other PDis

Table 3 presents the metabolites, their chemical shifts, p-values, and regulatory status when comparing the CP and Other PDis groups. From 385 bins, 16 bins and 17 unique metabolites were identified and deemed significant through VIAVC or MW tests. Among these significant bins, ten were upregulated, and six were downregulated in the CP group relative to the Other PDis group.

Table 3: Metabolites exhibiting statistically significant differences between the CP group (n=11) and the PDis group (n=9) as determined by MW tests and VIAVC analysis are organized by their chemical shift. Each row presents a metabolite's chemical shift, VIAVC/MW p-value, and regulatory status. Up-regulation and down-regulation are indicated by arrows, signifying positive and negative regulation, respectively, when comparing the CP group to the PDis group.

Metabolite	Chemical Shift (PPM)	VAIVC p- Value	Mann-Whitney p-Value	Regulation
Indoxyl sulfate	7.469	Not Sig.	5.74E-03	↑ 51.371
N-Phenylacetylglycine	7.393	3.10E-36	Not Sig.	↑ 22.213
Indoleacetic acid	7.253	5.72E-10	Not Sig.	↑ 47.846
N-Acetylmannosamine	5.016	8.10E-12	Not Sig.	↑ 29.929
Hippuric acid	4.954	1.18E-33	Not Sig.	↑ 49.604
Paraxanthine	3.989	1.35E-26	Not Sig.	↓ -10.589
Pseudouridine	3.721	1.16E-10	Not Sig.	↑ 23.667
Dimethylglycine	3.709	Not Sig.	6.75E-03	↑ 29.377
cis-Aconitic Acid	3.452	1.89E-18	Not Sig.	↓ -9.633
1,3-Dimethyluric Acid	3.431	4.41E-39	Not Sig.	↓ -19.018
Caffeine	3.319	3.40E-12	Not Sig.	↑ 13.985
Histidine	3.131	2.42E-46	Not Sig.	↓ -17.513
Serotonin, Malonic Acid	3.103	5.95E-22	Not Sig.	↓ -25.629
Deoxyadenosine	2.855	2.91E-137	5.30E-03	↑ 32.560
O-Acetylcarnitine	2.132	1.35E-17	Not Sig.	↑ 6.704
2-Hydroxy-2-methylbutyric acid	1.676	1.61E-23	Not Sig.	↓ -13.996

Figure 4 depicts the application of multivariate and univariate modelling techniques to analyze metabolomic data across two distinct groups. Initially, an unsupervised Principal Component Analysis (PCA) was conducted utilizing all bins, followed by a supervised Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA), which also incorporated all bins. However, the PCA showed no clear separation of the groups, and the OPLS-DA model did not meet validation criteria during permutation and cross-validation tests. Subsequently, PCA and OPLS-DA multivariate modelling was completed using the 16 bins identified as significantly altered by either the VIAVC or MW tests. Utilizing these bins, the supervised OPLS-DA model demonstrated a clear and real distinction between the groups, as evidenced by the quality of the model (Q2) and p-values, respectively, shown on the OPLS-DA scores plot presented in Figure 4. Figure 5 displays the Receiver Operator Characteristic (ROC) curve generated utilizing the bins that were part of the VIAVC best subset. This curve shows an excellent area under the curve value of 0.981 and a predictive accuracy of 94.2%, both representing almost perfect group classification based on the metabolites in the VIAVC best subset.

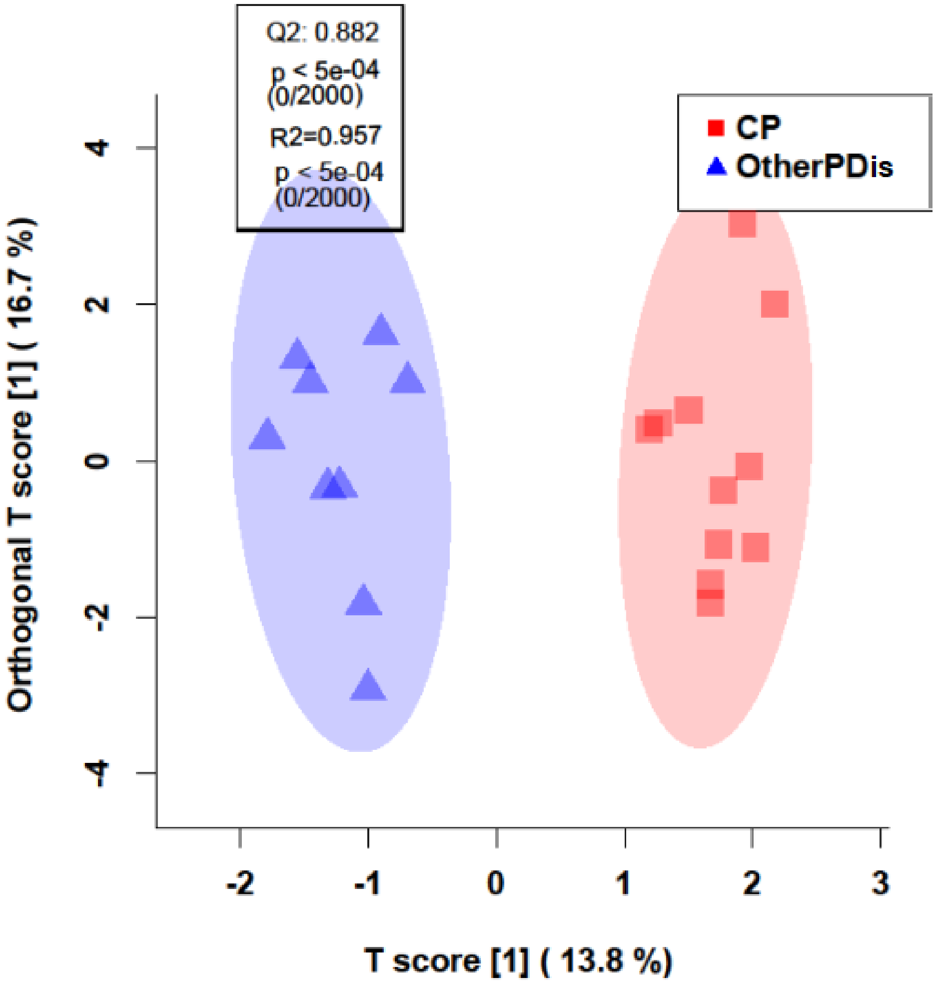

Figure 8: The plot of OPLS-DA scores for urine metabolites identified as statistically significant via VIAVC best subset testing is depicted. Red squares represent the CP group (n=11), and blue triangles represent the PDis group (n=9), with each shape corresponding to a participant. The shaded regions are the 95% confidence intervals. The x and y-axis represent predictive variation (between-group differences) and orthogonal variation (within-group variation). The graph includes the measure of cross-validation (Q2) and permutation testing (R2).

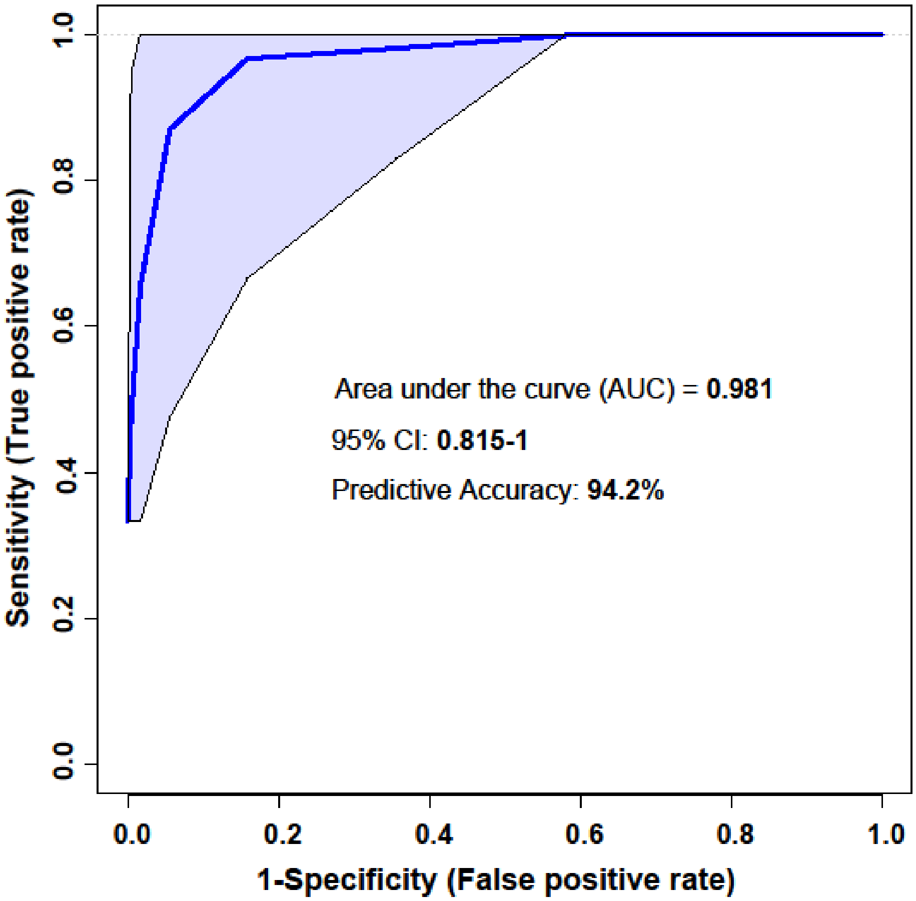

Figure 9: The ROC curve, based on the VIAVC best subset bins, is illustrated in the figure. It includes the related Area Under the Curve (AUC), the 95% confidence interval, and the model's predictive accuracy. This predictive accuracy is derived from 14 VIAVC bins, which are detailed in the figure to provide insights into the model's performance.

Figure 6 presents the results of the pathway topology analysis, which investigated possible disruptions in metabolic pathways that could result from the list of significantly altered metabolites. The metabolic pathways are displayed in order of their p-value, with the following pathways potentially impacted: (1) caffeine metabolism, (2) tryptophan metabolism, and (3) histidine metabolism. The metabolites utilized for this analysis were those identified as significant via the VIAVC or MW tests.

