

MATERNAL FACTORS AND THEIR IMPACT ON THE PLACENTAL METABOLOME

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DEDICATION

I dedicate this thesis to my parents, Juanita and Kevin, for their unconditional love and support since they brought me into this world.

ABSTRACT

Maternal stress during pregnancy has been correlated with adverse maternal and fetal health outcomes. The biological mechanisms underlying stress-linked risk and resilience factors, however, are still not fully understood. The placenta is a vital organ in mediating maternal-fetal interactions, and its function may reflect adaptations to psychological, environmental, and pharmacological exposures by the mother. This thesis includes two complementary studies that utilized nuclear magnetic resonance (NMR) spectroscopy-based metabolomics in conjunction with multivariate and univariate statistical approaches to examine whether distinct prenatal exposures are associated with metabolic signatures in placental tissue. The first study examined maternal prenatal depression (MPD), with or without selective serotonin reuptake inhibitor (SSRI) treatment, and its potential associations with placental metabolic profiles. This study identified unique placental metabolomic patterns associated with MPD without SSRI exposure, whereas placentas from mothers who were depressed and taking SSRIs were not statistically separable from non-depressed and non-SSRI-exposed controls. The second study explored prenatal maternal stress effects on placenta samples from mothers who were exposed to the 2011 Queensland Flood. Objective hardship and subjective distress measures were quantified using validated questionnaires to classify high and low stress groupings. Results indicated no discernable differences between high and low stress groups in either objective or subjective stress categories in placental metabolic profiles. Together, these studies demonstrate the utility of placental metabolomics for examining prenatal psychological, pharmaceutical, and environmental exposures with suggestions for improvement in future research.

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ETHICS STATEMENT

All research procedures were approved by institutional ethics boards. Secondary analysis of previously collected, de-identified placental tissue and associated clinical data for the VSSRI and QF2011 study chapters was approved by the University of Alberta Health Research Ethics Board – Health Panel (Molecular Signatures of Prenatal Maternal Stress and Fetal Programming, March 16, 2026, Pro00163708). Participants in the VSSRI/V-Norm cohorts were recruited under ethical approval from the University of British Columbia and the Children’s and Women’s Health Centre of British Columbia Research Ethics Board (certificate H12-0073340), and the respective metabolomics analyses were approved under UBC certificate H16-02280. Data collection and recruitment for the QF2011 study were approved by the Mater Health Services Human Research Ethics Committee (QF2011: The effects of the Queensland Flood on pregnant women, their pregnancies, and their children’s early development, HREC/13/MHS/36; amendment HREC/13/MHS/36/AM05 [1844M], December 8, 2014), the University of Queensland ethics committee (#2013001236), and the University of Lethbridge Human Subject Research Committee (HSRC Protocol #2015-75, October 7, 2015). All participants provided written informed consent.

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TABLE OF CONTENTS

Chapter 1: Introduction	1
1.1 General Introduction	1
1.2 The Developmental Origins of Health and Disease (DOHaD).....	3
1.3 Biological Mechanisms of Stress During Pregnancy.....	5
1.4 Adverse Effects of Stress in Pregnancy	8
1.5 Stress Classification and the Modern Context of Maternal Adversity.....	11
1.6 Modern Environmental Factors in Maternal Health and Stress.....	16
1.7 Metabolomics and Other Systems Biology Approaches to Studying Maternal Stress	20
1.8 Metabolomics Approaches in Placental Function and Biomarker Discovery	24
1.9 NMR Spectroscopy and Placental Analysis.....	27
1.10 ¹ H NMR Metabolomics Procedures for Placental Research.....	31
1.11 Biomarkers, Research Objectives and Study Design.....	34
1.12 References.....	40
Chapter 2: Maternal Prepartum Depression and Prenatal Exposure to SSRI Antidepressants Programs the Human Placental Metabolome.....	83
2.1 Abstract	85
2.2 Introduction.....	86
2.3 Materials and Methods.....	88
2.3.1 Subjects	88
2.3.2 Sample Collection.....	89
2.3.3 Metabolite Extraction.....	89
2.3.4 NMR Sample Preparation and Data Acquisition	90
2.3.5 Statistical Methods.....	90
2.3.6 Maternal Mood and SSRI Exposure Group Designation.....	91
2.3.7 Metabolite Identification and Pathway Topology Analysis	92
2.4 Results.....	92
2.4.1 Establishing a HAM-D Cut-off Value for Control Cohort.....	92
2.4.2 Comparisons Between Control Mothers and Mothers with Depression but no SSRI Treatment	93
2.4.3 Comparisons Between Control Mothers and Depressed Mothers with SSRI Treatment	94

2.4.4 Metabolite Correlations with HAM-D Score.....	95
2.5 Discussion.....	96
2.5.1 Placental Glutamate Regulation and Potential Impacts on Fetal Neurotransmission..	96
2.5.2 Lysine Metabolism Provides a Potential Mechanistic Link Between Maternal Depression and Fetal Development	97
2.5.3 Placental Serine and Potential Impacts on Fetal Nervous System Function	98
2.5.4 Placental Proline Homeostasis May Influence Fetal Development.....	99
2.5.5 D-Glutamine and D-Glutamate Pathway Regulation is Essential for Healthy Pregnancy Outcomes	99
2.5.6 Arginine and Proline Pathway Regulation May Directly Impact Placental Function	101
2.5.7 Limitations	102
2.6 Conclusion	102
2.7 References.....	104
2.8 Table and Figures	115
2.8.1 Tables	115
2.8.2 Figures.....	119
2.8.3 Supplemental Materials	124
Chapter 3: Natural Disaster Impacts on the Placental Metabolome: The QF2011 Queensland Flood Study.....	131
3.1 Abstract	132
3.2 Introduction.....	133
3.3 Materials and Methods.....	136
3.3.1 Participants.....	136
3.3.2 Sample Collection.....	137
3.3.3 Metabolite Extraction.....	138
3.3.4 NMR Sample Preparation and Data Acquisition	139
3.3.5 Statistical Methods.....	139
3.3.6 Group Creation for Analysis	140
3.3.7 Metabolite Identification and Pathway Topology Analysis	141
3.4 Results.....	141
3.5 Discussion.....	142
3.5.1 PNMS and the Placental Metabolome	143
3.5.2 Timing and Severity of Stress Exposure.....	143

3.5.3 Midwifery Care as a Protective Factor	143
3.5.4 Future Directions	144
3.6 Conclusion	144
3.7 References.....	146
3.8 Tables and Figures	153
3.8.1 Tables	153
3.8.2 Figures.....	155
3.8.3 Supplemental Materials	159
Chapter 4: Conclusion.....	161
4.1 Hypotheses and Results	161
4.2 Limitations	163
4.3 Future Directions	166
4.4 Overall Contributions.....	169
4.5 References.....	171

LIST OF TABLES

CHAPTER 2

Table 2-1: Cohort demographic characteristics.....	115
Table 2-2: Statistically significant placental metabolites for the comparisons of mothers with depression and no SSRI treatment versus control group.....	117
Table 2-3: Comparison of the correlation results between serine and HAM-D scores when using different cut-offs for the control and depression HAM-D scores.....	118
Supplemental Table 2-1. Statistical data for the pathway topology analysis.....	129
Supplemental Table 2-2. The results from VIAVC and Mann Whitney U Test statistical analysis for comparisons of the placental profile of control mothers against mother with depression with and without SSRI treatment.....	130

CHAPTER 3

Table 3-1. Sample distribution statistics.....	153
Table 3-2. Summarized results of 2 level stress comparisons.....	153
Supplemental Table 3-1. Sample distribution statistics based on supplemental analysis with 3 level stress classification and midwifery program participation.....	154
Supplemental Table 3-2. Summarized results of supplemental 3 level analysis for QFOSS and COSMOSS stress comparisons.....	154

LIST OF FIGURES

CHAPTER 2

Figure 2-1. Orthogonal Projections to Latent Structures Discriminant Analysis score plots comparing mothers with depression and no SSRI treatment to the control group	120
Figure 2-2. Receiver Operating Characteristic curves comparing mothers with depression and no SSRI treatment to the control group.....	122
Figure 2-3. Pathway topology analysis.....	123
Supplemental Figure 2-1. Orthogonal Projections to Latent Structures Discriminant Analysis score plots for placental tissues comparing the metabolomic signatures from mothers with depression and SSRI treatment to those of the control group	125
Supplemental Figure 2-2. Receiver Operating Characteristic curves for placental tissues comparing the metabolomic signatures from mothers with depression and SSRI treatment to those of the control group	127
Supplemental Figure 2-3. Serine concentration correlation with HAM-D scores plot.....	128

Chapter 3

Figure 3-1. Orthogonal Projections to Latent Structures Discriminant Analysis score plots for placental tissues that passed modeling from all comparisons ran in the 2 level QFOSS and COSMOSS high vs low stress tests	156
Figure 3-2. Receiver Operating Characteristic curves for placental tissues that passed modeling from all comparisons ran in the 2 level QFOSS and COSMOSS high vs low stress tests.....	158
Supplemental figure 3-1. Orthogonal Projections to Latent Structures Discriminant Analysis score plots for placental tissues that passed modeling from all comparisons ran in the subsequent 3 level QFOSS and COSMOSS high vs low stress tests.....	159
Supplemental figure 3-2. Receiver Operating Characteristic curves for placental tissues that passed modeling from all comparisons ran in the 3 level QFOSS and COSMOSS high vs low stress tests.....	160

LIST OF ABBREVIATIONS

11 β -HSD2	11 β -Hydroxysteroid dehydrogenase type 2
ACTH	Adrenocorticotrophic hormone
ADHD	Attention-deficit/hyperactivity disorder
ANS	Autonomic nervous system
ASD	Autism spectrum disorder
AUC	Area under the curve
CBT	Cognitive behavioural therapy
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
COSMOSS	COMposite Score for Mothers' Subjective Stress
COVID-19	Coronavirus disease of 2019
CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
D2O	Deuterium oxide
DAB	Dynamic adaptive binning
DOHaD	Developmental Origins of Health and Disease
DSS	3-Trimethylsilylpropanesulfonate
EI	Electron ionization
ESI	Electrospray ionization
FID	Free induction decay
GABA	Gamma-aminobutyric acid
GAD	Generalized anxiety disorder
GC-MS	Gas chromatography-mass spectrometry
HAM-D	Hamilton Depression Rating Scale
HMDB	Human Metabolome Database
HPA	Hypothalamic-pituitary- adrenal axis
IES-R	Impact of Event Scale-Revised

IPCC	Intergovernmental Panel on Climate Change
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS	Liquid chromatography–mass spectrometry
M@NGO	Midwives@New Group practice Options
MPD	Maternal prenatal depression
MS	Mass spectrometry
MS-MS	Tandem mass spectrometry
MW	Wilcoxon-Mann-Whitney U test
NMDA	N-methyl-D-aspartate
NMR	Nuclear magnetic resonance
	Orthogonal Projection to Latent Structures-Discriminant Analysis
OPLS-DA	Predictive adaptive response
PAR	Principal component analysis
PCA	Peritraumatic Dissociative Experiences Questionnaire
PDEQ	Peritraumatic Distress Inventory
PDI	Peritraumatic Distress Inventory Questionnaire
PDI-Q	Partial least squares-discriminant analysis
PLS-DA	Prenatal maternal stress
PNMS	Paraventricular nucleus
PVN	Queensland Flood Study (2011)
QF2011	

QFOSS	Queensland Flood Objective Stress Scale
RF	Radiofrequency
ROC	Receiver Operating Characteristic curve
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricarboxylic acid
TSP	3-Trimethylsilylpropionic acid
VIAVC	Variable importance analysis based on random variable combination

Chapter 1: INTRODUCTION

1.1 General Introduction

Pregnancy is one of the most sensitive periods of human life, during which maternal and environmental conditions can have profound effects on both the pregnant mother and the fetus. Several maternal stressors have been shown to affect fetal development, including anxiety and depression (Pinto et al., 2017), pharmaceutical and narcotic drug use (Hudson et al., 2023; Barry et al., 2021), nutritional deficiencies (Jain et al., 2022), maternal illness and disease (Yu et al., 2022), and environmental hardships such as natural disasters and toxic exposures (Ünsel-Bolat, 2024; Krausová et al., 2023). These factors have been linked with pregnancy complications, such as fetal growth restriction (Lewis et al., 2016), metabolic dysfunction (Rinaudo & Wang, 2012), immune dysregulation (Veru et al., 2014), and neurodevelopmental disorders in pre-exposed offspring (Makris et al., 2023). However, pregnancy complications are not limited to just the fetus. Pregnant individuals that experience excessive stress and adversity are also at an increased risk for health complications, such as preeclampsia (Zhang et al., 2013), gestational diabetes (Mishra et al., 2020), infection, and illness (Coussons-Read, 2013).

Stress during pregnancy may arise from various circumstances, including psychological trauma, chronic anxiety, economic hardship, medical complications, or a sudden severe change in living conditions caused by an environmental disaster. Notably, the recent coronavirus disease of 2019 (COVID-19) pandemic has exacerbated maternal stress exposure on a global scale (Padmaja & Behera, 2023; Caffieri et al., 2024; Liu et al., 2024; Zhou et al., 2021). Similarly, an increase in the occurrence of natural disasters due to climate change has been attributed to growing rates of displacement, loss of housing, and toxic environmental exposure, all of which

can significantly disrupt maternal well-being and pregnancy outcomes (Bekkar et al., 2020; Veenema et al., 2023).

Stress is not a uniform phenomenon and can be measured through both objective metrics (biological and physiological markers, financial loss, damage to property, etc.) and subjective metrics (cognitive appraisals of distress, fear, or loss of control). These dimensions, however, are not always commensurate with one another (King et al., 2015). On the other hand, *resilience*, which arguably lacks a uniform definition but can otherwise be described as the capacity of an individual to adapt positively to adversity, is a related but distinct feature in evaluating stress subjectively and objectively (O'Donohue et al., 2021). Some individuals may score high on objective stress measurements but report low subjective distress, while others may report high depression or anxiety in response to comparatively less severe exposures. The mediating factor in these contrasting examples is resilience, and therefore uncovering the relationship between stress and resilience is essential to developing effective interventions based on each individual's unique resilience factors.

To examine the biological impact of maternal stress, some researchers have focused on the placenta as a mediator of the maternal-fetal interface. The placenta is a metabolically active, hormone producing, and immune modulating organ, which also responds to maternal physiological conditions and adapts the fetus to them (Burton & Fowden, 2015; Sferruzzi-Perri & Camm, 2016). Placental metabolomics, particularly performed using nuclear magnetic resonance (NMR) spectroscopy, provides a methodological path for identifying and quantifying metabolic changes associated with maternal stress and resilience, and may even support the development of early biomarkers for at-risk pregnancies (Emwas et al., 2013).

1.2 The Developmental Origins of Health and Disease (DOHaD)

The concept of early-life conditions shaping long-term health outcomes has become a widely accepted framework in biomedical sciences, which is known as the Developmental Origins of Health and Disease (DOHaD) hypothesis (Faa et al., 2024). This idea emerged from the work of British epidemiologist David Barker, who in the late 1980s observed strong correlations between low birth weight and increased risk of ischemic heart disease-related deaths in adulthood (Barker & Osmond, 1986). From there, Barker's hypothesis emerged, which posited that adverse intrauterine conditions could *program* the structure and function of developing organs and consequently predispose individuals to later life health problems (Kwon & Kim, 2017). This theory has since sparked a paradigm shift in the scientific perspective and understanding of disease origins.

Early evidence supporting the DOHaD theory emerged from epidemiological studies, famously from those involving children born during the Dutch Hunger Winter (1944-1945). These studies reported that prenatal exposure to famine is linked with increased rates of metabolic and psychiatric disorders later in development (Lumey, 2016; Roseboom et al., 2011). Additional support came from studies of institutionalized Romanian orphans, whereby deprived early life conditions were correlated with persistent neurodevelopmental challenges despite adoption and environmental enrichment (Nelson et al., 2023). These findings, among several other seminal works, have contributed to the establishment of DOHaD as a major focus of research by the early 2000s, resulting in the formation of the International Society for Developmental Origins of Health and Disease in 2003 and a growing series of annual conferences and international collaborations.

While the DOHaD framework originally focused on nutrition, it has since expanded its interests to encompass a broad range of early exposures, including maternal stress and mental illness (Sugsihita, 2024), infection (Fitzgerald et al., 2020), environmental pollutants (Saldiva et al., 2023), and socioeconomic adversity (McEwen et al., 2019). In particular, maternal stress is recognized as a potential fetal programming factor. It has been associated with a multitude of maladaptive outcomes, such as preterm birth (Janssen et al., 2023), low birth weight (Matsas et al., 2023), and increased risk for neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD; Grizenko et al., 2008) and anxiety (McLean et al., 2018). Moreover, longitudinal studies have correlated prenatal stress exposure to an increased risk of cardiovascular disease (Gu & Guan, 2021), obesity (Lamichhane et al., 2020), and immune dysfunction (Veru et al., 2014) in developing offspring.

The biological interference of stress during gestation is thought to involve multiple interconnected systems (neuroendocrine, immune, metabolic, and epigenetic) which coordinate fetal adaptations to maternal conditions. Although these adaptations may confer short-term survival benefits, they may come at the cost of heightened vulnerabilities if postnatal environmental conditions do not match prenatal expectations. For example, a fetus programmed for nutrient scarcity may be maladapted to a postnatal environment of caloric abundance, thus increasing the risk for metabolic syndrome in later life: a phenomenon hypothesized as the *predictive adaptive response* (PAR), with unintended consequences (Gluckman et al., 2008). However, alternative theories, such as the *silver spoon model*, refute the logic of PAR by arguing that adverse health outcomes are caused by constrained prenatal development, which reduces the plasticity to adapt to later-life conditions (Hayward & Lummaa, 2013). Regardless, the interconnected systems of prenatal development do demonstrate adaptive functions in response

to maternal environmental conditions *in utero*, and whether these adaptations persist beyond parturition remains a subject for debate.

It is important to note that not all individuals exposed to prenatal stress develop abnormally, which underscores the role of resilience as a key moderating factor. Resilience can manifest biologically, psychologically, or socially, and may help buttress against the negative effects of prenatal adversity. Interventions designed to elevate maternal resilience, such as social assistance programs (Koch et al., 2021), midwifery care (Fikre et al., 2023), psychotherapy and mental health counseling (Nana et al., 2023; Metz et al., 2025), have shown promising results in improving birth outcomes, such as reduced risks for preterm birth and low birth weight. However, the precise mechanisms undergirding stress and resilience are not completely understood. In this context, the search for prenatal stress biomarkers has gained significant momentum.

Biomarkers may enable early screening of at-risk pregnancies and offer insight into how psychological, pharmaceutical, or structural interventions may alter maternal-fetal dynamics. This makes the examination of stress-responsive tissues, such as the placenta, a potential research priority. The placenta is a highly adaptable organ that interfaces between the maternal and fetal environments, which positions it as a unique target for insights into fetal programming. By understanding the molecular dynamics that occur at the placenta in response to maternal stress, we may uncover targets for early clinical intervention, thus guiding both preventative and therapeutic strategies.

1.3 Biological Mechanisms of Stress During Pregnancy

Stress can result in complex physiological processes, which evolved to help organisms respond to environmental threats. It incorporates an integrated network of neural, endocrine, and

autonomic responses, often initiated by the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS; Papadimitriou & Priftis, 2009). In temporally short periods, this system is adaptive, allowing the rapid mobilization of energy, increased awareness, and protection from bodily harm. However, during chronic periods of stress, the persistent activation of these systems can have potentially toxic physiological effects, especially during vulnerable states such as pregnancy.

Central to the biological stress response is the HPA axis. When a threat is perceived, the paraventricular nucleus (PVN) of the hypothalamus releases corticotropin-releasing hormone (CRH), stimulating the pituitary gland's secretion of adrenocorticotropin hormone (ACTH) into the bloodstream (Dedovic et al., 2009). ACTH then travels to the adrenal cortex to stimulate the release of cortisol, which is the body's primary glucocorticoid (Dedovic et al., 2009). In tandem, the sympathetic nervous system is engaged, simultaneously releasing catecholamines (epinephrine and norepinephrine) from the adrenal medulla (Khalil et al., 2024). These hormonal cascades result in the "fight-or-flight" response, causing increased heart rate, raised blood glucose levels, suppressed digestive and reproductive functioning, and heightened cognition and attention (Cannon, 1915/2010; Chu et al., 2024). Apart from acute survival needs, cortisol also supports several other functions, such as the wakening response and regulating the circadian rhythm (Law & Clow, 2020), modulating the immune response (Cain & Cidlowski, 2017), and regulating bone metabolism (Mallorie & Shine, 2022).

During typical conditions, the body returns to baseline arousal by engaging the parasympathetic nervous system as soon as the stimulus threat subsides. Cortisol exerts a negative feedback loop on the hypothalamus and pituitary to shut down the stress response and restore equilibrium, allowing the body to "rest and digest" (Porges, 2010). In the case of chronic

stress, this feedback loop becomes dysregulated, and cortisol remains elevated in the bloodstream for extended periods, which can then accumulate and damage organs and tissue in what is sometimes called *allostasis* or *allostatic load* (O'Toole et al., 2025). This shift from adaptive to maladaptive stress can become especially problematic during pregnancy.

Cortisol also plays a fundamental role in healthy fetal development. Low levels of cortisol from the maternal blood supply are permitted to cross the placenta's semi-permeable membrane under strictly regulated conditions until the third trimester, whereby increased supplies of cortisol pass to support the growth of vital fetal organs such as the lungs, while also aiding in neuronal maturation in the brain (Davis & Sandman, 2010). During the first two trimesters, low levels of maternal cortisol are permitted to cross the placenta's semi-permeable membrane. During the third trimester, however, cortisol supplies are increased in order to support the growth of vital fetal organs and aid neuronal maturation in the brain (Davis & Sandman, 2010). For example, Davis and Sandman discovered that elevated cortisol exposure in early gestation was associated with lower child mental development scores after one year post-partum (Davis & Sandman, 2010). On the other hand, increased maternal cortisol in late gestation produced the opposite effect, including accelerated cognitive development and higher mental development scores (Davis & Sandman, 2010). Nonetheless, excessive maternal cortisol during any trimester may overwhelm the placenta's protective mechanisms. Important to these mechanisms, the placental-bound enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) is responsible for converting biologically active cortisol into its inactive form, cortisone (Salvante et al., 2017). When maternal cortisol levels are chronically elevated, such as during prolonged periods of high stress, 11 β -HSD2 expression is downregulated, which reduces the

placenta's ability to occlude the fetus from excessive glucocorticoid exposure (Harris & Seckl, 2011).

1.4 Adverse Effects of Stress in Pregnancy

Unmitigated glucocorticoid exposure can impose significant detriments to a developing fetus. Elevated cortisol has been linked to lower birth weight (Bolten et al., 2011), preterm birth (Oaks et al., 2022), altered immune development (Von Hertzen, 2002), and long-term neurobehavioral changes in pre-exposed offspring (Sandman et al., 2011). Animal studies further demonstrated that excessive prenatal glucocorticoid exposure can induce alterations in hippocampal and amygdalar architecture (Charil et al., 2010), impair neurogenesis in dentate gyrus (Kino, 2015), and lead to hyperreactivity to stress postnatally (McGowan & Matthews, 2018). In humans, excess maternal cortisol has been associated with increased risk of emotional dysregulation (Korja et al., 2017), anxiety (Davis & Sandman, 2012), ADHD, and autism spectrum disorder (ASD) symptoms in prenatally exposed children (Ronald et al., 2011). Additionally, it is argued throughout animal and human epidemiological research that prenatal or early postnatal stress experiences pose epigenetic consequences (DNA methylation, miRNA expression, and histone modifications) leading to altered genetic regulation in the placenta and brain, with greater risks for ADHD, ASD, depression, and schizophrenia across generations (Babenko et al., 2015).

Compared to the general population, the maternal experience of stress during gestation is unique in several important ways. Pregnancy involves the reorganization of the maternal neuroendocrine system to support fetal development. As an example, the maternal HPA axis becomes progressively upregulated as gestation advances through trimester stages, with circulating maternal cortisol being increased two to four-fold compared to pre-pregnancy levels

(Irwin et al., 2021). This natural response may complicate the detection of abnormal cortisol elevations and makes it difficult to discern pathological stress in pregnancy. Moreover, it is possible that hormonal and immunological shifts during gestation may have individualized effects for how pregnant individuals respond to psychosocial challenges (Bjelica et al., 2018).

It is also important to consider the timing of maternal stressors due to the dynamically complex nature of gestation from the beginning stages of pregnancy until delivery. The fetus traverses three distinct phases of development (germinal, embryonic, and fetal) *in utero*, each with significant growth milestones often broken down into three roughly equal trimesters that total about 40 weeks. The first trimester (weeks 1-12) is characterized by the development of major organs and body structures, as well as the transition of the embryo becoming a fetus at week 9, with facial features, limbs, fingernails, and teeth buds forming (Donovan & Cascella, 2022; American College of Obstetricians and Gynecologists, 2018; Munteanu et al., 2021). In the second trimester (weeks 13-27), rapid growth and sensory development occurs, with the fetus starting to kick and suck its thumb, and the lungs progress through branching and canalicular development (American College of Obstetricians and Gynecologists, 2018; Rehman & Bacha, 2023; Schittny, 2017). Finally, during the third trimester (weeks 28–40), the fetus undergoes rapid maturation, accumulating more than 80% of total body fat and preparing for birth. Lung development accelerates as type II pneumocytes increase production of surfactant, reaching functionally sufficient levels near term to allow effective breathing after delivery (American College of Obstetricians and Gynecologists, 2018; Rehman & Bacha, 2023).