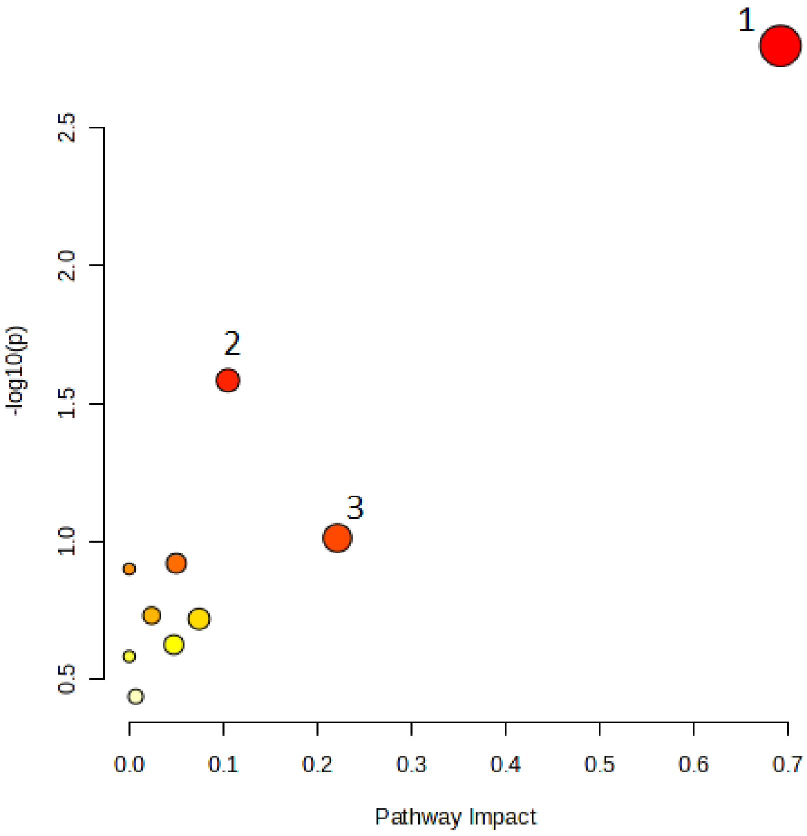

Figure 10: Metabolic Pathway Topology Analysis is depicted, where the x-axis and the size of each circle represent the impact factor, indicating the extent to which the identified metabolites influence each pathway. Each circle's y-axis and colour denote the p-value in -log10 format; a higher value signifies greater statistical significance, resulting in a darker shade. Pathways with p-values less than 0.1 are labelled. 1) Caffeine metabolism, p=.002, impact = .692, 2) tryptophan metabolism, p=.026, impact = .105, 3) histidine metabolism, p=.097, impact = .221.

3.3.4 Metabolite and Questionnaire Correlations

Figure 7 illustrates the correlations that were observed for the CP group. Negative correlations were found between (1) screen time and caffeine (p=.006, Rho=-.799), (2) PSS scores and hippuric acid (p=.008, Rho= -.778), and (3) PHQ scores and hippuric acid (p=.005, Rho= -.8). A positive correction was observed between (1) MDI scores and serotonin + malonic acid (p=.012, Rho=.756) and (2) GAD scores and caffeine (p=.011, Rho=.756). All observed correlations for the Other PDis group and metabolite concentrations were positive (Figure 8) and included (1) ΔPSS1 and dimethylglycine (p=.005, Rho=.837) (2), GAD score and dimethylglycine (p=.006, Rho=.826), (3) ΔPSS1 and serotonin + malonic acid (p=.002, Rho=.879), and (4) PSS scores during COVID-19 and 2-hydroxy-2-methyl-butyric acid levels (p=.002, Rho=.867).

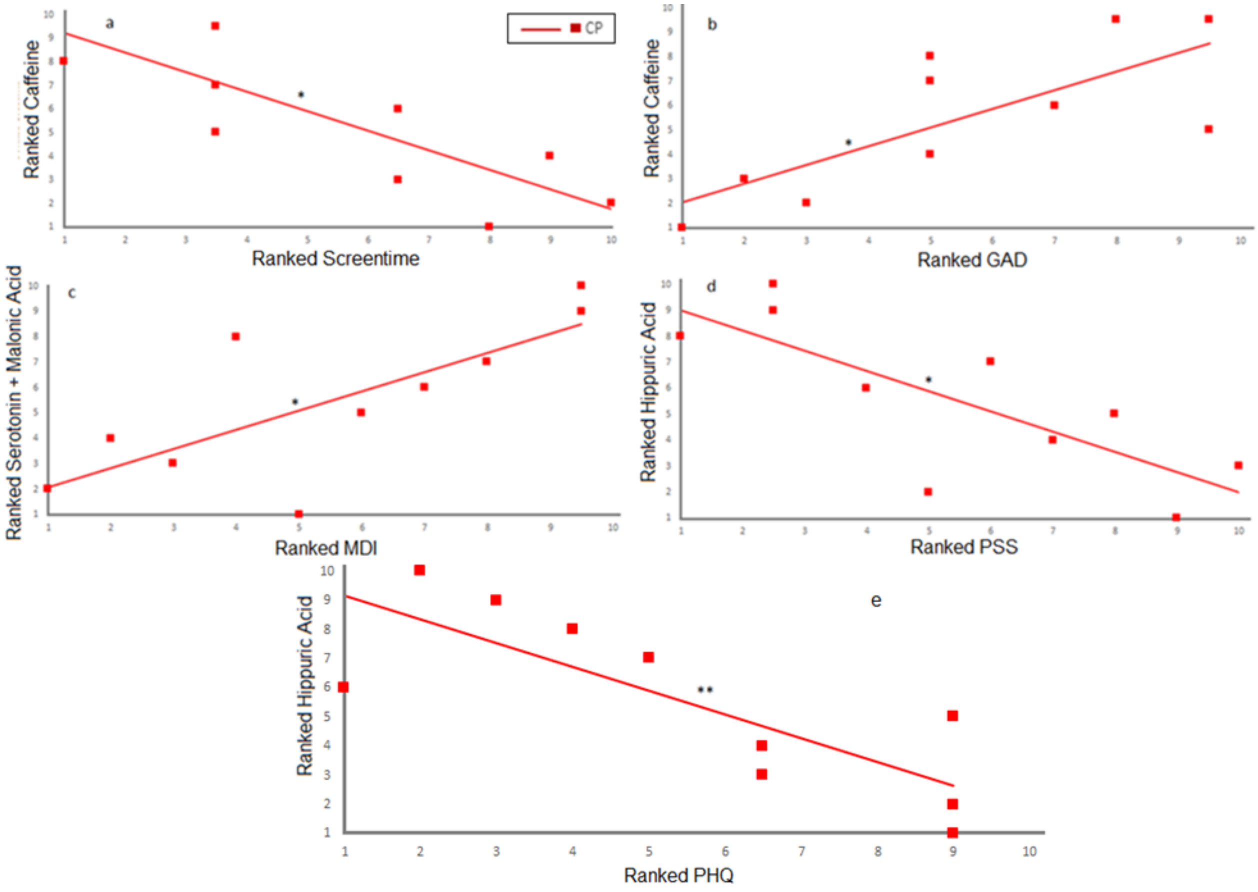

*Figure 11: Correlations between metabolite levels and questionnaire data are depicted in the analysis found within the CP group. The red solid line with squares represents the CP group (n=10), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and direction of the relationships between metabolites and questionnaire responses in each group. Each axis is ranked, questionnaire scores being on the x-axis and metabolite levels on the y-axis. Graph A shows that within the CP group, as screentime increases, caffeine levels have been found to decrease (p=.006, Rho=-.799). Graph B shows that as Generalized Anxiety Disorder Questionnaire (GAD) scores increase, caffeine levels have increased (p=.011, Rho=.756). Graph C shows that as Major Depression Inventory (MDI) scores increase, the bin with serotonin and malonic acid also increases (p=.012, Rho=.756). Graph D shows that as Perceived Stress Scale (PSS) scores increase, hippuric acid levels decrease (p=.008, Rho=-.778). Graph E shows that as Patient Health Questionnaire (PHQ) scores increase, hippuric acid levels decrease (p=.005, Rho=-.8). Both axes are ranked in descending order. Results with a * denote a p-value less than 0.05, and ** denotes a p-value of less than 0.005 and *** represents the Bonferroni corrected 0.0016 p-values based on the total number of compairisons.*