The unique growth trajectory of the fetus *in utero* creates time-specific windows of heightened vulnerability, meaning that exposure to adversity, such as maternal stress, can have different impacts depending on which physiological systems are developing at the moment of

exposure. In one example, maternal stress caused by an earthquake in Chile during the first trimester was correlated with smaller head circumferences and shorter body lengths in pre-exposed offspring (Palmeiro-Silva et al., 2018). It is inferred that early phase exposure is an exceptionally vulnerable period for stress interference with profound consequences for organ and structural development (Tadanki et al., 2025). However, other studies argue that developmental programming is most sensitive in the third trimester, known as the *window of vulnerability*, because maternal stress experienced in this phase is frequently associated with premature delivery with underdeveloped neurological systems, as well as socioemotional problems, such as anxiety, depression, and aggression later in life (Lammertink et al., 2021; Berthelon et al., 2021).

Common causes of stress during pregnancy include prepartum depression and anxiety (Field et al., 2010; Field, 2017), interpersonal conflict (Westdahl et al., 2007), financial insecurity (Seely & Mickelson, 2019), and physical discomfort like nausea, chronic pain, and fatigue caused by physiological changes associated with the progressing stages of pregnancy (Bai et al., 2016; Beyazit & Sahin, 2018). It is important to address that these stressors may exert biological effects regardless of whether they are internally perceived or externally measured. For this purpose, the distinction between subjective and objective experiences of stress is crucial to determine inter-individual differences in fetal outcomes. Some individuals demonstrate remarkable resilience even when faced with objectively extreme degrees of hardship, while others may exhibit high physiological stress responses to comparatively low hardship (King et al., 2015).

In recognition of inter-individual variation, researchers have sought nuanced approaches in investigating maternal stress (Levinson et al., 2023), aiming to compartmentalize subjective and objective appraisals while giving special consideration to factors that promote resilience,

such as social support (Shishehgar et al., 2015), access to healthcare (Lobel & Preis, 2025), and midwifery care (Kildea et al., 2018). This is especially relevant given today's global challenges. Faced with climate change, the aftermath of a global pandemic, and rising mental health burdens, there is a growing urgency to understand the biological effects of prenatal stress and develop effective prevention, screening, and intervention strategies. Hence, clarifying the nuances of stress will directly enhance the effectiveness of future clinical and social interventions, helping to mitigate the growing health challenges for individuals and their communities.

1.5 Stress Classification and the Modern Context of Maternal Adversity

Understanding the complexities of prenatal maternal stress requires a thorough exploration of its many forms. To reiterate, stress is not a singular phenomenon. Rather, it is better conceptualized as a multifaceted experience shaped by numerous influences, such as individual perception, preexisting health conditions, and environmental or social circumstances. This diverse array of factors has prompted some researchers to develop classifying frameworks for subjective (internal experience or perception of distress) and objective (quantifiable measures of hardship) stresses. These perspectives may sometimes overlap yet are not identical and overall enhance the observation and understanding of risk and resilience in pregnancy outcomes.

Subjective stress often involves psychological symptoms such as anxiety, depression, or fear that is reported by the individual. These features are typically measured using standardized assessments, often as questionnaires, such as the Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960) or the Peritraumatic Distress Inventory (PDI; Brunet et al., 2001), which calculate the severity of a target symptom by assessing a participant's responses to various symptom-specific questions. By contrast, objective stress may be assessed using questionnaires that calculate quantifiable dimensions of hardship that are independent of internal emotional

appraisal but are measurable through external data. These tests can also be tailor-made to address the particular stressor or event of research interest. For example, the Queensland Flood Objective Stress Scale (QFOSS) scores participants' experience of objective hardship following the devastating 2011 Queensland flood in Australia, and is measured among parameters of financial loss, damage to property, and difficulty with insurance companies (King et al., 2015). Importantly, two individuals exposed to the same stressor may respond differently. One may demonstrate remarkable resilience while the other may exhibit strong biological and behavioural signs of distress. This variability reflects the psychological differences in coping and biological differences in stress reactivity, which underscores the need for research approaches that utilize both perspectives (King & Laplante, 2005). Among the many causes of maternal stress, depression is one of the most widely studied and clinically relevant. Depression affects approximately 20.7% of pregnant mothers worldwide, with rates rising since the onset of the COVID-19 pandemic and climate related disasters (Yin et al., 2021). Maternal prenatal depression (MPD) is problematic because of its demonstrable harm towards both the mother and the developing fetus. For mothers, MPD reduces quality of life by increasing the risk for sleep disturbances, appetite changes, fatigue, and reduced engagement in parental care (Carlson et al., 2025). There are also risks for preeclampsia, prolonged labour (Quiñones et al., 2023), ischemic heart disease, arrhythmia, cardiac arrest, and cardiomyopathy, especially postpartum (Ackerman-Banks et al., 2023). The fetus is also significantly impacted by MPD, with increased risks for preterm birth, low birth weight, intrauterine growth restriction (Grote et al., 2010; Babu et al., 2020), abnormal brain development and organization (Reissland et al., 2018; Sandman et al., 2015; Roos et al., 2022), colic, infections (Coburn et al., 2018), and delayed motor and cognitive neurodevelopment (Chen et al., 2025). Given the broad variety of complications that can arise

for both mother and child, it is crucial that mothers are equipped with effective strategies to allay the impacts of MPD.

A particularly complicated issue in perinatal medicine is the use of antidepressants to treat MPD, due to controversy over potential effects these drugs may have on the fetus. Selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed antidepressants throughout the general population, with 2023 CDC data indicating that 11.4% of adults 18 years of age or older had them prescribed in the United States (National Center for Health Statistics, 2024). Furthermore, women (15.3%) were more than twice as likely to be prescribed SSRIs compared to men (7.4%; National Center for Health Statistics, 2024). Recent estimates for antidepressant usage among pregnant women in the US range from 6% to 8% (Suarez et al., 2022). These medications increase the availability of the neurotransmitter serotonin in neuronal synaptic clefts by inhibiting its reuptake, modulating mood and cognitive function (Harmer et al., 2017).

While SSRIs are generally well tolerated by most patients, the contraindications they may pose for pregnant individuals and their pre-exposed offspring are still uncertain. On one hand, serotonin is recognized across numerous animal studies to be an essential modulator for neuronal cell migration and proliferation during fetal development (Bonnin et al., 2011; Côté et al., 2007; Migliarini et al., 2013). On the other hand, SSRIs taken by mothers have been shown to readily cross the placental barrier and can expose the fetus to elevated levels of serotonin, which may have detrimental effects on development (Ellenberger et al., 2025). Human epidemiological studies have associated maternal SSRI use with an increased prevalence of preterm delivery, intrauterine growth restriction, and abnormal neurobehavior in some infants (Oberlander et al., 2009). Additionally, these associations include increased risks for lowered Apgar scores, a test

used since the 1950's to assess heart rate, respiratory effort, reflex irritability, muscle tone, and colour of newborns at birth (Apgar, 1953; Rubarth, 2012; Jensen et al., 2013). Other complications include persistent pulmonary hypertension (Alwan et al., 2016), and ASD (Houssari et al., 2017); however, these risks are relatively low on a population scale and some findings remain mixed and highly context-dependent (Alwan et al., 2016; Houssari et al., 2017).

Importantly, reaching consensus of antenatal antidepressant usage safety is stymied by inconsistent results throughout the literature. A large population-based Finnish study of over 845,000 pregnancies between 1996 and 2010 found asymmetrical effects of antidepressants, including lower risk for late preterm birth and very preterm birth in SSRI-exposed offspring compared to non-exposed offspring from mothers who had psychiatric conditions (Malm et al., 2015). Meanwhile, offspring born to SSRI-treated mothers were at a higher risk for neonatal complications, such as low Apgar scores, while the risk among unexposed mothers was significantly lower compared to SSRI-treated mothers and untreated mothers with psychiatric conditions (Malm et al., 2015). A follow up study in 2016 by the same authors found a cumulative 8.2% incidence of depression diagnoses in SSRI pre-exposed adolescents compared to 1.9% of mothers in the untreated group, while rates of ASD and ADHD were not affected by treatment status (Malm et al., 2016). These results raise the important question of whether risks associated with antenatal SSRI usage offset the risks associated with untreated antenatal psychiatric conditions.

Prescribed SSRIs represent a large and varied group of pharmaceutical drugs. Depending on the brand of drug, there are differences in chemical composition and pharmacodynamics, such as half-life, potency in placental permeability, and metabolic processing, which may result in distinct fetal exposures and effects (Zakaraya et al., 2024; Hendrick et al., 2003; Domingues et

al., 2023). For example, fluoxetine has an elimination half-life of about 1 to 4 days, whereby its active metabolite, norfluoxetine, has a half-life of 7 to 15 days (Altamura et al., 1994). In placental studies, fluoxetine shows relatively higher cord : maternal ratios compared to other SSRIs (Hendrick et al., 2003). Sertraline has a shorter half-life (~22 to 36 hours) and a less metabolically active metabolite (desmethyl-sertraline), which tends to demonstrate comparatively lower cord : maternal ratios at delivery (Hendrick et al., 2003; De Vane et al., 2002). Furthermore, paroxetine has a half-life of ~21 to 24 hours and is a strong cytochrome P450 2D6 (CYP2D6) inhibitor, while also showing relatively lower placental passage compared to fluoxetine and citalopram but only marginally less than sertraline (Hemeryck & Belpaire, 2002; Hendrick et al., 2003; Schoretsantis et al., 2021). Citalopram and escitalopram are in-between in terms of half-life (~35 hours and ~27 to 33 hours, respectively) and exhibit substantial placental transfer (Heikkinen et al., 2002; Rao, 2007; Hendrick et al., 2003). Altogether, these compounding complexities of treated versus untreated and unique chemical composition for each drug exacerbate the confusion and hesitancy mothers face when deciding what is best for them and their child.

Based on the different pharmacological responses, clinicians prescribing SSRIs face a challenging dilemma. Treating maternal depression is crucial for preventing maternal and fetal harm yet doing so with drugs may introduce separate risks. This conundrum highlights the need for individualized treatment methods that are supported by unambiguous biological data, such as metabolomic and genetic biomarkers. It also underscores the need to understand both the mechanistic pathways through which maternal depression and SSRIs act and the potential markers that could be used to inform clinical treatment options.

1.6 Modern Environmental Factors in Maternal Health and Stress

Beyond individual psychological conditions, maternal stress is influenced by global and environmental events and crises. For example, the recent COVID-19 pandemic forced governments worldwide to rapidly implement public-health measures, which often disrupted access to healthcare, social supports, employment, and overall mental and physical well-being (Padmaja & Behera, 2023; Zhou et al., 2021; Li et al., 2023; Liu et al., 2024). Arguably, pregnant mothers faced disproportionate levels of mental health struggles and isolation. One study reported that generalized anxiety disorder (GAD) rates were 3- to 4-fold higher in pregnant women than the general population during the first wave of the pandemic (Berthelot et al., 2020). In a 2023 umbrella study of 25 systematic reviews and 12 meta-analyses from 45 countries during the beginnings of the COVID-19 pandemic up to April 2023, the pooled prevalence of antenatal depression was 29% and 31% for antenatal anxiety, equating to roughly 1 in 3 pregnant women affected globally (Caffieri et al., 2024). While these numbers are not confirmed to be caused by the pandemic directly, a 2021 study of 2 systematic reviews and meta-analyses up to February 2019 showed a pooled prevalence of antenatal depression across 173 studies and 182 reports at 20.7%, indicating a marked increase from pre-pandemic rates (Yin et al., 2021).

Like COVID-19, climate change and global environmental emergencies have become a new frontier for perinatal science. Events such as ice storms (King & Laplante, 2005), forest fires (Evans et al., 2022), and floods (King et al., 2015) are increasing in frequency and severity according to the most recent Intergovernmental Panel on Climate Change (IPCC) report (2023), posing significant threats to maternal physical and mental health as the crisis progresses (Olson et al., 2019). Pregnant individuals exposed to natural disasters have exhibited varying physiological distress markers, such as higher levels of cortisol found in hair (Gao et al., 2014; Howells et al., 2023) and saliva (Kotozaki & Kawashima, 2012), which are indicative of HPA

axis dysregulation. Moreover, pregnant mothers affected by climate-related disasters were at significantly higher risk for high blood pressure and preeclampsia (Kyoze et al., 2018; Rezaei et al., 2022), as well as gestational diabetes (Preston et al., 2020). Pre-exposed fetuses were also found to be disproportionately at risk for preterm birth and immune dysfunction (Moghadam et al., 2024).