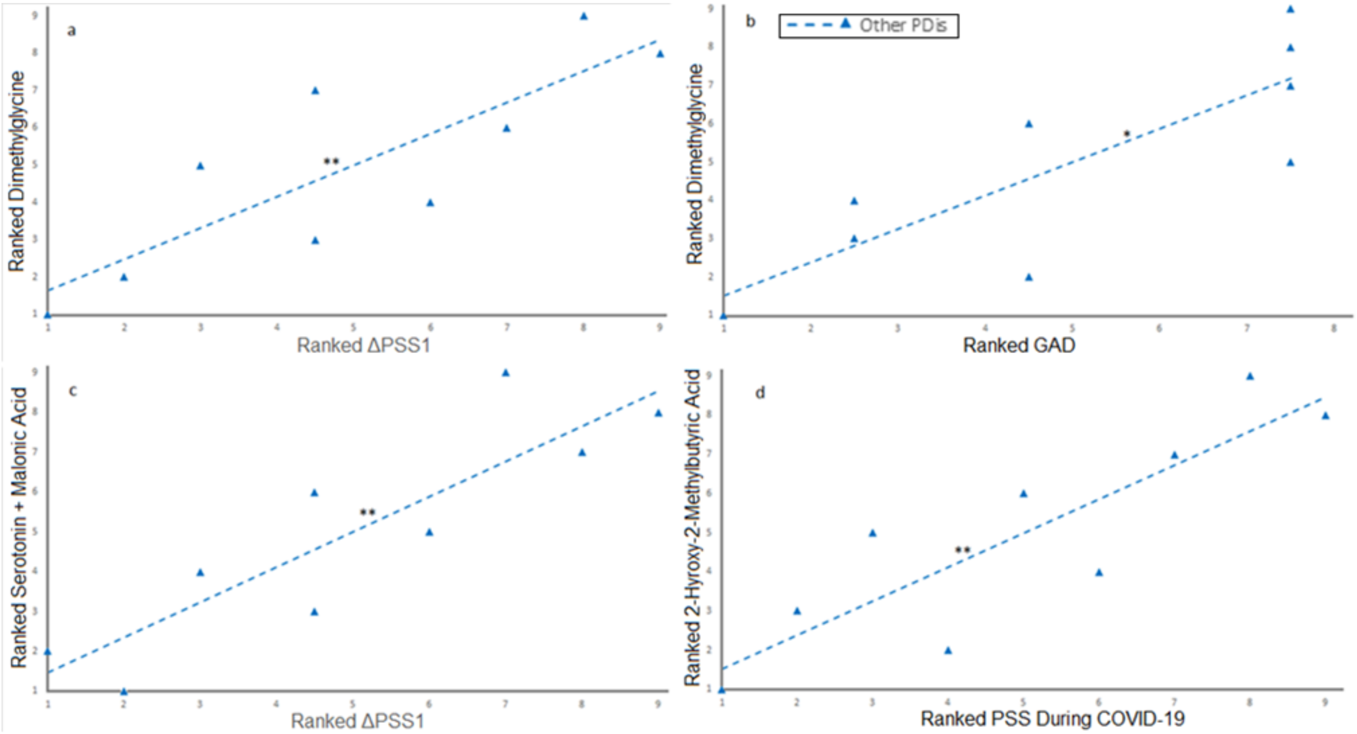

*Figure 8: Correlations between metabolite levels and questionnaire data are depicted in the analysis found within the Other PDis group. The red solid line with squares represents the CP group (n=10), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and direction of the relationships between metabolites and questionnaire responses in each group. Each axis is ranked, questionnaire scores being on the x-axis and metabolite levels on the y-axis. Graph A shows that dimethylglycine levels also increase as Δ Perceived Stress Scale (PSS)1 increases (p=.005, Rho=.837). Graph B shows that as Generalized Anxiety Disorder Questionnaire (GAD) scores increase, dimethylglycine levels are seen to increase (p=.006, Rho=.826). Graph C shows that as ΔPSS1 levels increase, the bin with serotonin and malonic acid also increases (p=.002, Rho=.879). Graph D shows that as PSS during COVID-19 increases, 2-Hydroxy-2-methylbutyric acid levels also increase (p=.002, Rho=.867). Both axes are ranked in descending order. Results with a * denote a p-value less than 0.05, and ** denotes a p-value of less than 0.005 and *** represents the Bonferroni corrected 0.0016 p-values based on the total number of compairisons.*

3.4 Discussion

Individuals living with spastic CP may experience different levels of psychological distress and mental health challenges than populations with other types of non-spastic PDis. Here, we delivered a comprehensive questionnaire and metabolomic assessment to identify specific perceived stress burdens among individuals living with spastic CP. The findings show that those with spastic CP show similar adverse mental health symptoms, perceived stress and stress responses when compared to non-spastic PDis. The spastic CP group showed greater physical activity levels, perceived stress during COVID-19 and unique correlations within questionnaire scores for each group. Within urine, differences were found between the two groups' metabolite levels and their potential impact on biochemical pathways. Unique correlations between metabolite concentrations and questionnaire scores were also found for each group.

3.4.1 Overview of Patient Characteristics

The demographics data indicate proportion similarities between the two groups in age, employment status, and partial post-secondary education; however, there was a notable distribution difference regarding sex ratios, education levels, religiosity, mobility levels, and those living with caretakers. Demographic differences, including sex, age, religiosity, education, and mobility, may significantly impact mental health outcomes, thus influencing the results of this study. Notably, the distribution variety of non-spastic PDis is relatively equal across AMC, SCI, SMA and stroke.

The sex differences revealed a higher proportion of males. Only three females were included in this study, all of them in the CP group. Due to the small sample size of females, metabolomic comparisons focused on male participants; however, the data indicated no significant sex difference. The present study does not consider sex differences. The literature indicates a significant impact of sex on lived experiences with PDis, as women record depressive symptoms that impact emotional reliance, while men experience depressive symptoms that impact functional impairment (Brown, 2014). Furthermore, the non-PDis literature suggests that females generally record higher perceived stress and more significant adverse mental health morbidity than males (Mayor, 2015; Weekes et al., 2005). Males and females have also been found to exhibit different neuronal responses to stress, where men show higher cerebral blood flow to the prefrontal cortex while women show more significant activity to the limbic system (Wang et al., 2007). Aside from their psychological impacts, these changes may manifest in significant metabolomic sex differences.

The consideration of education levels outside of partial post-secondary education suggests that the CP group on average had a higher proportion of individuals with a high school degree, whereas the Other PDis group had a higher proportion of individuals with a post-secondary degree. Previous literature has shown that higher education levels are associated with improved mental health outcomes (Bjelland et al., 2008). The Other PDis group recording higher levels of education may have implications on the metabolomic and questionnaire results regarding the mental health results. The CP group recorded a significantly higher proportion of religious participants, which may have implications for mental health outcomes. Previous research has shown that religiosity has a positive impact on mental health and it may act as a buffer for stress; however, these results are context- and population-dependent (Clements & Ermakova, 2012; Connor et al., 2003; Moreira-Almeida et al., 2006). These kinds of considerations have focused on non-PDis individuals; however, one study showed that religious practices in disabled war veterans contributed to improved mental health and lower incidence of post-traumatic stress disorder, beyond other predictors like physical function and social support (Aflakseir & Coleman, 2009). The religious difference between the two groups may alter psychological and perceived stress results and coping strategies that may impact metabolite levels.

Furthermore, the CP group revealed higher levels of mobility, including the ability for assisted walking or the ability to hold oneself upright on the side of a bed. This difference was expected since individuals with non-spastic PDis within this study had more extensive lower body limitations. This difference in mobility may have implications for the Godin scores and metabolomics results since it is well-established that physical exercise and sedentary behaviours affect cell metabolism (Greer et al., 2015). In addition, the CP group recorded a much higher rate of individuals living with a caretaker than the Other PDis group. As seen in the previous chapter, this group of participants was investigated for the implications of those with PDis and their living situation and found that the presence of a caretaker did not alter mental health, stress or social support levels in comparison to those who lived alone. Living with a caretaker, however, did impact several metabolites and biological pathways within the body compared to the absence of a caretaker.

The results of the questionnaire data regarding the CP and Other PDis groups show that significant differences were found in the Godin activity scores. This difference suggests that individuals with spastic quadriplegia CP engaged in more physical activity than those with other non-spastic PDis, suggesting differences in the use of mobility aids, therapeutic interventions, or structured physical activity programs specifically designed for individuals with CP. This difference in physical activity may have implications for mental health and metabolomics results within this study (Greer et al., 2015; Vankim & Nelson, 2013).

3.4.2 Mental Health, Stress and COVID-19 Impact

The perceived stress PSS scores revealed a sizable difference between the spastic CP group and the non-spastic PDis group, indicating that the complexity of the PSS effectively captures specific changes in perceived stress over time among the two different PDis populations. The PHQ scores between the groups were comparable, yet the MDI scores showed greater group separation, even though both questionnaires screened for depressive symptoms. Again, this observation suggests that the MDI, which is designed for diagnostic purposes aligned with DSM-IV and ICD-10 criteria, captures broad mental health changes, whereas the PHQ-9 instead focuses on screening and monitoring depressive symptoms over time in a clinical setting and is not used as a diagnosis tool (Bech et al., 2001; Kroenke et al., 2001). Even with the slight differences in mental health symptoms, the results are not statistically significant, and these observations should be interpreted cautiously.

The retrospective PSS scores revealed perceived stress responses to the COVID-19 pandemic. Before the pandemic, both CP and Other PDis groups reported a similar PSS score. During the pandemic, both groups reported a sharp increase in perceived stress, with the CP group experiencing a larger increase than the Other PDis group. Both groups then reported a reduction in perceived stress levels following the COVID-19 pandemic in their “last month” stress rating. Here, the CP group achieved levels similar to the global average and had similar stress levels prior to COVID-19. In contrast, the Other PDis group rated their perceived stress levels during the last month as lower than both the global average and their stress levels prior to COVID-19. Overall, the CP group recorded a relatively larger change in perceived stress both in their increase from before to during COVID-19 and their decrease from during COVID-19 to their last month’s PSS; however, these relative changes between time points (ΔPSS1 and ΔPSS2) were not statistically significant compared to the Other PDis group, but the trend may indicate that individuals with CP may be more susceptible to stress than those with other types of PDis.

When comparing the two groups at individual time points, the CP group showed higher perceived stress levels during the COVID-19 pandemic than those with other types of PDis, and this may be due to unique and multifaceted challenges, including specific comorbidities or lifestyle differences. Comorbidities may exist among the Other PDis group, but since this group is composed of a range of four different PDis, the comorbidities may not be shared throughout the group. The CP group may share specific spasticity-related comorbidities, such as impairments in communication, vision, hearing, and sleep complications, which may have caused additional stress and limited healthcare accessibility during the COVID-19 pandemic (Viswanath et al., 2023). In addition, the CP group also recorded higher levels of exercise than the Other PDis group; during COVID-19, individuals with CP may have lost opportunities to engage in physical activity, such as physiotherapy, specific interventions, and access to a gym or trainers. Physical activity may improve mental health and reduce stress (Vankim & Nelson, 2013).

The correlation tests between questionnaire scores were calculated independently for each group. The CP group showed a negative correlation between Godin and GAD scores, indicating that higher levels of physical activity were associated with lower levels of anxiety among these individuals. As considered previously in the COVID-19-related section, physical activity might serve as a protective factor against anxiety in this group (Cribb et al., 2023). Engaging in physical activity may alleviate anxiety by providing physical health benefits, social interaction, and a sense of achievement (Vankim & Nelson, 2013). Physical activity has known benefits of increased cognitive abilities and hippocampal/ prefrontal cortex neurogenesis (Di Liegro et al., 2019; Erickson et al., 2013). The observations that this correlation existed for the CP group but not the Other PDis group may suggest that the structured nature of the physical activity, physiotherapy or intervention programs is better tailored to the specific needs of individuals with spasticity and potentially less effective for individuals with non-spastic PDis. Effective engagement in physical activity may reduce spastic symptoms for individuals with CP. The Other PDis group showed a correlation between analog stress score and ICU mobility, indicating that higher levels of mobility are associated with lower perceived stress among individuals with non-spastic PDis. Improved mobility likely enhances independence and increases access to social and recreational activities, which become effective interventions for mitigating stress (Walsh, 2011). This correlation existing within the Other PDis group and not the CP group may be due to the diverse nature of the non-spastic PDis types, where mobility improvements may directly and significantly impact daily functioning, resulting in the possibility of reduced stress via increased mobility independence. It is possible that increased mobility within the CP group may not increase independence or access to social and recreational activities to the same degree since the spastic symptoms may still provide a social barrier to participating in activities fully.

3.4.3 Pathway Analysis

The pathway analysis illustrates potential biochemical pathway differences between those with spastic CP and those with non-spastic other types of PDis. Caffeine metabolism showed the highest impact and statistical significance with caffeine's upregulation and paraxanthine's downregulation. The significant difference in caffeine metabolism between the CP and Other PDis groups could indicate differences in caffeine intake, metabolism, or excretion, potentially influenced by variations in lifestyle, dietary habits, or metabolic capabilities associated with different types of PDis. Based on previous literature (Roehrs & Roth, 2008), the differential regulation of the caffeine pathway may even suggest that CP individuals are at a greater risk of sleep difficulties, potentially leading to a greater caffeine intake. However, one would expect that higher long-term caffeine intake would also lead to greater caffeine tolerance, creating a faster caffeine metabolism due to increased enzyme activity and adenosine receptors (Lau & Falk, 1995; Varani et al., 2000; Viswanath et al., 2023). Suppose this difference in caffeine metabolism was not linked to changes in dietary intake. In that case, altered enzyme activity in the CP group may lead to altered caffeine clearance rates and differential metabolite production, potentially affecting physiological responses to caffeine, including its stimulant effects and influence on alertness and sleep patterns (Varani et al., 2000). Moreover, it has been reported that common medications, such as antispasmodics, muscle relaxants, and anticonvulsants, may impact CYP1A2 activity, which is an enzyme associated with caffeine metabolism (Monostory et al., 2019; Stallings et al., 1996). While individuals with CP recorded higher activity levels and experienced significant muscle spasticity, caffeine metabolism may be expected to increase (LeBlanc et al., 1985). By contrast, the present data suggest that caffeine metabolism decreased, indicating reduced metabolic conversion of caffeine to paraxanthine via lower CYP1A2 enzyme activity, possibly influenced by genetic factors, medication use, physiological differences, or lifestyle factors. Conversely, individuals with non-spastic other PDis may have upregulated caffeine metabolism, leading to lower rates of caffeine excretion in the urine.

The tryptophan metabolism pathway showed moderate impact and statistical significance based on the downregulation of serotonin and upregulation of indoleacetic acid. The observed differences in tryptophan metabolism suggest that individuals with CP may have altered serotonin synthesis and degradation pathways compared to those with Other PDis (Young et al., 1980). This finding points to implications for mental health states, as serotonin levels are closely linked to mood and anxiety disorders (Myint et al., 2013). Serotonin's relationship with adverse mental health outcomes is complex and dependent upon multiple factors, such as a decrease in synaptic serotonin levels, decreased receptor sensitivity and increased synaptic enzymatic activity levels (Dell'Osso et al., 2016; Kahn et al., 1990; Myint et al., 2013). Additionally, indoleacetic acid, a breakdown product of serotonin, may vary due to differences in gut microbiota composition and function, which can influence tryptophan metabolism (Young & Gauthier, 1981). Lastly, changes in tryptophan metabolism may be linked to variations in enzyme activity, such as monoamine oxidase (MAO), which plays an essential role in the breakdown of serotonin into indoleacetic acid (Adolfsson et al., 1978). Future work should focus on determining whether changes in gut microbiome composition or enzyme activity cause the observed change in metabolite levels. A better understanding of the mechanistic reasons for alterations in tryptophan metabolism between these two groups and its direct impact on serotonin levels may have further implications on non-recordable mental health differences between those with spastic CP and those with non-spastic PDis.

3.4.4 Correlation of Metabolites with Questionnaire Data

Significant correlations between questionnaire scores and metabolic signatures occurred within each group, demonstrating that metabolic profiles may serve as robust biological indicators of mental health. Within the CP group, there is a correlation between caffeine concentration and both screen time and anxiety levels. The correlation between screen time and caffeine levels could be a coping mechanism for increased screen-related stress or fatigue. The correlation between anxiety and caffeine may have to do with individuals with CP showing slightly higher anxiety levels; furthermore, they might consume more caffeine as a coping mechanism to deal with fatigue or to maintain alertness. It is noteworthy that caffeine may exacerbate anxiety symptoms, creating a cyclical pattern where anxiety leads to increased caffeine intake, which in turn might increase symptoms of anxiety (Klevebrant & Frick, 2022). In addition, these correlations in the CP group might be attributed to specific behavioural, lifestyle, and environmental factors unique to individuals with spastic quadriplegia CP. The absence of this correlation in the Other PDis group may reflect the broader diversity in disabilities, routines, and coping strategies within this group.

The CP group also showed a positive correlation between the bin containing both serotonin and malonic acid and MDI scores. Based on the literature, one may expect a negative correlation between serotonin and MDI scores, with higher levels of serotonin being linked to a lower risk of depressive symptoms (Dell'Osso et al., 2016). It remains, therefore, speculative to propose that the positive correlation may be linked to compensatory mechanisms or dysregulation in serotonin receptors (Kahn et al., 1990; Savitz et al., 2009). Malonic acid may be related to depression; however, previous literature has corresponded that malonic acid is more likely to impact cognition due to it being an inhibitor of mitochondrial enzyme complex II (Li et al., 2020). Due to this, the accumulation of malonic acid has been speculated to cause cognitive impairments, and with the known relationship between cognition and depression, that may explain this interaction between MDI scores and malonic acid (Skonieczna-Żydecka et al., 2018; Son et al., 2018). Interestingly, this correlation was not observed for the Other PDis group, even though this group recorded slightly higher MDI scores than the CP group. This discrepancy may be linked to the more homogenous symptoms and experiences of the CP group compared to the Other PDis group, as direct physiological and psychological challenges may result in more precise correlations between depression symptoms and these biochemical markers.

Negative correlations within the CP group between hippuric acid and both PSS and PHQ scores suggest a further candidate for metabolic biomarkers of mental health. Hippuric acid metabolism is directly linked to dietary factors, the metabolism of specific amino acids, or exposure to toluene. It can be used as a liver detoxification marker directly connected to gut microbiota activity (Toromanović et al., 2008). Previous literature has shown that adverse mental health outcomes and stress can alter gut microbiota, which is crucial in producing metabolites such as hippuric acid (Westfall et al., 2021). Future research should look into these mechanisms within hippuric acid and its relationship to spasticity while measuring and quantifying the gut microbiota differences between those with spastic CP and those with non-spastic PDis. The possible microbiota activity differences should also be related to stress and depressive symptoms to further understand hippuric acid and its possible relationship with depressive symptoms and perceived stress.

Within the Other PDis group, correlational analyses revealed a positive relationship between dimethylglycine (DMG) levels and both ΔPSS1 and anxiety symptoms. Choline metabolism, which involves DMG, has been shown to have both immunomodulating properties and a protective effect on glucose metabolism (Magnusson et al., 2015). DMGs role during choline metabolism is an intermediary in the methylation cycle, supporting the synthesis of essential compounds, such as homocysteine and methionine, and maintaining cellular homeostasis (Nitter et al., 2014). Previous work has shown that glucose metabolism can be impacted by chronic stress or high-stress life events during adulthood (Tosato et al., 2021). A relative increase in stress before versus during COVID-19 may have impacted glucose metabolism to the degree where DMG levels increased along with higher levels of experienced stress. The potentially protective effects of DMG may also be the reason for the correlation between DMG and anxiety symptoms within the Other PDis group. DMG has been tested as a supplement for individuals with autism to improve mental and physical states, with mixed results, nonetheless illustrating a growing interest in the potential therapeutic effects of DMG (Bolman & Richmond, 1999). Interestingly, the correlation between DMG and ΔPSS1 was not seen in the CP group, possibly because the CP group recorded a greater change in stress and significantly higher perceived stress levels during the COVID-19 pandemic. Thus, DMG protective effects may not have been utilized or may have been overutilized. Accordingly, similar rationales might explain why the CP group did not display a correlation between DMG levels and anxiety as well.

A positive correlation was observed between ΔPSS1 and the bin corresponding to both serotonin and malonic acid for the Other PDis group. The relationship between serotonin and psychological stress was expected to be negative based on the previously mentioned connections between serotonin and mental health. Several factors may have influenced this correlation, including synaptic serotonin levels, receptor sensitivity, and synaptic enzymatic activity levels (Kahn et al., 1990; Savitz et al., 2009). Interestingly, malonic acid was correlated to ΔPSS1 and MDI in the Other PDis and CP groups, respectively, further supporting the need to explore this metabolite as a biomarker of mental health and stress impact in these populations. In addition, both questionnaires were designed for individuals with full body mobility, and there may be stressors unique to each of these groups that the selected questionnaires failed to account for. Further investigating these differences may help to advance the understanding of the intricacies characteristic of spastic CP versus non-spastic PDis.

Within the Other PDis group, the positive correlation between 2-hydroxy-2-methylbutyric acid and PSS scores during COVID-19 continues to show a trend of how high-impact stressful events such as COVID-19 uniquely impacted each of these groups. 2-Hydroxy-2-methylbutyric acid is involved in the catabolism of branched-chain amino acids, such as leucine, isoleucine and valine (Liebich & Först, 1984; Sutton et al., 2003). This pathway may reflect variations in enzyme activity, hormonal regulation, or other metabolic processes that are uniquely affected by stress in the Other PDis group (Collins et al., 2012). The CP group not displaying this correlation further showcases the difference in the response of each group to high-impact stressful events such as COVID-19. Further research should focus on this discrepancy in stress response among those with spastic CP and those with non-spastic PDis.