The 2011 Queensland Flood in Australia represents a particularly well-documented case of prenatal disaster exposure. This flood took place in early January of 2011 and was reputed to be one of the worst in 30 years, affecting more than 200,000 people across 70 townships (Van den Honert & McAneney, 2011). Three quarters of the province were declared a disaster zone with more than \$2 billion of damages reported and 23 lives lost (Van den Honert & McAneney, 2011). The Queensland Flood Study (QF2011) was established to investigate the impacts of prenatal maternal stress (PNMS) caused by this sudden yet devastating disaster, adapting and expanding on the methods of the similarly themed Project Ice Storm (King et al., 2015).

The QF2011 project measured objective hardship and subjective stress using uniquely crafted questionnaires, which allows for the differentiation between tangible effects of disaster and the mother's emotional and cognitive response, both of which were predicted to influence maternal physiology and fetal development (King et al., 2015). Additionally, study participants volunteered in biological sampling, placental tissue collection, and repeated developmental assessments of offspring at time points ranging from 6-12 weeks up to 4 years of age (King et al., 2015). So far, findings from the QF2011 project extend beyond the scope of this thesis but are worth noting. For example, Simcock et al. in 2017 found that prenatal exposure to flood-induced stress was linked with alterations in infant neurodevelopment, such as motor and cognitive domains, within the first year of life (Simcock et al., 2017). Also, McLean et al. in 2018 showed

that different elements of PNMS (objective hardship, cognitive appraisal, and subjective distress) had distinct and additive associations with anxiety symptoms in early childhood (McLean et al., 2018).

In response to the challenges of maternal stress, regardless of its sources, various intervention strategies have been proposed and tested to reduce stress and bolster maternal resilience. One of these strategies includes expressive writing, a method first proposed in the 1980s by James W Pennebaker (Pennebaker, 1995). This method involves relatively brief sessions of expressive writing (about 15-20 minutes), with the intention to reduce anxiety symptoms by off-loading worries from working memory (Olson et al., 2019). Observed benefits from this approach include relative improvements in maternal biochemical markers involved in immune and physiological functioning (Olson et al., 2019), as well as improved cognitive outcomes for offspring (Arabin et al., 2021).

Another promising maternal intervention strategy is a combined gynecological and psychotherapy approach. Published results from a German cohort study found that regular prenatal psychotherapeutic and gynecological care was associated with a lower rate of preterm birth compared with standard care (Metz et al., 2025). Infants born to mothers in the psychotherapy group also reported greater birth weights, heights, and head circumferences. Specifically, this study included routine sessions of gynecological examination and various types of counseling, such as personalized system-oriented psychosomatic therapy, solution focused therapy, salutogenesis, and couple therapy (Metz et al., 2025). Other forms of counseling can include cognitive behavioural therapy (CBT). CBT is an approach attributed to Dr. Aaron T. Beck in the 1960s and is the first talking therapy proven to be more effective than medication for treating depression (Beck & Fleming, 2021; Blackburn et al., 1981). This method focuses on an

individual's interpretations of situations and how they influence their emotional, behavioural, and physiological reactions, rather than the situations themselves (Beck & Fleming, 2021).

Maladaptive underlying beliefs that individuals may have about themselves and the world around them are challenged, which helps correct distorted thinking patterns and modifies behaviour, which ultimately improves symptoms of anxiety and depression (Beck & Fleming, 2021).

Numerous studies over the past decade support CBT as an intervention in prenatal care for anxiety, depression, insomnia, and stress (Burns et al., 2013; Manber et al., 2019; Li et al., 2022; Pettman et al., 2023). Furthermore, other studies have linked these maternal benefits with improved birth outcomes. One neuroimaging study found that prenatal CBT was associated with a decrease in overall child DNA-methylation and white matter fiber-bundle cross-section in areas involved with cognitive function and stress response, as well as increased cortical thickness (Bleker et al., 2020). Other studies have shown promising links between prenatal CBT and a reduction in low birth weight and short gestational age rates (Siebach et al., 2024), as well as improved newborn Apgar scores (Karamoozian & Askarizadeh, 2015).

Music therapy, which involves actively or passively listening to music, is another simple yet effective intervention that has been studied for decades. This activity offers several physiological benefits, such as promoting the release of serotonin and acetylcholine to lower heart rate and blood pressure (Betegón et al., 2017), as well as a reduction in the secretion of cortisol into the bloodstream (Garcia-Gonzalez et al., 2018), which altogether help induce states of relaxation (Shimada et al., 2021). A recent systematic review of 23 studies on musical therapy for pregnant mothers from 2009 to 2019 reported numerous benefits, including decreased maternal anxiety, pain from labour, stress, depression, as well as improvements in quality of sleep and fetal bonding (Shimada et al., 2021).

One more promising intervention that was broached in a previous section is midwifery care, which emphasises continuity of care, emotional support, and informed decision-making throughout pregnancy and childbirth (Peters et al., 2020). Midwifery models, including those elected in the Midwives @ New Group practice Options (M@NGO; Tracy et al., 2011), have been associated with perceived improvements in emotional support and quality of care (Allen et al., 2019), as well as fewer caesarean sections before the onset of birth and higher unassisted vaginal birth rates (Hartz et al., 2013). Furthermore, mothers affected by the QF2011 flood who also opted for midwifery care showed improved offspring outcomes at 6 months of age in fine motor and problem-solving Ages and Stages Questionnaire (ASQ-3) domains (Simcock et al., 2018). In this manner, these prevention models may act as a buffer against chronic stress, improving maternal well-being and mood while also benefiting birth and fetal outcomes.

The alarming trends of global stressors, paired with the advances in maternal health care and research, make it essential to identify early biological signs of distress that could inform screening and intervention efforts. Metabolomics, a scientific study that can detect subtle biochemical alterations across a variety of substrates, may offer a promising route forward. By examining placental metabolites in response to stress, scientists may be able to identify molecular signatures of vulnerability and resilience, thus opening a lab-based path for personalized medicine in obstetrics.

1.7 Metabolomics and Other Systems Biology Approaches to Studying Maternal Stress

Emerging over two decades ago, systems biology is an integrative framework that examines how multiple layers of biological information interact across genes (genomics), transcripts (transcriptomics), proteins (proteomics), and metabolites (metabolomics; Chuang et al., 2010). Due to its ability to capture a more comprehensive picture of underlying cellular

mechanisms, researchers have increasingly adopted systems biology approaches into their investigations of health and disease, with some focusing on maternal stress and fetal development specifically (Yadav et al., 2022; King et al., 2025; Sookoian et al., 2013). As a major pillar of the systems biology framework, metabolomics is the study of small molecules that represent the downstream products of gene expression, enzyme activity, and protein synthesis (Chen et al., 2022; Putri et al., 2013). Metabolites can offer a lens into the dynamic physiological state of a cell, tissue, or organism at a specific moment in time, and may be indicative of biomarkers for stress exposure and the biochemical pathways involved in fetal programming.

Metabolites are defined as small molecules ($< 1,500$ daltons) that are involved in or produced by metabolic reactions (Badillo-Sanchez et al., 2023). This definition can fit a wide range of chemical compounds, including amino acids (alanine, lysine, tyrosine, etc.; Lopez & Mohiuddin, 2024), sugars (glucose, fructose, lactose, etc.; Holesh et al., 2023), lipids (triglycerides, phospholipids, sterols, etc.; Chandel, 2021a), nucleotides (purines, pyrimidines, deoxyribonucleotides; Chandel, 2021b), ketones (acetoacetate, beta-hydroxybutyrate, acetone; Huang et al., 2024), and alkaloids (morphine, caffeine, nicotine, etc.; Aniszewski, 2015), among numerous others (Johnson et al., 2016). Metabolites act as substrates, intermediates, and end products within biochemical pathways that underpin cellular function. They are integral to energy production by way of glycolysis and the tricarboxylic acid (TCA) cycle (Kierans & Taylor, 2024; Arnold & Finley, 2023), intracellular and intercellular signaling through molecules such as cyclic adenosine monophosphate (cAMP) and nitric oxide (Aslam & Ladilov, 2022; Lundberg & Weitzberg, 2022), and the detoxification of xenobiotics via conjugation pathways in the liver (Surendradoss et al., 2023). Moreover, fluctuations in metabolite concentrations can

reflect and influence cellular states, making them both functional regulators and dynamic indicators of physiological and pathological conditions (Patti et al., 2012). The metabolome, meanwhile, refers to the entire complement of metabolites that can be identified within a specific biological system or substrate (e.g., plasma and urine; Zamboni et al., 2015). As such, metabolomes are constantly shifting in response to environmental factors, dietary intakes, disease states, pharmacological interventions, and psychological and physiological stress.

While genomics, transcriptomics, and proteomics offer insights into the regulation of biological systems, they primarily indicate potential or intermediate upstream changes in cellular function. By contrast, metabolomics determines the small-molecule end products and intermediates of metabolism, which provide a direct capture of the organism's current biochemical state (Patti et al., 2012). While stress-responsive genes may be transcriptionally upregulated, metabolomics can test whether those upstream signals translate into functional changes. For example, functional metabolic changes may be observed in altered glycolytic flux and glucose utilization (Monteiro et al., 2019; Lee et al., 2023); rerouting or depletion of amino acids such as tryptophan (via kynurenine-pathway induction) or branched-chain amino acids (Boyle et al., 2015; Manoli & Venditti, 2016); and lipid signatures of disrupted mitochondrial β -oxidation (e.g. acylcarnitine patterns; Guerra et al., 2022; Trongtrakul et al., 2021). The functional proximity of metabolomics therefore makes it suitable to detect real-time effects of maternal stress on placental function and fetal development, where adaptations in energy metabolism (Vaughan & Fowden, 2016), redox state (Schoots et al., 2018), and neurotransmitter precursors can have lasting developmental impacts (Rosenfeld, 2021; Sferruzzi-Perri & Camm, 2016; Burton & Fowden, 2016).

Important distinctions between metabolomics and other “omic” approaches include temporal sensitivity and context dependence. DNA sequences are relatively stable and protein abundance is dynamically renewed on characteristic timescales, but metabolite profiles can shift within minutes to hours based on dietary intake (Weinisch et al., 2022), circadian phase and sleep deprivation (Hancox et al., 2021), or acute/chronic stress exposure (Kuti et al., 2022; Gary et al., 2024). This responsiveness is essential to the detection of subtle physiological changes on short timescales, but it also introduces potential confounding factors such as recent food intake, caffeine (Cornelis et al., 2018), diurnal variation (Gu et al., 2019; Hancox et al., 2021), pharmaceutical drug use (e.g. SSRIs and antibiotics; Bhattacharyya et al., 2019; Jaskiw et al., 2020), and ambient pollution (Liang et al., 2025). A robust experimental design is therefore important, which should include standardized sampling (e.g. time-of-day, prandial state), matched controls, pooled quality-control samples, and explicit strategies for pre-analytical handling planned a priori.

Metabolomic analyses can be conducted on a variety of biological substrates, including blood and serum (Dunn et al., 2011; Gowda et al., 2008; Duarte et al., 2014; Psychogios et al., 2011), urine (Beckonert et al., 2007; Bouatra et al., 2013), cerebrospinal fluid (CSF; Yan et al., 2021; Wishart et al., 2008), saliva (Dame et al., 2015), and tissue (Lin et al., 2007), each offering unique advantages and limitations depending on the research objectives and circumstances (Wishart, 2019). Blood and urine are readily obtainable substrates, with urine collected non-invasively and blood-based assays considered to be minimally invasive approaches that can reflect the instantaneous metabolic status of the donor (Mal, 2016; Wawrzyniak et al., 2024; Gowda et al., 2008). Saliva can index neuroendocrine status (e.g. free cortisol) in circadian contexts (Kawanishi et al., 2019), and CSF can detect neuroinflammation in central nervous

system disease (Yan et al., 2021). Yet, to uncover how maternal stress specifically affects the maternal-fetal interface, placental tissue is increasingly prioritized because it integrates maternal and fetal signals whose metabolome directly reflects intrauterine conditions (Mohammad et al., 2021).