3.4.5 Future Directions and Limitations

This study's limitations are the small sample size, the retrospective data, the data collected over two periods, and the not ideal alignment of the groups. The differences in the matching of sex, education levels, socio-economic status, religiosity, and employment may generate the possibility of confounding variabilities persisting within the study. The collection across two summers increased the possibility of extra factors impacting metabolomic data and also created variability in the displacement of time since the referenced time points in the retrospective PSS data. Another limitation of this study is that it does not measure physical activity levels, such as VO2 max, accelerometers, or heart rate monitors. ICU mobility scores could have been physically measured as well instead of being a self-reported assessment.

Future studies should aim for a more balanced female/male ratio to ensure that gender-specific differences in stress, depression, and metabolic markers are accurately captured and analyzed. This could also lead to an understanding of sex differences within the specific symptoms and metabolic mechanisms affected by CP and Other PDis. Furthermore, expanding the study to include other subgroups of CP, such as ataxic or dyskinetic types, will provide a more comprehensive understanding of the relationship between psychological stress, depression, and metabolic processes across different types of CP. Incorporating cognitive tests can offer additional insights into how cognitive function interacts with stress, depression, and metabolic changes, particularly in individuals with PDis. Examining additional biofluids such as saliva, blood, and cerebrospinal fluid could help identify a broader range of biomarkers related to adverse mental health outcomes, providing a more detailed metabolic profile and accurate diagnostic and prognostic tools for these conditions. Conducting data collection during different periods, not close to significant events such as the end of COVID-19 lockdowns, would also help ensure that the findings are not biased by external stressors specific to a particular time frame and by retrospective assessments of subjectively experienced health outcomes. Increasing the patient population would be necessary to enhance the study's statistical power, allowing for more robust and generalizable conclusions regarding the correlations observed. Ensuring a more representative caretaker/alone ratio would assist in explaining the impact of social support and caregiving on psychological and metabolic health in individuals with PDis. Given the relevance of policymaking and health care, a more exhaustive consideration of the determinants of the quality of life and lived experiences among individuals with PDis is critical. By addressing these areas, future research can provide a deeper and more nuanced understanding of the interactions between psychological stress, depression, and metabolic processes in individuals with PDis, ultimately leading to better-targeted interventions and support strategies.

3.5 Conclusion

The findings of this proof-of-concept study highlight the complex interplay between psychological stress, mental health, and metabolite levels in individuals with spastic quadriplegia CP and other non-spastic PDis. Questionnaire data revealed higher physical activity levels in individuals with CP, with potential trends in perceived stress and anxiety levels. The retrospective analysis of perceived stress before, during, and after the COVID-19 pandemic revealed a substantial stress increase, specifically in the CP group, reflecting their heightened vulnerability to high-impact stressors. This susceptibility may be related to the unique challenges individuals with CP face, including comorbidities and disruptions to physical activity routines during the pandemic. Pathway analysis identified significant metabolomic changes pointing to altered biochemical functions in the CP group relative to the Other PDis group. These findings suggest potential neurotransmitter levels and enzyme activity variations, possibly influenced by genetic factors, medication use, or lifestyle differences. The correlation of metabolite levels with questionnaire scores further reveals a close relationship between specific metabolites and adverse mental health outcomes. Thus, the present study provides a foundation for personalized approaches to managing mental and metabolic health in individuals with PDis. The specific pathways and correlations may aid in developing targeted interventions and support strategies that improve the quality of life for individuals living with PDis.

3.6 Supplemental Figures

Table 4: Correlations between questionnaire scores. The red solid line with squares represents the CP group (n=11), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and direction of the relationships between metabolites and questionnaire responses in each group.

Group	Sample Size	Questionnaire	Questionnaire	Spearman Rho	P value	Assigned Letter
CP	10	Godin	GAD	-0.673	0.023	a
Other PDis	9	Mobility	Stress Scale	-0.709	0.033	b

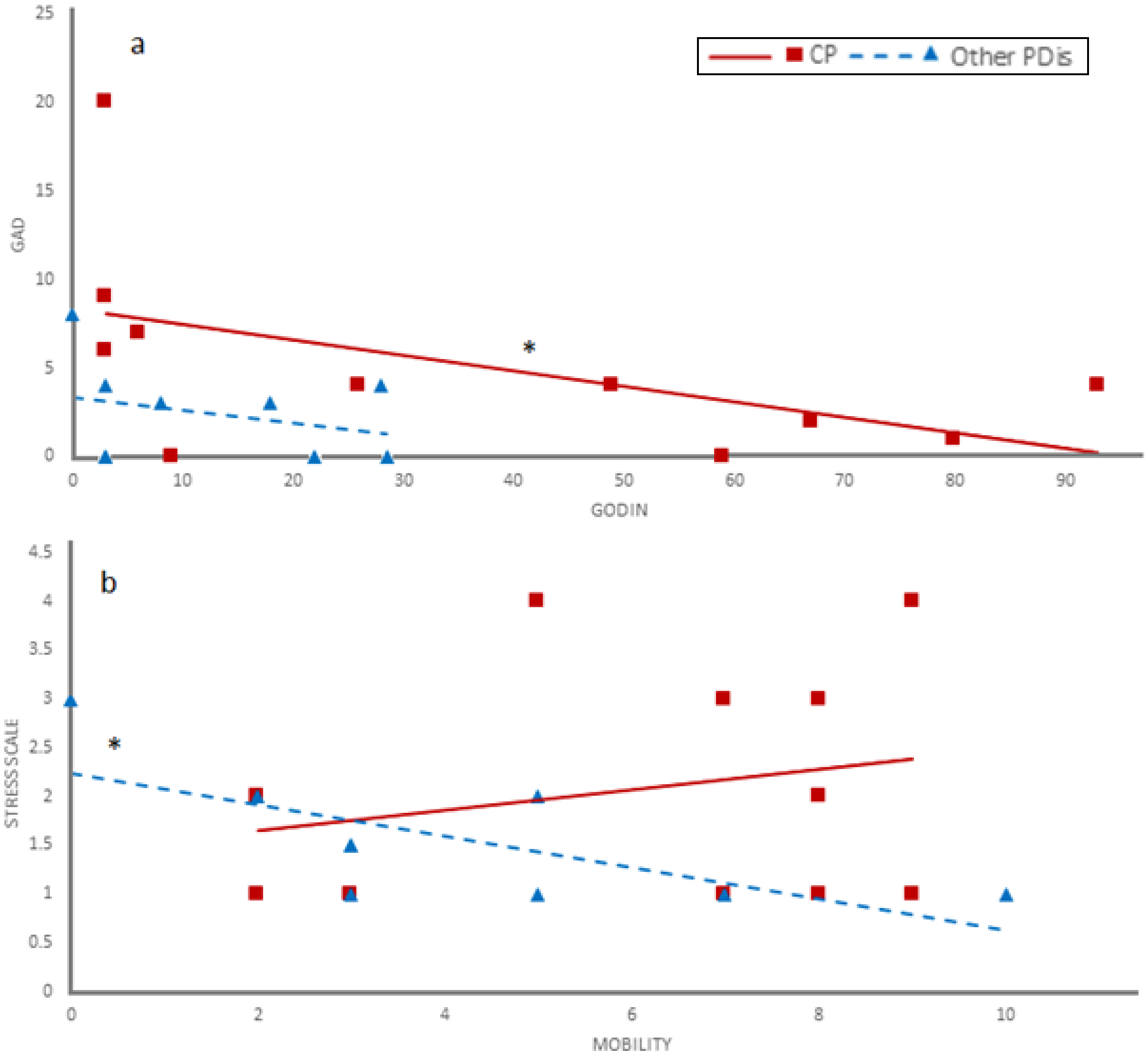

Figure 9: Correlations between questionnaire scores. The red solid line with squares represents the CP group (n=11), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank

correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and direction of the relationships between metabolites and questionnaire responses in each group. Graph A shows that within the CP group, as Godin scores increase, GAD scores decrease (p=.023, Rho=-.673). Graph B shows that within the Other PDis group, the stress scale score decreases as the ICU mobility score increases (p=.033, Rho=-.709).

Table 5: Metabolic Pathway Topology Analysis

Pathway	Total	Hits	P-Value	Impact	Metabolite(s)
Caffeine metabolism	10	2	0.002	0.692	Caffeine, Paraxanthine
Tryptophan metabolism	41	2	0.026	0.105	Serotonin, Indoleacetic acid
Histidine metabolism	16	1	0.097	0.221	Histidine

Table 6: Correlations between metabolite levels and questionnaire data are depicted in the analysis found within the CP group.

Group	**Sample Size**	**Questionnaire**	**Metabolite**	**Spearman Rho**	**P value**	**Assigned Letter**
CP	10	Screentime	Caffeine	0.799	0.006	a
CP	10	GAD	Caffeine	0.756	0.011	b
CP	10	MDI	Serotonin + Malonic Acid	0.754	0.012	c
CP	10	PSS	Hippuric Acid	-0.778	0.008	d
CP	10	PHQ	Hippuric Acid	-0.8	0.005	e
Other PDis	9	ΔPSS1	Dimethylglycine	0.837	0.005	a
Other PDis	9	GAD	Dimethylglycine	0.826	0.006	b
Other PDis	9	ΔPSS1	Serotonin + Malonic Acid	0.879	0.002	c
Other PDis	9	PSS During COVID-19	2-Hydroxy-2-methylbutyric acid	0.867	0.002	d