1.8 Metabolomics Approaches in Placental Function and Biomarker Discovery

The placenta is a metabolically active, highly specialized organ whose functions govern nutrient and gas transfer, endocrine signaling, immune protection, and fetal programming (Burton et al., 2016; Elshenawy et al., 2020). Hence, its functional state is tightly linked to offspring health and development. Metabolic profiling of placental tissue can reveal how maternal stressors are translated into biochemical alterations with developmental implications (Walejko et al., 2018). For example, the placenta synthesizes serotonin from maternally supplied tryptophan early in gestation and provides 5-HT to the fetal brain (Bonnin et al., 2011; Karahoda et al., 2020). This pathway is highly sensitive to fluctuations in maternal condition. Inflammatory states can reduce placental serotonin, and SSRIs have been shown to disrupt signaling of placental 5-HT (Bondarenko et al., 2024; Rosenfeld, 2020). Similarly, the placenta adjusts how it transports and uses fatty acids to match the maternal lipid environment. In maternal obesity or diabetes and during inflammation, these processes are disrupted, whereby transport shifts and β -oxidation is reduced (Duttaroy & Basak, 2022; Calabuig-Navarro et al., 2017). Moreover, oxidative stress markers that indicate glutathione-mediated defenses shift in placental disorders such as preeclampsia (Aouache et al., 2018).

Previous studies demonstrated that placental metabolite signatures differ in pregnancies affected by sundry maternal health conditions compared to controls (Yang et al., 2021; Fattuoni et al., 2018; Lien et al., 2020; Zhang et al., 2022; Yeramilli et al., 2023; Monni et al., 2021; Wong et

al., 2021; Troisi et al., 2023). In one study by Wong and colleagues, targeted lipidomics of placental tissue in prenatal depression identified unique phospholipid and sphingolipid signatures that also predicted early socio-emotional behaviours in offspring (Wong et al., 2021). Another study found placental lipids and lipid-like molecules involved in the biosynthesis of fatty acids, especially linoleic acid and α -linolenic acid, were significantly associated with gestational diabetes mellitus diagnoses (Yang et al., 2021). Further, a maternal obesity study by Fattuoni et al. showed differences in placental metabolites involved in nucleotide production, antioxidant defenses, and lipid synthesis among obese mothers, reflecting intrauterine environment changes that may enhance the risk for developmental abnormalities later in life (Fattuoni et al., 2018). Complementary disaster-exposed cohorts from Hurricane Sandy and Project Ice Storm also showed that prenatal natural disaster exposure reprograms placental molecular signatures via reorganization of the placental transcriptome in immune, endocrine, and vascular pathways, and were associated with altered child stress-hormone phenotypes, such as child HPA dysfunction, anxiety, and aggression (Nomura et al., 2021; Ping et al., 2020). Together, the above studies' findings support the plausibility of disruptions in tryptophan/serotonin metabolism, fatty-acid oxidation, and glutathione-centered antioxidant defenses being stress-sensitive placental pathways.

Apart from identifying detrimental patterns, metabolomics also enables biomarker discovery, which is the identification of single or multiple metabolite panels (including ratios) that differentiate exposure states or predict adverse obstetric outcomes such as preeclampsia and fetal growth restriction (Kenny et al., 2010; Lee et al., 2020; Sovio et al., 2020; Conde-Agudelo et al., 2024). Specifically, early pregnancy and mid-trimester metabolic signatures have demonstrated predictive power for preeclampsia in independent participant groups and even

sometimes outperform traditional endothelial biomarkers (like anti- and pro-angiogenic factors ratios, placental growth factor, and soluble fms-like tyrosine kinase-1; Kenny et al., 2010; Lee et al., 2020). Moreover, the ratio of the products for the relative concentrations of positively associated 1-(1-enyl-stearoyl)-2-oleoyl-GPC and 1,5-anhydroglucitol to the product of the relative concentrations of negatively associated 5α -androstane- $3\alpha,17\alpha$ -diol disulfate and N1,N12-diacetylspermine may significantly predict fetal growth restriction at term (Sovio et al., 2020). Finally, a 2019 study reliably classified three distinct placental dysfunction phenotypes (normal, moderate, and severe) using metabolomics in correlation with Flt-1 (FMS-like tyrosine kinase receptor 1) and preeclampsia and/or fetal growth restriction, remarkably providing information for high-risk pregnancies that traditional diagnostic approaches previously could not identify (Austdal et al., 2019).

Multiple analytical instruments are available for metabolomics, notably liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy (Gika et al., 2014; Garcia et al., 2010; Emwas et al., 2019). As a brief overview, LC/GC-MS generally provide superior sensitivity and broader coverage of low-abundance compounds, often detecting hundreds to thousands of features in a single sample (Dunn et al., 2011). In contrast, NMR excels in absolute quantification, reproducibility, and non-destructive analysis with minimal sample preparation, which are invaluable qualities for tissue studies where minimum sample weight requirements may be difficult to meet and for scalable clinical pipelines (Markley et al., 2017; Emwas et al., 2019; Huang et al., 2022). Despite NMR being less sensitive than LC/GC-MS, continuing advancements in instrument power, portability, and quantitative workflows are expanding dynamic range and metabolic coverage, reinforcing the viability and practicality of NMR for

future metabolomics research (Nagana Gowda & Raftery, 2022; Yu et al., 2021; Laurencin & Ashbrook, 2021).

1.9 NMR Spectroscopy and Placental Analysis

NMR is an analytical technique that can be utilized to identify and quantify metabolites with minimal sample preparation and highly reproducible results (Emwas et al., 2019; Mal et al., 2020; Crook & Powers, 2020). In placental metabolomics, NMR provides a reliable and non-destructive method to acquire a placental metabolic fingerprint, preserving limited tissue specimens for downstream analyses. In contrast, mass-spectrometry workflows often require extensive sample manipulation like derivatization, may be susceptible to variability between runs, and are destructive of the sample (Dunn et al., 2011). The aforementioned qualities of NMR analysis are especially valuable for rare, finite tissues such as human placenta, where specimen conservation is of cardinal importance.

Fundamentally, NMR exploits the intrinsic magnetic properties of atomic nuclei (Zia et al., 2019). Nuclei with even atomic number (number of protons) and even atomic mass (number of protons and neutrons) have zero spin, meaning they do not rotate physically around their axes (no angular momentum), while others, like ^1H , ^{13}C , ^{31}P , and ^{15}N , possess nuclear spin and exist in discrete spin states (Zia et al., 2019; Emwas et al., 2019; Crook & Powers, 2020). When nuclei with non-zero spin are placed inside a strong external magnetic field (B_0), they align either with or against the field, producing a net magnetization along the magnet axis (Bloch, 1946). Usually, the most observed nucleus in metabolomics is ^1H , owing to its biomolecular ubiquity and high NMR sensitivity (Emwas et al., 2019).

When nuclei with non-zero spin are placed in B_0 , the nuclear magnetic moments precess around the direction of the B_0 at the Larmor frequency, which is proportional to B_0 and the

gyromagnetic ratio of the nucleus (Bloch, 1946). At equilibrium, the net magnetization vector aligns with the direction of the external field (longitudinal or z-axis). Applying a short radiofrequency (RF) pulse perpendicular to B_0 rotates this magnetization 90 degrees into the transverse x-y plane and excites the nuclei (Bloch, 1946; Kiselev & Novikov, 2018). After the RF pulse is turned off, the magnet moment of the rotating transverse magnetization induces a decaying current into the receiver coil, known as the free induction decay (FID), as spins relax back towards equilibrium along the static magnetic field (Kiselev & Novikov, 2018).

The FID is digitized and subsequently converted from the time domain to the frequency domain using a Fourier transform mathematical operation, yielding a digital spectrum with signal peaks that are distinctly positioned by chemical shift (in ppm) corresponding to protons in unique chemical environments (Jameson, 1996). These unique chemical environments result in an NMR “fingerprint” for each molecule that is determined by both electron density and bonding effects. In the case of the former, electrons orbiting nuclei create their own induced magnetic field that opposes B_0 ; hence, features of the chemical environment like the electron density and magnetic anisotropy contribute to a phenomenon known as electron shielding (Muhammad et al., 2014; Nardelli et al., 2020). Electron shielding effectively “shields” or guards nuclei from the full strength of B_0 , meaning they experience a weaker effective field and therefore rotate at a lower frequency. Thus, shielding results in lower chemical shifts (upfield) and deshielding (the removal of electron density due to electronegativity effects of neighbouring functional groups) results in higher chemical shifts (downfield) (Muhammad et al., 2014). This enables molecular discrimination and structural inference based on electrical and chemical properties (Muhammad et al., 2014; Nardelli et al., 2020). J-coupling or scalar coupling is an indirect through-bond interaction between two neighboring nuclei with non-zero spin that causes splitting of NMR

spectral peaks into multiplets (doublets, triplets, etc.) (Marion, 2013; Dona et al., 2016). In metabolomics, this splitting helps distinguish otherwise overlapping signals (Marion, 2013). This, in practice, provides connectivity and geometrical constraints that are complementary to chemical shift, making it a useful source of structural specificity for annotating metabolites in crowded biological mixtures (Reif et al., 2021; Dona et al., 2016). Altogether, chemical-shift positions, multiplicities, and integrated peak areas obtained from NMR spectra enable both identification and absolute/relative quantification of a broad range of metabolites with proficient reproducibility across test runs (Crook & Powers, 2020; Emwas et al., 2019). The reproducibility of these spectra makes NMR especially suitable for longitudinal studies and for comparing metabolic profiles across experimental groups.

While NMR is widely regarded as a highly reliable and sufficiently sensitive technique for metabolomic analyses, it is important to contrast it with other leading platforms such as LC-MS and GC-MS in greater detail than previously described. LC-MS and GC-MS achieve high sensitivity and wide metabolite coverage, but the way these instruments are built and operate introduces distinct sources of variability that users must closely manage (Perez et al., 2016; Kwon et al., 2012). In LC-MS, a high-pressure liquid chromatograph (pump and column) first separates analytes (Metz et al., 2007; Grebe & Singh, 2011). At the mass spectrometer inlet, an atmospheric pressure ion source [typically electrospray (ESI) or atmospheric pressure chemical ionization (APCI)] creates gas-phase ions that are directed into the mass analyzer and detector (Grebe & Singh, 2011). Triple-quadrupole LC-MS systems are especially common, whereby quadrupole 1 (Q1) selects a precursor ion, Q2 (collision cell) fragments it, and Q3 filters the product ions for detection, yielding sensitive and selective quantification (Yost, 2022). However, LC-MS sensitivity can be undermined by matrix effects/ion suppression during ESI, marring

accuracy and reproducibility (Perez et al., 2016; Kwon et al., 2012). Therefore, long, multi-batch LC-MS studies rely on rigorous quality control design and post acquisition drift/batch correction to stabilize signals (Wehrens et al., 2016). In GC-MS, a heated injector volatilizes the sample and an oven-programmed capillary column resolves compounds before electron ionization (EI; typically 70 eV) in the MS (Morrison & Clowers, 2019; Stan, 2005). EI produces highly reproducible, fragment rich spectra that are matched with massive libraries, but many polar/thermolabile metabolites must be chemically derivatized to become sufficiently volatile, thus adding extra labor and potential biases into the process (Stan, 2005; Halket et al., 2005; Peisl et al., 2018). It is important to highlight that for both LC-MS and GC-MS, the MS portion of the analysis that ionizes and breaks apart molecules means that the samples are effectively destroyed and cannot be used for further non-MS analyses. For tandem MS (MS-MS), structural certainty is added by isolating a precursor and recording its fragment ions after collision-induced dissociation (CID), which is becoming a staple for metabolite and lipid annotation in many LC-MS pipelines (Heiles, 2021).

Opposed to mass spectroscopy approaches, NMR detects nuclei in a strong magnetic field using radiofrequency excitation and induction coils (Emwas et al., 2019; Mal et al., 2020; Crook & Powers, 2020). It is non-destructive, highly reproducible, and intrinsically quantitative with minimal sample preparation. Despite being less sensitive than MS, these features make NMR a suitable option when preservation of rare tissue (like placenta), cross-batch comparability, and straightforward quantification are experimental priorities (Emwas et al., 2019; Crook & Powers, 2020).

1.10 ^1H NMR Metabolomics Procedures for Placental Research

In placental research, the sample preparation process begins with careful collection of whole chorionic villi at the time of delivery. To reduce heterogeneity and minimize maternal blood contamination, sampling is often restricted to multiple sites on the fetal side of the placental disc, with the removal of visible blood prior to processing (Yuan et al., 2021; Inkster et al., 2022; Blair et al., 2013). After excision, placental tissue is rapidly snap-frozen in liquid nitrogen and later stored at -80 degrees Celsius to preserve molecular integrity (Roberts et al., 2019; Blair et al., 2013). To extract metabolites from placental tissue, multiple methods such as ultrafiltration, Bligh-Dyer, and direct sample-to-buffer with vortex and centrifugation are serviceable options (Brown et al., 2022). Initially, frozen samples are grounded using a mortar and pestle that is pre-chilled using liquid nitrogen. Pulverized tissue is then typically mixed with a phosphate buffer solution (e.g. $\text{KH}_2\text{PO}_4:\text{K}_2\text{HPO}_4$), a bacteriostatic agent (e.g. sodium azide; NaN_3), and calibrated to a standard pH ($\sim 7.35\text{-}7.45$; Lindon et al., 2000; Wishart, 2008; Beckonert et al., 2007). Buffered samples are then homogenized, often with glass or zirconium beads and a tissue homogenizer. To remove proteins and colloids that broaden resonances, the homogenate is ultrafiltered by a centrifuge using pre-rinsed filters (Beckonert et al., 2007).