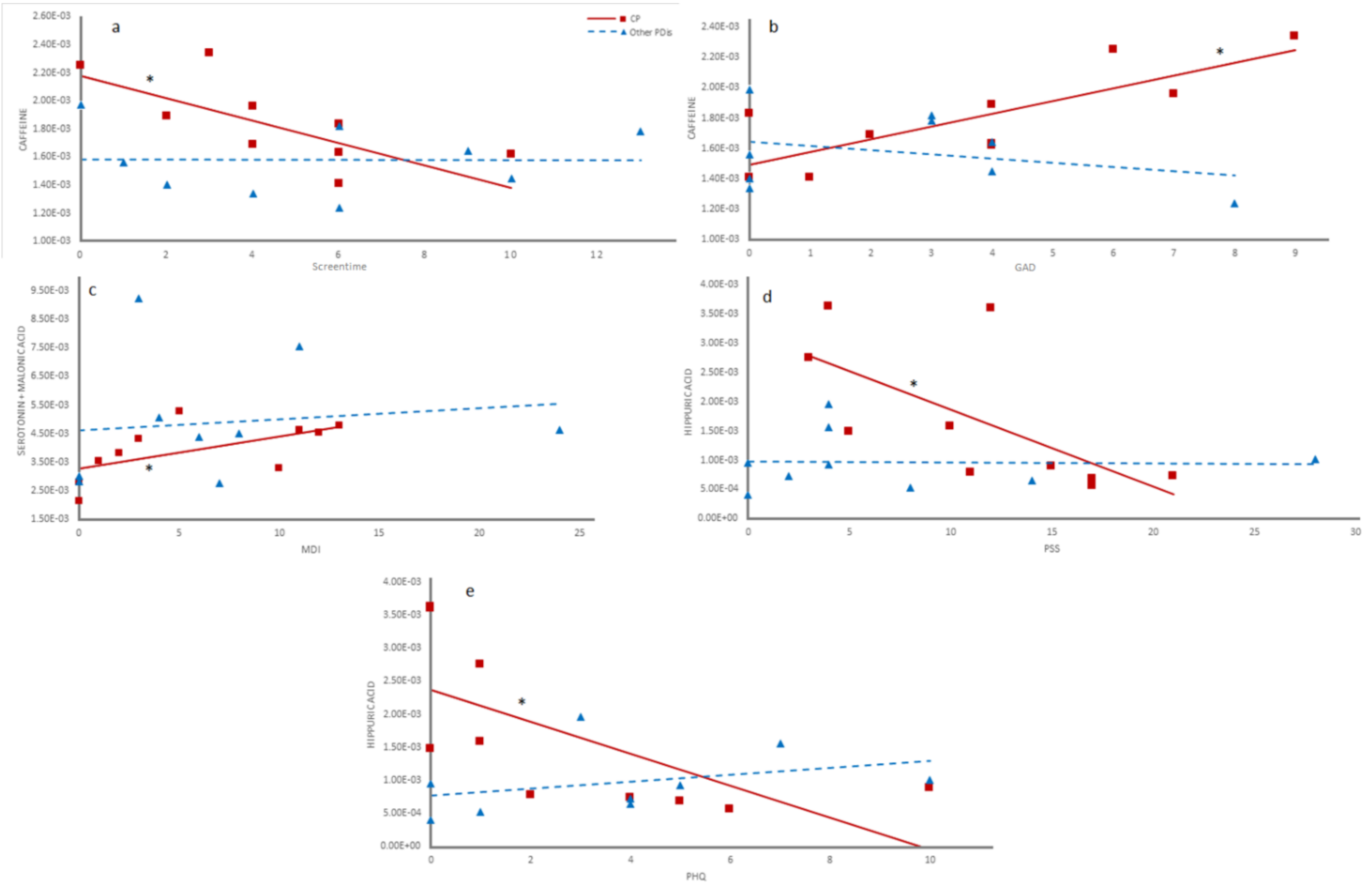

Figure 10: Correlations between metabolite levels and questionnaire data are depicted in the analysis found within the CP group. The red solid line with squares represents the CP group (n=10), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and direction of the relationships between metabolites and questionnaire responses in each group. Graph A shows that within the CP group, as screentime increases, caffeine levels have been found to decrease (p=.006, Rho=-.799). Graph B shows that as GAD scores increase, caffeine levels have increased (p=.011, Rho=.756). Graph C shows that as MDI scores increase, the bin with serotonin and malonic acid also increases (p=.012, Rho=.756). Graph D shows that as PSS scores increase, hippuric acid levels decrease (p=.008, Rho=-.778). Graph E shows that as PHQ scores increase, hippuric acid levels decrease (p=.005, Rho=-.8).

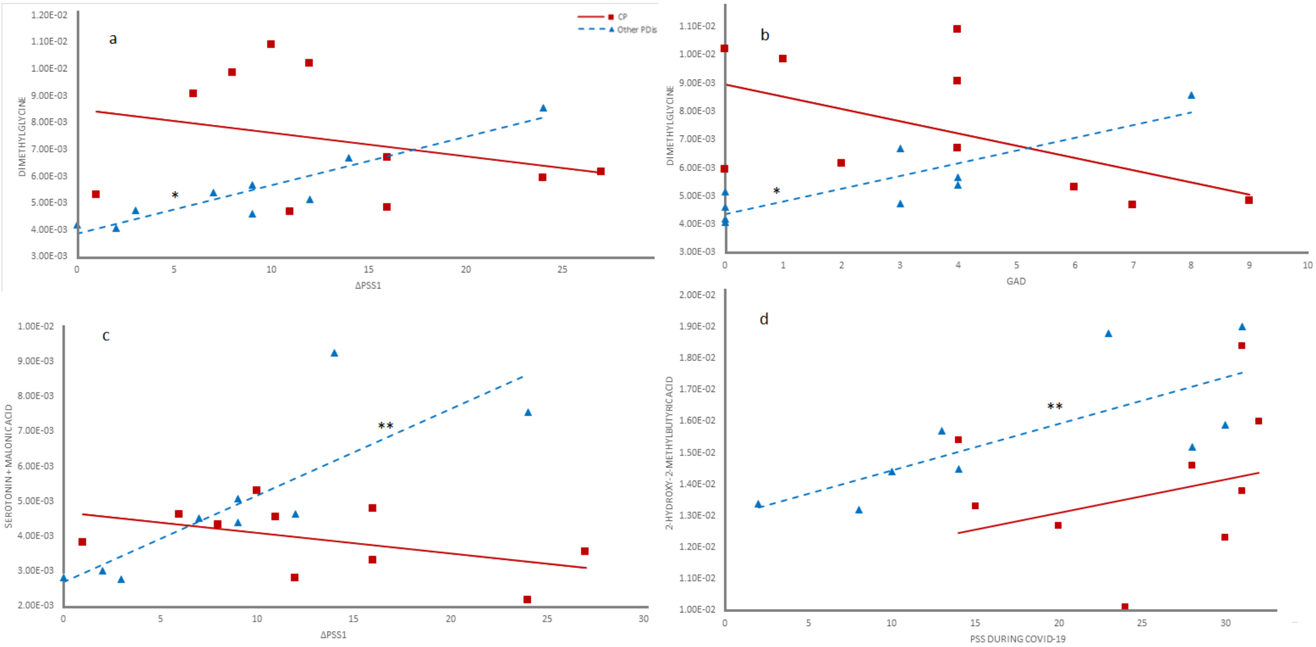

Figure 11: Correlations between metabolite levels and questionnaire data are depicted in the analysis found within the Other PDis group. The red solid line with squares represents the CP group (n=10), while the blue dotted line with triangles indicates the PDis group (n=9). All correlations were determined using Spearman's rank correlation coefficient, which is appropriate for non-parametric data. This method was chosen to assess the strength and direction of the relationships between metabolites and questionnaire responses in each group. Graph A shows that as ΔPSS1 increases, dimethylglycine levels also increase (p=.005, Rho=.837). Graph B shows that as GAD scores increase, dimethylglycine levels are seen to increase (p=.006, Rho=.826). Graph C shows that as ΔPSS1 levels increase, the bin with serotonin and malonic acid also increases (p=.002, Rho=.879). Graph D shows that as PSS during COVID-19 increases, 2-Hydroxy-2-methylbutyric acid levels also increase (p=.002, Rho=.867).

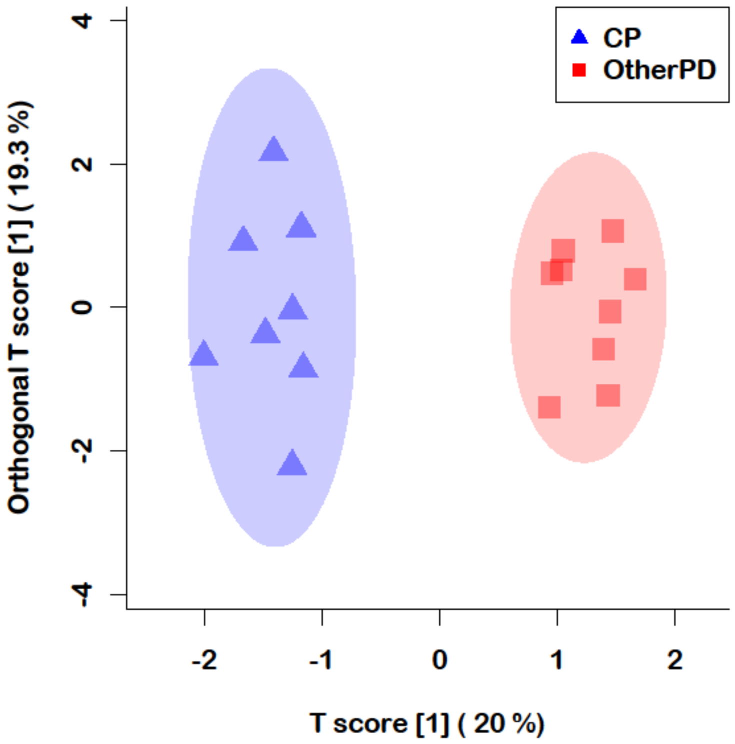

Figure 12: The plot of OPLS-DA scores for urine metabolites identified as statistically significant via VIAVC best subset testing is depicted with the female participants removed. Red squares represent the CP group (n=11), and blue triangles represent the PDis group (n=9), with each shape corresponding to a participant. The shaded regions are the 95% confidence intervals. The x and y-axis represent predictive variation (between-group differences) and orthogonal variation (within-group variation). The measure of cross-validation and permutation testing is Q2= .805. R2= .963

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Chapter 4: Conclusion

4.1 Hypotheses and Results

The goal of this thesis was to advance the understanding of those with a physical disability (PDis), their perceived stress, mental health, perceived social support levels and metabolic differences. The hypothesis of Chapter 2 posited that individuals with PDis who live with a caretaker record increased social support levels, reduced stress, fewer adverse mental health symptoms and metabolic differences relative to those with a PDis who live alone. The results showed that the two living situations did not significantly differ in the levels of perceived stress, mental health outcomes, and the amount of perceived social support. There was a trend toward altered stress levels throughout the COVID-19 pandemic between the two groups, but the differences were not statistically significant. Both groups also showed a metabolomic difference concerning metabolites that furthermore may impact biochemical pathways. Finally, specific correlations between questionnaire scores and specific metabolites were found for each group, serving as possible future interventions and treatment options.