NMR samples are prepared by mixing filtrate with deuterium oxide (D_2O) and an internal chemical shift reference and quantitation standard, such as trimethylsilylpropanesulfonate (DSS) or 3-trimethylsilylpropionic acid (TSP; Beckonert et al., 2007) and are transferred to glass NMR tubes. Spectra are acquired on high-field spectrometers (typically 600-900 MHz) using water suppressed one dimensional ^1H experiments, such as NOESY-presaturation (e.g. `noesygppld`), to balance water suppression with quantitative accuracy (Poplawski et al., 2020).

To accommodate minor chemical-shift variation and reduce feature convolution, spectra are binned, commonly using dynamic adaptive binning (DAB) with subsequent manual

inspection, the water peaks are excluded, and each spectrum is normalized (e.g., constant sum) prior to Pareto scaling and log transformation to stabilize variance, all using computer software such as MATLAB (The MathWorks, MA, USA; Anderson et al., 2011; Craig et al., 2006; Kiss, 2016). Statistical assessment then proceeds with univariate and multivariate analyses. Normality is assessed (e.g. Shapiro-Wilk test) and non-parametric tests (e.g. Mann-Whitney-U, Welch's t test) are applied with corrections (e.g. Bonferroni) following decision tree algorithms (Goodpaster et al., 2010). Unsupervised principal component analysis (PCA) is applied for quality assessment, while supervised partial least squares-discriminant analysis (PLS-DA) and orthogonal projections to latent structures-discriminant analysis (OPLS-DA) improve classification, and complement feature discovery following cross-validation (e.g. single or double cross validation) and receiver operating characteristics (ROC) visualization (Westerhuis et al., 2008; Szymańska et al., 2012; Wiklund et al., 2008; Fawcett, 2006). Machine learning algorithms such as variable importance analysis based on random variable combination (VIAVC) facilitate detection of significant bins, and downstream ROC analysis summarizes performance by way of area under the curve metrics, with repeated double ten-fold cross-validation to prevent overfitting the model (Paxman et al., 2018; Yun et al., 2015; Fawcett, 2006). From there, bins that are calculated to be significantly altered between comparison groups are further examined using a variety of different software programs. Tools such as Chenomx can be used to map significant bins on to spectral databases (e.g. Human Metabolome Database; HMDB), where matching signatures are used to identify and quantify specific metabolic entities (Wishart et al., 2022). Finally, following identification, online tools such as MetaboAnalystR can be chosen for metabolomic pathway topology analysis using pathway libraries like the Kyoto Encyclopedia of

Genes and Genomes (KEGG), which determines significantly associated metabolic pathways based on the metabolites identified in the study (Xia & Wishart, 2010; Kanehisa et al., 2023).

Across species and cohorts, ^1H metabolomics reliably assesses the biochemical footprint of prenatal stress and pregnancy-related metabolic dysfunction, providing a quantitative and highly reproducible view into pathway level change. ^1H NMR boasts strengths in standardized, system-wide profiling and suitability for longitudinal and population studies, albeit with sensitivity trade-offs relative to MS. Consistent with these features, Ice Storm and QF2011 studies show durable urinary fingerprints in children exposed in utero to disaster-related stress, implicating amino acid pathways and energy metabolism, several years after birth (Heynen et al., 2023; Paxman et al., 2018). Concurrent work in pregnancy demonstrated that maternal urinary ^1H NMR profiles differentiate obesity and women who later develop gestational diabetes mellitus (pre-GDM) from controls, highlighting glycine, serine, and threonine pathway as well as aromatic amino-acid pathway alterations relevant for early risk assessment (Scott et al., 2021). What's more, complementary comparative mice studies correlate early-life stress to persistent metabolic remodeling in the heart and kidneys, with ^1H NMR-resolved shifts in branched-chain amino acid and energy metabolism that are associated with cardiorenal and mental-health complications (Poplawski et al., 2020). Altogether, these examples support NMR metabolomics as a powerful tool that can map prenatal exposure onto downstream physiology, which is squarely in line with the DOHaD framework.

Within the placenta, ^1H NMR studies are beginning to interpret biochemical signatures of adaptation and pathology that may serve as candidate biomarkers. For example, ^1H NMR of term placentas delivered by cesarian section identified distinct metabolic profiles between maternal- and fetal-side tissues (namely serine, threonine, aspartate, taurine, and alanine), differences that

reinforce the necessity to control sampling layer and provide biological context for preconditions such as diabetes or hypertension during pregnancy (Walejko et al., 2018). Beyond tissue layers, ¹H NMR analysis of paired maternal-cord plasma in placenta-mediated disorders shows distinct metabolic fingerprints in preeclampsia compared to fetal growth restriction, demonstrating elevated pro-atherogenic and pro-inflammatory profiles compared to controls, including alterations in choline containing compounds and lipoprotein related features that are either unique or dependent to maternal-fetal pathological status (Youssef et al., 2021). In accordance with the DOHaD framework, linking early exposures to lifelong risk (Faa et al., 2024; Gluckman et al., 2008), placental NMR metabolomics offers a plausible route to develop biomarkers that link maternal stress biology to fetal programming and could undergird screening, targeted intervention, and public-health initiatives.

1.11 Biomarkers, Research Objectives and Study Design

Understanding the prolonged impacts of maternal stress on fetal development requires more than recording correlations; rather, it demands strategies that allow for measurement and intervention. Within the DOHaD conceptual framework, biomarkers have emerged as promising targets for early detection, clinical observation, and therapeutic targeting because they directly index real-time biological processes (via metabolites, proteins, enzyme activity, transcripts, etc.) rather than mere downstream outcomes like symptoms, functional status, morbidity/mortality that are not direct measures of biology (Davis et al., 2020; Vo & Trinh, 2024). Accordingly, metabolites, proteins, and signaling molecules provide snapshots of functioning at the pathway level. When sampled from the placenta, these outputs reflect the maternal-fetal interface itself, thus closing the gap between exposure and effect while elucidating how prenatal experiences (e.g. psychological distress and environmental catastrophe) are biochemically translated during gestation (Faa et al., 2024; Gluckman et al., 2008; Cifkova et al., 2024).

It follows that placental stress biomarkers can serve numerous functional purposes. First, they may assist in the early identification of at-risk pregnancies which inform rapid and individualized interventions (Estrela et al., 2025). Second, they may uncover mechanisms of fetal programming by coupling specific pathways (e.g. amino acid and energy transport) with adversity and altered maternal or offspring phenotypes (Shimada et al., 2024; Li et al., 2025). Third, they provide unbiased metrics to monitor the impact of therapies, pharmaceutical (e.g. antidepressants) and non-pharmaceutical (e.g. midwifery care), by measuring whether physiological disruptions persist or resolve despite being under different treatment conditions (Inkster et al., 2022; Ellenberger et al., 2025; Staal et al., 2024; King et al., 2015). In sum, biomarkers are not static flags but dynamic indicators with diagnostic utility. For metabolomics specifically, ^1H NMR offers reproducible and quantitative coverage that is well suited for longitudinal and population studies, regardless of known limitations in sensitivity compared to mass spectroscopy (Emwas et al., 2019; Crook & Powers, 2020).

The present thesis aims to apply placental metabolomics to identify signatures of prenatal stress across two complementary studies that focus on increasingly common stressors: maternal prenatal depression (MPD) and natural disaster exposure. Both studies target fetal programming, biomarker discovery, and the placenta as mediators of maternal-fetal transmission. However, they differ by the modality of stress and context, which enables comparison across unique types of physiological and environmental adversity. This design is based on current and prior metabolomics work from fellow colleagues at the University of Lethbridge who demonstrated that prenatal stress and pregnancy-related metabolic dysfunction imprint biochemical fingerprints into biofluids and tissues (Paxman et al., 2018; Scott et al., 2021; Poplawski et al., 2020; Heynen et al., 2023; Ambeskovic et al., 2022; Inkster et al., 2022; Ellenberger et al., 2025;

Staal et al., 2024; King et al., 2015) as well as collaborators at the University of British Columbia and the University of Queensland, who direct some of the best established human pregnancy cohorts (King et al., 2015; Kildea et al., 2018; Oberlander et al., 2006).

The first study presented in this thesis examined the effects of MPD (with and without pharmaceutical treatment) on placental metabolic profiles. For background, depression during pregnancy is a common health problem that is growing at an increasing rate over recent years. Pooled estimates of antenatal depression range between 10-20% globally, with numerous factors like stress, lack of social support, history of abuse, marital conflict, and physical health status believed to be strong contributing factors (Yin et al., 2021; Jhawar et al., 2022; Razurel et al., 2013). SSRIs are the most commonly prescribed antidepressants in pregnancy; however, the long-term impacts of taking these medications during a period when many other drugs are considered too risky remains controversial with mixed results (Huybrechts et al., 2014; Oberlander et al., 2006; Malm et al., 2015; Malm et al., 2016). Evidence suggests potential benefits of SSRI treatment for certain obstetric risks, although neonatal adaptation issues have also been reported (Malm et al., 2015). Large cohort studies underscore the importance of weighing medication risks against the significant dangers of untreated maternal psychiatric illness (Howard et al., 2014; Malm et al., 2016). While concerns such as preterm birth, intrauterine growth restriction, and later neurodevelopmental difficulties remain important considerations in antenatal pharmacotherapy, these risks must be interpreted in the context of maternal mental health needs (Oberlander et al., 2009). Using ¹H NMR metabolomics, this study examined whether placentas from untreated MPD, SSRI-treated MPS, and non-depressed controls show unique metabolomic fingerprints. The goal was to determine whether SSRI

treatment mitigates or exacerbates stress-related metabolic alterations and to identify metabolite biomarkers associated with these differences.

The second study investigated acute environmental stress caused by the 2011 Queensland Flood (QF2011 Study), a massive natural disaster that disrupted housing, income, and infrastructure and produced a sudden and uncontrollable stressor with psychological and physiological ramifications. Continuing the work initiated by Dr. Suzanne King and colleagues' quasi-experimental paradigm, this study used validated and complementary measures of objective stress (QFOSS; quantifying exposure across threat, loss, scope, and change) and subjective stress (COSMOSS; derived from principal components of the IES-R, PDI, and PDEQ scales; King et al., 2015). Distinguishing between objective hardship and subjective appraisal is essential for parsing resilience versus vulnerability in prenatal contexts and aligns with downstream offspring outcomes reported across the QF2011 projects (McLean et al., 2018; McLean et al., 2019; Simcock et al., 2017). An important addition to this project is that a subset of participants belonged to the M@NGO randomized controlled trial, which compared caseload midwifery to standard prenatal care. This linkage allowed for the evaluation of midwifery care as a potential resilience factor to protect against stress-linked placental metabolic alterations. In M@NGO, midwifery for women of any risk was found to be safe and cost-effective, showing lower elective cesarean section rates compared to standard care (Tracy et al., 2011). Drawing parallels to pharmaceutical therapy in the first MPD study, midwifery support offers an alternative route for care that might also normalize stress-related metabolic profiles at the maternal-fetal interface.

These two studies together provide a dual lens on maternal stress: one representing chronic, psychiatric stress (depression) and the other capturing acute, environmental stress

(disaster exposure). They also span a continuum of real-world conditions that shape perception and response, including objective versus subjective stress, medicated versus unmedicated presentations, and supported versus unsupported models of care. By applying ^1H NMR metabolomics to placental tissue, this thesis aims to identify chemical biomarkers associated with each stress condition and to evaluate how metabolic profiles vary according to stress type, treatment status, and supportive care context. This approach is grounded in the DOHaD framework, which asserts that early-life experiences shape health trajectories across the lifespan, and leverages the standardized, quantitative, and systems-level strengths of metabolomics as a clinical science (Gluckman et al., 2008; Wishart, 2008; Emwas et al., 2019).

The unifying objective for this thesis is to illuminate how maternal stress is biologically translated to the fetus via the placenta, while utilizing metabolomics to detect, differentiate, and interpret those outputs. I hypothesize that both MPD and disaster exposure will be associated with unique placental metabolomic signatures, and that these profiles will be different based on treatment (SSRI use) and social support (midwifery care). Moreover, I expect that resilience factors, such as pharmacologic, psychological, and social, will be reflected in more normalized or buffered placental profiles, indicating measurable protective effects of intervention. Finally, I propose that ^1H NMR metabolomics is well suited for biomarker discovery under the DOHaD framework, building on the growing evidence that prenatal stress disrupts specific metabolic pathways linked to later life risk, which can be captured and observed through standardized NMR pipelines.

In conclusion, the research presented in this thesis was expected to show that ^1H NMR metabolomics of the placenta offers a proficient strategy for closing crucial gaps in obstetric medicine. By examining metabolic correlates of stress and resilience, this thesis aims to identify

early biomarkers of risk, inform interventions, and ultimately improve pregnancy outcomes in an increasingly unpredictable world.

1.12 References

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CHAPTER 2: MATERNAL PREPARTUM DEPRESSION AND PRENATAL EXPOSURE TO SSRI ANTIDEPRESSANTS PROGRAMS THE HUMAN PLACENTAL METABOLOME

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2.1 Abstract

One in ten pregnant women take selective serotonin reuptake inhibitors (SSRIs) to treat depression; however, the downstream effects of maternal depression and SSRIs on placental function and fetal development are incompletely understood. This pilot study characterized the placental metabolome to explore the influence of both depression and SSRIs on the prenatal environment. Pregnant women were recruited and divided into three groups: 1) non-depressed, non-SSRI treated mothers (controls), 2) mothers with depression without SSRI treatment, and 3) mothers with depression and SSRI treatment. The presence and severity of depressive symptoms in pregnant mothers was assessed using the Hamilton Depression Rating Scale (HAM-D) and were categorized as control or depressed under different sets of increasingly discriminant values. Placenta samples were collected after parturition and analyzed for metabolite concentrations using proton nuclear magnetic resonance (^1H NMR) spectroscopy. The analysis of 58 placenta samples revealed significant differences between depressed mothers with no SSRI treatment ($\text{HAM-D} \geq 10$) versus controls ($\text{HAM-D} < 5$). Significantly altered metabolites in depressed mothers with no treatment included glutamate, lysine, proline, and serine. Interestingly, placentas from depressed mothers treated with SSRIs show no separation when compared to controls, suggesting that the treatment may have minimized or altered depression-related placental programming. Thus, SSRI treatment may counteract metabolomic disruptions associated with prenatal maternal depression. These findings suggest that the placenta may have a role as a biomarker for personalized risk assessment and replication of these placental programming effects is needed in a larger cohort.

2.2 Introduction

Pregnancy is a highly sensitive period for both the mother and the fetus, with biological and environmental factors influencing maternal wellbeing and child development. Maternal prepartum depression (MPD), which affects 7-20% of pregnant women (Rodriguez et al., 2021), may increase the risk for pregnancy complications, such as preeclampsia and gestational diabetes (Grote et al., 2010). Moreover, MPD is associated with a higher risk of birth complications, including preterm birth and low birth weight, which further increase the likelihood for neurodevelopmental impairments in the offspring (Heynen et al., 2023; King et al., 2025). The rising prevalence of MPD invokes urgency for effective intervention and treatment strategies (Adrianto et al., 2022).

Among antidepressant treatments for MPD, selective serotonin reuptake inhibitors (SSRIs) are the most prescribed (Millard et al., 2017); however, the risks for the developing fetuses make antidepressant treatment challenging for mothers and their clinicians alike. Maternal SSRI use during pregnancy has been linked to adverse birth outcomes, including increased risk for preterm birth, low birth weight, and neurobehavioral deficits (Oberlander et al., 2009). Furthermore, prenatal exposure to SSRIs have raised questions about links to heightened risk for behavioural problems, anxiety, depression, autism spectrum disorder (ASD), and attention-deficit hyperactivity disorder (ADHD) in childhood (Millard et al., 2017; Hutchison et al., 2021, 2023), though many of these SSRI effects are attenuated when accounting for maternal mood (confounding by indication). Current research has yet to identify the underlying biological mechanisms that unequivocally demonstrate that SSRIs are indeed teratogenic (Leshem et al., 2021) and maternal mood disturbances continue to remain a key determinant of developmental outcomes (Pawluski & Oberlander, 2025).

One promising avenue for investigating biological mechanisms and potential effects of MPD and prenatal SSRIs is the placental metabolome. A metabolome is the complement of metabolites

in a biological sample, which represent the end products of gene expression, transcription, and enzyme modification (Heynen et al., 2022). Dynamic changes in metabolic profiles reflect organ functioning and gene-environment responses to diet, disease, and drug exposures (Monni et al., 2021). The placenta plays a key role in fetal development by exchanging nutrients, removing waste products, and activating hormones (Donnelly & Campling, 2014); therefore, the placental metabolome may offer unique biomarkers and mechanistic insights into how MPD and SSRIs individually or synergistically influence fetal development. A powerful technique that can be used to analyze the placental metabolome is proton nuclear magnetic resonance (^1H -NMR) spectroscopy, which offers a comprehensive view of metabolic pathways and intricate cellular function during pregnancy (Scott et al., 2021; Karaer et al., 2022). Recent findings in the present cohort, a prospectively recruited cohort with SSRI exposure and clinician ascertained mood assessments, have described a lack of large-scale up-stream changes in DNA methylation patterns in response to MPD or SSRI treatment (Inkster et al., 2022; Khan et al., 2023). However, it is possible that other epigenetic changes may occur in MPD and SSRI treatment that are ultimately reflected in down-stream changes to the metabolome and, unlike the epigenome, the metabolome is perhaps more sensitive to the environmental factors that are key to the developmental and functional roles of placenta. Thus, a ^1H -NMR spectroscopy-based metabolomic study of the placenta may provide comprehensive insights into regulatory pathways and cellular functions that influence the perinatal environment.

The objective of this study was to apply ^1H -NMR spectroscopy to examine the placental metabolome and its response to MPD and SSRI exposure during pregnancy. This approach aimed to identify potential biomarkers associated with MPD and prenatal SSRIs use by comparing the metabolic profiles of placental tissue from non-depressed mothers to depressed mothers with and

without SSRI treatment. The findings show characteristic metabolomic profiles linked to MPD, which are partially reversed and normalized by SSRI treatment. Ultimately, these findings provide a new perspective of the influence of MPD and SSRIs on the developing fetus and suggest biomarkers for personalized risk assessment.

2.3 Materials and Methods

2.3.1 Subjects

The present study was based on data collected in the existing V-SSRI project, which included a population of 67 pregnant women with/without clinical depression and with/without the use of selective serotonin reuptake inhibitors (SSRIs; Inkster et al., 2022), recruited by the BC Women's Hospital in Vancouver, Canada in the 20th week of gestation. Exclusion criteria were pregnancies with multiple fetuses or chromosomal abnormalities, mothers with bipolar illnesses, hypertension, current substance abuse, diabetes, or infants with congenital brain malformations or fetal growth restriction. Depression symptoms in each mother were assessed at the 26th week and the 36th week of gestation using the clinician-rated Hamilton Depression Rating Scale (HAM-D; Hamilton, 1960; Williams, 2001). Of the 67 participants in the study, 58 placenta samples were available for NMR-based metabolomic analysis. This reduction of 9 samples was due to the exclusion of 3 samples corresponding to younger siblings of participants already included in the study and 6 samples from non-depressed mothers treated with an SSRI antidepressant. These 58 placentas were assigned to one of three groups: (1) mothers with no depressive symptoms and not treated with an SSRI (controls; HAM-D < 8, n = 23); (2) mothers with depressive symptoms (HAM-D ≥ 8) and not treated with an SSRI (n = 21); and (3) mothers with depressive symptoms (HAM-D ≥ 8) and receiving SSRI treatment (n = 14). Mothers who received treatment were administered SSRIs for at least 75 days, including the third trimester, with one of the following medications: venlafaxine (Effexor), sertraline (Zoloft), citalopram (Celexa), escitalopram

(Lexapro), paroxetine (Paxil), or fluoxetine (Prozac). Table 2-1 provides the demographics for the 58 placentas utilized in this study.

2.3.2 Sample Collection

Placental tissues were collected in Vancouver, Canada, according to previously described sampling methodology (Blair et al., 2013). Samples of chorionic villi (1.5-2cm³) were taken from four unique cotyledons/placental sites found below the surface of the fetal-facing side of the placental disc at a depth that targeted intermediate and tertiary villi (Khan et al., 2023). Briefly, all membranes (amnion and chorion) were removed, the samples were washed thoroughly to remove blood, and any thick vessels were removed. The processed samples were then frozen at -20°C and subsequently stored at -80°C. Finally, the samples were shipped in dry-ice to the University of Lethbridge, Canada, for ¹H NMR spectroscopy. All samples were then stored frozen at -80°C until metabolite extractions were conducted.

2.3.3 Metabolite Extraction

Placental tissue was removed from -80°C storage and ground while frozen in a mortar and pestle that was pre-chilled with liquid nitrogen. 150 mg of ground placental tissue was then mixed with 375 µL of buffer (0.5M 4:1 KH₂PO₄:K₂HPO₄, 3.75 mM NaN₃, and 0.375M KF; pH 7.4) and 150 mg of glass beads in a 2 mm centrifuge tube with a snap-tight lid for each sample. This mixture was then homogenized for one minute in a bullet blender homogenizer at an intensity setting of 8. After samples were homogenized, a 365 µL aliquot of the homogenized mixture and 135 µL of buffer top-up was transferred into a pre-rinsed Amicon 3 kDa Centrifugal Filter (Sigma-Aldrich, ON, Canada). The filters were pre-rinsed with milliQ water (Sigma-Aldrich, ON, Canada) ten times to remove residual glycerol from the filter (Tiziani et al., 2008). Each filter was then centrifuged at 14,000 x gravity for 30 minutes at 4°C and the resulting filtrate was used for NMR sample preparation.

2.3.4 NMR Sample Preparation and Data Acquisition

350 μ L of the filtrate, 280 μ L of buffer, and 70 μ L of D₂O with 0.03% w/v trimethylsilyl propanoic acid (TSP) were added to a 2 mL centrifuge tube and centrifuged at 12,000 x g and 4°C for five minutes. 550 μ L of the resulting supernatant was pipetted into a 7" glass NMR tube for NMR data collection. Spectra were collected on a Bruker 700 MHz Avance III HD spectrometer (Bruker, ON, Canada) equipped with a TBO-Z probe using the noesygppr1d pulse sequence (Poplawski et al., 2020). Data were collected from each sample using 512 scans and a total acquisition size of 128 k. The resulting spectral data were then processed in TopSpin 4.1 using zero-filling to 256k points, line broadening of 0.3 Hz, automatic phase- and baseline-correction, and referenced to TSP at zero ppm. The processed spectra were then exported to MATLAB (The MathWorks, MA, USA) for statistical analysis.

2.3.5 Statistical Methods

Statistical analysis was completed using both MATLAB (The MathWorks, MA, USA) and MetaboAnalystR software version 3.0 (Pang et al., 2020) running inside R version 3.5.3. First, inside MATLAB, the spectra were binned using dynamic adaptive binning (Anderson et al., 2011), followed by manual inspection and correction for errors (Kiss et al., 2016). In total, 404 bins were created for the analysis of the placenta samples. Each binned spectrum was then normalized to the total unit area of all bins (the region corresponding to water was removed) and subsequently pareto scaled and log transformed (Craig et al., 2006).

Differences between the placental metabolite profiles were investigated using both univariate and multivariate statistical testing. In the case of univariate analysis, the decision tree algorithm outlined by Goodpaster et al. (2010) was utilized. In brief, each dataset was first assessed for normality using the Shapiro-Wilk test, and all variables were found to be non-normally distributed (Goodpaster et al., 2010). Subsequently, a Wilcoxon-Mann-Whitney U (MW) test was

utilized to test for significance ($p < 0.05$). All univariate tests underwent Bonferroni-Holm correction for multiple comparisons. Multivariate statistical analysis was conducted using Variable Importance Analysis based on random Variable Combination (VIAVC; Yun et al., 2015). This machine learning algorithm was performed in MATLAB and enabled the detection of significant variables and synergistic changes in the metabolites found in the ^1H NMR spectra through the Receiver Operating Characteristic (ROC) test, followed by analysis of the Area under the Curve (AUC; Fawcett, 2006). Furthermore, the bins in the spectral data underwent binary matrix resampling, which provided a more robust approach to randomly sample data. Finally, MetaboAnalystR was utilized to carry out unsupervised principal component analysis (PCA), supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA), and ROC modelling. In the case of the PCA and OPLS-DA, modelling was completed first using all bins from the data set, followed by modelling using only the bins identified as significantly altered by either VIAVC or univariate MW analysis. ROC models were completed using the subset of bins determined to be significant based on VIAVC modelling ($p < 0.05$). All supervised multivariate models were subjected to double ten-fold cross-validation (Wiklund et al., 2008; Szymanska et al., 2012) to ensure their validity.