Chapter 2 showed that individuals with PDis and their living conditions may record similar mental health and social support scores. The data showed, however, detectable differences in metabolite levels, which related to specific biochemical pathway differences between the two groups. Notable metabolic differences in the histidine, beta-alanine, alanine, aspartate, and glutamate pathways indicate potential treatment or intervention tracking areas when investigating those with PDis and their living conditions. The questionnaire and metabolite correlations also displayed psychological and biological adaptive mechanisms to specific aspects of mental health. Aspartic acid, l-isoleucine, inosine, and 3-hydroxy isovalerate metabolites may be potential biomarkers to guide therapeutic interventions. These metabolite and pathway differences may aid in developing objective assessments in clinical decision-making processes. Through this proof-of-principle study, the impact of living situations in individuals with PDis can be better understood, and this knowledge may enable the identification of ways to optimize their mental and physical well-being.

Chapter 3 hypothesized that both spastic quadriplegia CP and non-spastic PDis would exhibit comparable levels of perceived stress and adverse mental health symptoms but symptom-specific differences in metabolites and biochemical pathways. The results indicated that both groups showed comparable severity in mental health outcomes, but those with CP recorded higher perceived stress levels relating to the peak of the COVID-19 pandemic; they also performed more exercise and showed differences regarding metabolites and biochemical pathways implications compared to individuals with non-spastic PDis.

Chapter 3 revealed that individuals with spastic CP show different perceived stress and physical exercise levels but similar adverse mental health outcomes and metabolomic pathway perturbations relative to the non-spastic PDis group. The metabolic differences in caffeine, tryptophan, and histidine metabolism suggest potential avenues for biomarker discovery or therapeutic targets for clinical monitoring of PDis symptoms over time. The correlations between questionnaire scores and metabolite levels suggested that spasticity particularly impacts biochemical functions. The metabolic signatures may reflect the body's adaptation to the spastic symptoms of the PDis and the specific lived experiences. Within the CP group, the correlations with caffeine, serotonin + malonic acid and hippuric acid show possible spasticity-specific biomarkers, whereas within the group with types non-spastic PDis, the correlations with the metabolites of dimethylglycine, serotonin + malonic acid, and 2-hydroxy-2-methylbutyric acid may serve as biomarkers for specific motor disabilities not linked to muscle spasm, but rather their specific metabolic adaptations to the pathological underpinnings of their conditions or adaptations to physical constraints. Through understanding the mental health and metabolomic consequences of spasticity in various types of PDis, future research may guide strategies that enhance the well-being of both spastic and non-spastic PDis.

4.2 Limitations and Future Directions

The limitations of this thesis include a relatively low sample size, lack of female study participants and lack of matching control groups for various PDis symptoms and lifestyles. Another limitation of this study is that it does not include direct measurements of physical activity levels, such as VO2 max, accelerometry, or heart rate monitoring. Additionally, ICU mobility scores could have been assessed through objective measurements rather than relying on self-reported data. As a proof-of-principle study, the present thesis involved a cohort of individuals with PDis to address the critical gap in understanding how lived experiences with a PDis will affect mental health and quality of life. These studies will provide a solid foundation for further studies that engage more substantial sample sizes and matched controls. Due to the lack of previous studies in this area of research, the present thesis pioneers the analysis of PDis and its impacts on mental health, stress and metabolomics while attempting to identify critical needs and opportunities for possible treatments and interventions to improve the quality of life for individuals living with a PDis.

Future study comparisons within this cohort will include a PDis and non-PDis, low and high stress conditions, low and high depression symptoms, low and high anxiety symptoms, a powerchair sport intervention and differences in education and employment levels. This data will initiate a future research program that can begin to break down and conceptualize PDis, the factors that impact the PDis community and identify the most urgent areas where they lack or need more support. Using metabolomics, studies in the future will provide potential robust biomarkers for diagnosing PDis, tracking their pathway through recovery and rehabilitation efforts, and prognosticating long-term outcomes.

Future studies outside of this PDis cohort should focus on adolescent PDis, other biofluids, metabolomic differences within specific interventions, other PDis that were not included in this study, other forms of CP, and other forms of neurodevelopmental disorders and their possible comparisons to PDis. A focus should also be placed on the need for a control group with lived experience with wheelchair use and their spectrum of individual cases; through this, the variability within mobility, living conditions, and PDis can be appropriately conceptualized for their given PDis while also strengthening the understanding of how being in a wheelchair impacts an individual's mental, and biological heath. Future research into individuals with PDis, including biomarkers, mental health, stress, social support, and metabolomics, should focus on increasing the overall well-being and quality of life for those with PDis. This starts with a more robust biomarker identification process across larger sample sizes. Once biomarkers or metabolic fingerprints of those with a PDis are better understood, research should turn to interventions and treatments centred around those biomarkers. Interventions should use a longitudinal study format with several questionnaires and biological sample collection periods; the previous biomarkers and metabolites should then be tracked throughout the intervention while cross-validating with the questionnaire scores. Depending on how these interventions impact those with a PDis, measurements in the questionnaires and biological samples will determine if these interventions should be long-term community programs or areas for healthcare workers to focus on when working with those with a PDis. These interventions could hold high importance in congenital

PDis during adolescence and those who acquire a PDis immediately after the injury. The adolescence and immediate post-injury periods are critical since they are the time frames in which those with PDis are in the greatest need of adaptation, understanding, and accepting their PDis.

The area of research regarding those with a PDis, their mental health, stress, and metabolism is an area that lacks research yet is plentiful in data and possible outcomes that could help academia, mental health and health care professionals understand and conceptualize those with a PDis to lead these individuals to the most optimized versions of their lives.

Appendices

Questionnaires:

Ethics Approvals:

Physical Disabilities Health and Wellness Questionnaire

SECTION #1

Demographics

These questions will give us a better picture of the people and families who take part in this study. All information will be treated highly confidential and will not leave this study. We are trying to learn more about your personal story. Please provide us with some information that help us to understand your background. Please answer as much as you feel comfortable:

Name: ________________________________

Assigned Study Code: ______________

Age: ____________________

Sex: ____________________

Please indicate how much **stress** you have *at this moment* by marking the line between the two extremes.

|____|
1 2 3 4 5

No Stress **Stress as bad as it could possibly be**

1. What is your physical disability diagnosis? Please check.

____ Cerebral Palsy

If so what kind? ___________

____ Spinal Muscular Atrophy

If so what kind? ___________

____ Multiple Sclerosis

If so what kind? ___________

____ Stroke

If so what kind? ___________

____ Arthrogryposis

____ Spina Bifida

Other: _____________

Is there any additional information regarding your physical disability and symptoms we should be aware about?

____

____

____

At what time did you receive this diagnosis? Please provide the date as close as you can

remember. ____________________________

Other disabilities:______________________________

2. What is your current marital status? Please check.

____ Married
____ Living with a partner / Common law
____ Separated, divorced or widowed
____ Single and never married

3. What is your living situation? Please check.

_____ Living alone

_____ Living with children

_____ Living with partner/spouse

_____ Living with parents/siblings/other relatives

_____ Living with caretakers

4. If you have caretakers, how often do you need them? Please check.

_____ Always

_____ Always other than nights

_____ Only to eat and use the bathroom

_____ Only to use the bathroom

_____ Only to eat

_____ Only at night

_____ Only for transportation (driving or movement in/out of chair)

Other: __________________

5. How do you move around the majority of the time? Please check

_____ Manual Wheelchair

_____ Powerchair

_____ Walker

_____ Crutches

_____ Walking

Other _______

6. How often per week do you leave the house? Please check

_____ Rarely (never to once or twice)

_____ Sometimes (three to four times)

_____ Often (five to six times)

_____ Very frequently (seven or more times)

7. What is your employment status? Please check.

____Student / school
____ Full-time employment
____ Part-time employment
____ Minijob
____Unemployed with Unemployment Benefits
____Unemployed with Goverment subsidy

8. Are there other less common forms of transformation you use? Please check.

____ Yes
If so what is it? (ex. walker, manual wheelchair) ____________
____ No

9. What is your current occupancy?

____ House
____ Apartment
____ Group home
Other ______

10. What is your highest level of education completed?

____ Grade 9 or less
____ Some high school
____ High school diploma
____ Some college or university
____ College or university degree

11. What is your faith?

1) Christianity
2) Islam
3) Hinduism
4) Buddhism
5) Judaism
6) Other: ______________________________
7) None

12. Are you practicing your faith?

____ Yes
____ No

13. If applicable, how long have you participated in a sport (powerchair or other)?

____
If so which sports? ______
_______ Never
_______ Use to but have not for one year +

SECTION #2

Mobility Scale

Circle which number best represents your mobility. Select the highest number applicable.

	Classification	**Definition**
0	Nothing (lying in bed)	Passively rolled or passively exercised by staff, but not actively moving.
1	Sitting in bed, exercises in bed	Any activity in bed, including rolling, bridging, active exercises, cycle ergometry and active assisted exercises; not moving out of bed or over the edge of the bed.
2	Passively moved to chair (no standing)	Hoist, passive lift or slide transfer to the chair, with no standing or sitting on the edge of the bed.
3	Sitting over edge of bed	May be assisted by staff, but involves actively sitting over the side of the bed with some trunk control
4	Standing	Weight bearing through the feet in the standing position, with or without assistance. This may include use of a standing lifter device or tilt table.
5	Transferring bed to chair	Able to step or shuffle through standing to the chair. This involves actively transferring weight from one leg to another to move to the chair. If the patient has been stood with the assistance of a medical device, they must step to the chair (not included if the patient is wheeled in a standing lifter device).
6	Marching on spot (at bedside)	Able to walk on the spot by lifting alternate feet (must be able to step at least 4 times, twice on each foot), with or without assistance.
7	Walking with assistance of 2 or more people	Walking away from the bed/chair by at least 5 metres (5 yards) assisted by 2 or more people.
8	Walking with assistance of 1 person	Walking away from the bed/chair by at least 5 metres (5 yards) assisted by 1 person.
9	Walking independently with a gait aid	Walking away from the bed/chair by at least 5 metres (5 yards) with a gait aid, but no assistance from another person. In a wheelchair bound person, this activity level includes wheeling the chair independently 5 metres (5 yards) away from the bed/chair
10	Walking independently without a gait aid	Walking away from the bed/chair by at least 5 metres (5 yards) without a gait aid or assistance from another person.

Number:________

SECTION #3

Physical Activity Questionnaire

During a typical **7-day period** (a week), how many times on average, do you do the following kinds of exercise for **more than 15 minutes**.

1. **STRENUOUS EXERCISE (HEART BEATS RAPIDLY)** Examples – sweat inducing activities: Weightlifting, balance exercises, difficult physio tasks, walking/assisted walking for long periods of time.	Times per week: ____ Hours per week: ____
2. **MODERATE EXERCISE (NOT EXHAUSTING)** Examples – difficult talks but non sweat inducing: walking/ assisted walking for moderate period of time, moderately difficult physio tasks.	Times per week: ____ Hours per week: ____
3. **MILD EXERCISE (MINIMAL EFFORT)** Examples – easy tasks that still require effort: walking/ assisted walking for a short time, wheelchair sports, easy physio tasks.	Times per week: ____ Hours per week: ____

SECTION #4

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts before COVID, during COVID, and over the last month. In each case, you will be asked to indicate by circling how often you felt or thought a certain way.

0 = Never, 1 = Almost Never, 2 = Sometimes, 3 = Fairly Often, 4 = Very Often

1. How often have you been upset because of something that happened unexpectedly?

   Before COVID _____ During COVID _____ In the last month _____

2. How often have you felt that you were unable to control the important things in your life?

   Before COVID _____ During COVID _____ In the last month _____

3. How often have you felt nervous and "stressed"?

   Before COVID _____ During COVID _____ In the last month _____

4. How often have you felt confident about your ability to handle your personal problems?

   Before COVID _____ During COVID _____ In the last month _____

5. How often have you felt that things were going your way?

   Before COVID _____ During COVID _____ In the last month _____

6. How often have you found that you could not cope with all the things that you had to do?

   Before COVID _____ During COVID _____ In the last month _____

7. How often have you been able to control irritations in your life?

   Before COVID _____ During COVID _____ In the last month _____

8. How often have you felt that you were on top of things?

   Before COVID _____ During COVID _____ In the last month _____

9. How often have you been angered because of things that were outside of your control?

   Before COVID _____ During COVID _____ In the last month _____

10. How often have you felt difficulties were piling up so high that you could not overcome them?

    Before COVID _____ During COVID _____ In the last month _____

SECTION #5

Patient Health Questionnaire (PHQ-9)

Over the last 2 weeks, how often have you been bothered by any of the following problems?

	Not at all (0)	**Several days (1)**	**More than half the days (2)**	**Nearly every day (3)**
a. Little interest or pleasure in doing things.	☐	☐	☐	☐
b. Feeling down, depressed, or hopeless.	☐	☐	☐	☐
c. Trouble falling/staying asleep, sleeping too much.	☐	☐	☐	☐
d. Feeling tired or having little energy.	☐	☐	☐	☐
e. Poor appetite or overeating.	☐	☐	☐	☐
f. Feeling bad about yourself, or that you are a failure, or have let yourself or your family down.	☐	☐	☐	☐
g. Trouble concentrating on things, such as reading the newspaper or watching TV.	☐	☐	☐	☐
h. Moving or speaking so slowly that other people could have noticed. Or the opposite; being so fidgety or restless that you have been moving around more than usual.	☐	☐	☐	☐
i. Thoughts that you would be better off dead or of hurting yourself in some way.	☐	☐	☐	☐

If you checked off any problem on this questionnaire so far, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

☐ Not difficult ☐ Somewhat difficult ☐ Very difficult

☐ Extremely difficult

SECTION #6

General Anxiety Disorder (GAD-7) Questionnaire

Over the <u>last 2 weeks</u>, how often have you been bothered by the following problems? *(Use "✓" to indicate your answer")*	Not at all	Several days	More than half the days	Nearly every day
1. Feeling nervous, anxious or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid as if something awful might happen	0	1	2	3

If you checked off <u>any</u> problems, how <u>difficult</u> have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
☐	☐	☐	☐

SECTION #7

Mental Wellness Inventory (MDI)

Please check each box that identifies how you have felt over the past two weeks.

	All The Time	Most of The Time	Slightly More Than Half The Time	Slightly Less Than Half The Time	Some of The Time	At No Time
Have you felt low in spirits or sad?						
Have you lost interest in your daily activities?						
Have you felt lacking in energy and strength?						
Have you felt less self-confident?						
Have you had a bad conscience or feelings of guilt?						
Have you felt that life wasn't worth living?						
Have you had difficulty in concentrating?						
Have you felt very restless?						
Have you felt subdued or slowed down?						
Have you had trouble sleeping at night?						
Have you suffered from reduced appetite?						
Have you suffered from increased appetite?						

SECTION #8

Brief Resilience Scale (BRS)

Please respond to each item by marking one box per row	**Strongly Disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
I tend to bounce back quickly after hard times					
I have a hard time making it through stressful events.					
It does not take me long to recover from a stressful event.					
It is hard for me to snap back when something bad happens.					
I usually come through difficult times with little trouble.					
I tend to take a long time to get over set-backs in my life.					

SECTION #9

Social Support Questionnaire (SSQ)

Please respond to each item by marking one box per row	**definitely false**	**probably false**	**probably true**	**definitely true**
If I wanted to go on a trip for a day (for example, to the country or mountains), I would have a hard time finding someone to go with me.				
I feel that there is no one I can share my most private worries and fears with.				
If I were sick, I could easily find someone to help me with my daily chores				
There is someone I can turn to for advice about handling problems with my family				
If I decide one afternoon that I would like to go to a movie that evening, I could easily find someone to go with me.				
When I need suggestions on how to deal with a personal problem, I know someone I can turn to				
I don't often get invited to do things with others.				
If I had to go out of town for a few weeks, it would be difficult to find someone who would look after my house or apartment (the plants, pets, garden, etc.)				
If I wanted to have lunch with someone, I could easily find someone to join me.				
If I was stranded 10 miles from home, there is someone I could call who could come and get me.				
If a family crisis arose, it would be difficult to find someone who could give me good advice about how to handle it				
If I needed some help in moving to a new house or apartment, I would have a hard time finding someone to help me.				

SECTION #10

Visual Analog Scales

1) Please indicate how much **stress** you have *at this moment* by marking the line between the two extremes.

1 2 3 4 5

No Stress

Stress as bad as it could possibly be

2) How would you rate your overall well-being at this moment?

1 2 3 4 5

Excellent well-being

I do not feel well at all

3) Approximately how many hours of screen time (smartphone, TV, computer, video games) did you have yesterday?

0 1-2 3-4 5-6 7-8 9-10 11-12 >13

Hours of screen time

Fanshawe College Research Ethics Board Review

Approval Notification of Proposed Research

Involving Human Participants at Fanshawe College

Protocol Number:	23-06-12-1
Principal Researcher(s):	Dr. Gerlinde Metz / Chase Petruska
Research Protocol Title:	Mental Health and Metabolomic Biomarkers in Individuals with Physical Disabilities
Research Project Start Date:	June 2022
Expected Date of Termination:	January 2025
Documents Reviewed:	Protocol; Consent Form; Questionnaire; Project Description

Based solely on the ethical considerations raised by the research proposed in the application, the Research Ethics Board has completed its delegated review of the above research proposal and **Approved** the project on June 27, 2023.

Comments and Conditions:

Please note that the REB requires that you adhere to the protocol reviewed and approved by the REB. The REB must approve any modifications to the protocol before they can be implemented.

Researchers must report to the Fanshawe REB:

a) any changes which increase the risk to the participants;
b) any changes which significantly affect the conduct of the study;
c) all adverse and/or unexpected experiences in the course of carrying out the study;
d) any new information which may adversely affect the safety of the participants or the conduct of the study.

Ethics approval of this protocol is for a period of one (1) year from the approval date above.

☐ Researchers must submit an REB Amendment/Extension form if research continues beyond this period. ☐ Upon completion, researchers must submit an REB Annual Review/Status Update form.

ETHICS APPROVAL DOES NOT CONSTITUTE PERMISSION TO CONDUCT THE RESEARCH; OTHER INSTITUTIONAL APPROVALS MAY BE REQUIRED TO CONDUCT THE RESEARCH PROJECT.

Members of the FCREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the FCREB.

June 27, 2023

Steve Crema, BA, MA
Date

Chair, Research Ethics Board

Fanshawe College

Approval Form

Date:	May 14, 2024
Study ID:	Pro00120950
Principal Investigator:	Gerlinde Metz
Study Title:	Mental Health and Metabolomic Biomarkers in Individuals with Physical Disabilities
Approval Expiry Date:	May 13, 2025
Sponsor/Funding Agency:	Alberta Innovates Health Solutions

Thank you for submitting the above study to the Health Research Ethics Board - Health Panel. Your application has been reviewed and approved on behalf of the committee.

Approved Documents:

Recruitment Materials

Recruitment Emails

Consent Forms

PD Consent Form.pdf

Questionnaires, Cover Letters, Surveys, Tests, Interview Scripts, etc.

PD Questionnaire.pdf

Protocol/Research Proposal

PD Project Description

Any proposed changes to the study must be submitted to the REB for approval prior to implementation. A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date, you will have to re-submit an ethics application.

Approval by the REB does not constitute authorization to initiate the conduct of this research. The Principal Investigator is responsible for ensuring required approvals from other involved organizations (e.g., Alberta Health Services, Covenant Health, community organizations, school boards) are obtained, before the research begins. Sincerely,

Anthony S. Joyce, PhD.
Chair, Health Research Ethics Board - Health Panel