2.3.6 Maternal Mood and SSRI Exposure Group Designation

Metabolomic profiles for 58 placental tissues were compared between prenatal exposure groups based on (1) varying levels of prenatal maternal depressive symptoms (based on mean HAM-D score from the 26th and 36th week of gestation) and (2) SSRI treatment. The creation of several different groups based on varying HAM-D cut-off scores was done to account for the subjective nature of the HAM-D assessment, as this may lead to misclassifications of mild depression around the score of 7 (Zimmerman et al., 2012; Sawamura et al., 2018). As such, three different control groups were created utilizing HAM-D score cut-offs of < 5 ($n = 8$), < 6 ($n = 16$),

and < 8 ($n = 23$), each of which had no treatment with SSRIs. Similarly, six different depression groups were created based on whether the mother received SSRI treatment and the following HAM-D score cut-offs: ≥ 8 (w/o SSRI, $n = 21$; w/ SSRI, $n = 14$), ≥ 9 (w/o SSRI, $n = 16$; w/ SSRI, $n = 14$), and ≥ 10 (w/o SSRI, $n = 10$; w/ SSRI, $n = 13$). Statistical analysis was then completed by comparing each of three control groups to each of the 6 depression groups (18 total comparisons).

2.3.7 Metabolite Identification and Pathway Topology Analysis

Metabolites corresponding to significantly altered bins were identified using Chenomx Chemical Profiler software (Chenomx, AB, Canada) and the following libraries: 700 MHz version 11 metabolite library from Chenomx and the Human Metabolome Database (HMDB_700) metabolite library (Wishart et al., 2022). Upon identification, the complete list of significantly altered metabolites for each comparison was used to carry out metabolomic pathway topology analysis in MetaboAnalystR (Xia & Wishart, 2010). Pathway analysis was completed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway library (Kanehisa et al., 2023), the hypergeometric test for over-representation analysis, and relative-betweenness for the pathway topology analysis.

2.4 Results

2.4.1 Establishing a HAM-D Cut-off Value for Control Cohort

Multivariate and univariate statistical analyses were utilized to investigate the use of different control group (non-depressed and non-treated mothers) HAM-D cut-off scores. This was achieved by comparing the placental metabolomic profiles from mothers with HAM-D scores < 5 ($n = 8$), < 6 ($n = 16$), and < 8 ($n = 23$) (all without SSRI treatment) against mothers with HAM-D scores ≥ 8 ($n = 21$), ≥ 9 ($n = 16$), and ≥ 10 ($n = 10$) (all without SSRI treatment). The comparison of placental profiles from mothers with HAM-D score < 6 and < 8 and no SSRI treatment (controls) against mothers with HAM-D scores greater than or equal to 8, 9, and 10 (depressed) showed no

unsupervised group separation in the PCA scores plot and failed both cross validation and permutation testing for supervised OPLS-DA modelling (data not shown). However, the comparison of mothers with a HAM-D cut-off score < 5 and no SSRI treatment (controls) against mothers with a HAM-D cut-off score ≥ 8 , ≥ 9 , and ≥ 10 and no SSRI treatment displayed varying degrees of separation in the OPLS-DA scores plots and passed both permutation and cross-validation testing (Figure 2-1). Based on this, any reference hereafter to the control group will be referring to mothers with a HAM-D cut-off score of less than 5 and no SSRI treatment ($n = 8$).

2.4.2 Comparisons Between Control Mothers and Mothers with Depression but no SSRI Treatment

As the HAM-D cut-off score utilized for the depression group increased, so did the quality of separation observed between the control group and depressed mothers without SSRI treatment, as indicated by increasing values for both the quality of the model (Q^2 equal to 0.328, 0.415, and 0.577 for Figures 2-1A, 2-1B, and 2-1C, respectively) and the amount of variation explained by the model (R^2 equal to 0.422, 0.592, and 0.745 for Figures 2-1A, 2-1B, and 2-1C, respectively). These figures were created using the list of bins identified as significant by either the univariate Mann-Whitney or multivariate VIAVC test. The number of bins that were identified as significant by each test for each of the three comparisons are provided in Supplemental Table 2-1 with the metabolites and statistical result corresponding to these bins provided in Table 2-2. Comparing the metabolome of placenta tissue from the control group against the profiles obtained from women with a HAM-D score ≥ 8 , ≥ 9 , and ≥ 10 resulted in the identification of 12, 6, and 7 metabolites, respectively (Table 2-2). Interestingly, glutamate and serine were found to be significantly altered in all three comparisons, while lysine, proline, and betaine were common to at least two of the comparisons (Table 2-2); however, the direction of regulation was not consistent for lysine and proline.

ROC curves (Figure 2-2) were created using the list of metabolites identified as significant from the VIAVC best subset analysis (Table 2-2) and validated the identified metabolites as contributing to the observed placental metabolomic differences. Furthermore, and in agreement with the OPLS-DA models, an increase in both the AUC and predictive accuracy was observed with an increase in HAM-D cut-off score utilized to classify depression, with the best classification achieved when utilizing a HAM-D score cut-off of less than 5 and greater than or equal to 10 for the control and non-SSRI treated depression groups, respectively (AUC: 0.964, Predictive accuracy: 89.5%, Figure 2-2 C).

Pathway topology analysis was completed utilizing all metabolites found to be significantly altered for the comparison of control mothers and depressed mothers with a HAM-D score greater than or equal to 10 (Figure 2-3 and Supplemental Table 2-1). This comparison was chosen as it had the best quality of model (Figure 2-1) and the strongest predictive accuracy (Figure 2-2) of the three significant comparisons. The resulting pathway analysis indicated that the following eight pathways have potentially been altered: (1) aminoacyl-tRNA biosynthesis; (2) arginine and proline metabolism; (3) nitrogen metabolism; (4) D-glutamine and D-glutamate metabolism; (5) biotin metabolism; (6) arginine biosynthesis; (7) butanoate metabolism; (8) histidine metabolism.

2.4.3 Comparisons Between Control Mothers and Depressed Mothers with SSRI Treatment

The effect of SSRI treatment on the metabolomic profile of placental tissue was investigated by comparing control mothers against mothers with a HAM-D cut-off score of 8 or greater, 9 or greater, and 10 or greater, all with SSRI treatment. For all three comparisons no separation was observed in the supervised OPLS-DA and all tests failed both permutation and cross-validation testing (Supplemental Figure 2-1). In addition, the AUC for the ROC curve for all three tests was less than 0.5 with predictive accuracies below 50% representing random chance (Supplemental Figure 2-2). Lastly only one, one, and two bins, respectively, were significant based

on the univariate Mann-Whitney test for the comparison of the control mothers and depressed mothers with SSRI treatment based on a HAM-D cut-off score of greater than or equal to 8, 9, and 10 (Supplemental Table 2-2). Similarly, for the same three comparisons, there were only three, three, and seven significant bins based on VIAVC best subset analysis, respectively (Supplemental Table 2-2). However, the VIAVC bins were utilized to carry out the ROC analysis for these comparisons and resulted in poor predictive accuracy. This is in stark contrast to the number of bins identified by the comparisons of the control mothers to depressed mothers without SSRI treatment.

2.4.4 Metabolite Correlations with HAM-D Score

Spearman correlation analysis with Bonferroni correction was performed for each significantly altered metabolite (Table 2-2) and the HAM-D scores. This analysis was initially completed using the data from participants included in the comparison of the control mothers with depressed mothers with a HAM-D cut-off score of greater than or equal to 10 and no SSRI treatment. Serine was the only metabolite found to have a significant correlation (Spearman rho = 0.658, $p = 0.003$, $n = 18$; Supplemental Figure 2-3). Subsequently, a further analysis was carried out investigating the strength of this correlation when using all three HAM-D score cut-offs for the control group (< 5 , < 6 and < 7) and each of the three cut-off scores for the depression with no SSRI treatment group (≥ 8 , ≥ 9 , and ≥ 10) resulting in a total of 9 correlation and p-values (Table 2-3). The strength of the correlation improved as the cut-off value was both decreased for the control group and increased for the depression group. This agrees with the results presented earlier with respect to both establishing a HAM-D cut-off score for the control cohort and the group classification observed as the HAM-D cut-off score utilized to establish the depression group is increased (Figures 1 and 2).

2.5 Discussion

The molecular pathways through which MPD and SSRIs regulate placental function and fetal development are poorly understood. The present study demonstrated unique placental metabolomic signatures linked to MPD during pregnancy, supporting the association between specific biochemical pathways and adverse developmental outcomes. We identified several significantly upregulated metabolites: glutamate, lysine, proline, and serine. Particularly, these metabolites were significantly altered when comparing the control group with a HAM-D cut-off of less than 5 with the depression group with a HAM-D cut-off of 10 or greater. The potentially altered metabolic pathways involved in the downstream effects of MPD on fetal development mainly involved D-glutamine and D-glutamate metabolism and arginine and proline metabolism pathways. The findings are consistent with the documented impacts of the maternal metabolism on pregnancy outcomes (Field, 2011; Heazell et al., 2011). A significant finding is the notable alteration in the placental metabolic profiles of mothers with untreated MPD compared to controls, an effect that appears to be modulated with the use of SSRIs. This observation strengthens the notion that SSRIs may have protective or restorative effects on the placental metabolome in depressed mothers, thereby potentially alleviating the impact of MPD on the developing fetus.

2.5.1 Placental Glutamate Regulation and Potential Impacts on Fetal Neurotransmission

Elevated levels of glutamate, a neurotransmitter and a significant component of the D-glutamine and D-glutamate metabolism pathway, were observed in the placentas obtained from depressed mothers. Glutamate serves as the primary excitatory neurotransmitter in the mammalian central nervous system, with a key role in synaptic plasticity, an essential process for learning and memory (Collingridge & Abraham, 2022). Altered glutamate signaling has been implicated in various neurodevelopmental and neuropsychiatric disorders, such as autism (Nisar et al., 2022), schizophrenia (Egerton et al., 2020), and major depressive disorder (Sarawagi et al., 2021). As

expected, major depressive disorder shares several commonalities with MPD, suggesting that the observed changes in glutamate levels could be a physiological response to maternal depressive symptoms. Beyond its role as a neurotransmitter, glutamate is an integral factor in cellular metabolism, supporting the synthesis of proteins, nucleotides, and other amino acids (Walker and van der Donk, 2016). In the placenta, glutamate contributes to the transfer of amino acids between the mother and fetus, a process crucial for furnishing the fetus with the nutrients necessary for development (McIntyre et al., 2020). In the context of MPD, alterations in maternal mood could potentially disrupt these metabolic processes, leading to modified glutamate levels. Therefore, the observed alterations in placental glutamate levels in mothers with MPD could influence an array of developmental processes in the fetus, potentially leading to long-term impacts on child development.

2.5.2 Lysine Metabolism Provides a Potential Mechanistic Link Between Maternal Depression and Fetal Development

Lysine, an amino acid, plays a significant role in protein synthesis and is a key component in the production of carnitine, a molecule that facilitates the conversion of fatty acids into energy (Tomé & Bos, 2007; Adeva-Andany et al., 2017). Lysine also aids in collagen formation, which is essential for providing structure to skin and bones (Yamauchi & Shiiba, 2008), as well as encouraging the formation of new blood vessels (Datta et al., 2001). Animal studies suggest that lysine might offer anxiolytic effects by enhancing γ -aminobutyric acid (GABA)-receptor activity (Chang & Gao, 1995), reducing the breakdown of serotonin (Jalal et al., 2022), and obstructing specific serotonin receptors within the gastrointestinal tract (Smriga & Torii, 2003). In the context of MPD, changes in lysine levels might influence both the physical and emotional aspects of the disorder. For example, the observed increase in lysine levels in the present study may represent a physiological response to depressive symptoms, potentially altering energy metabolism and

impacting the structural integrity of tissues like the placenta. This could, in turn, influence the functioning of the placenta and the development of the fetus. The potential anxiolytic properties of lysine (Chang & Gao, 1995) also add another layer of complexity to the present understanding of the interaction between maternal MPD and fetal development. Nonetheless, further human studies are required to understand the implications of altered lysine levels in the context of MPD on energy metabolism, structural tissue integrity, placental functioning, and fetal development.

2.5.3 Placental Serine and Potential Impacts on Fetal Nervous System Function

Serine is a non-essential amino acid that plays a critical role in the development of the central nervous system. It contributes to the synthesis of nucleotides and proteins (Tabatabaie et al., 2010) and serves as a potent neurotrophic factor (El-Hattab, 2016). L-Serine is readily converted into D-serine, a co-agonist of the NMDA type of glutamate receptors, thereby influencing neurotransmission (Wolosker et al., 1999; Neame et al., 2019; Wolosker et al., 2013). This conversion highlights D-serine as a dynamic regulator of NMDA receptor function in the brain, with implications for learning, cognition, and the pathophysiology of fear and anxiety-related disorders (Wolosker & Balu, 2020). In the present study, elevated levels of serine were observed in the placentas of mothers with untreated MPD. This elevation was further observed in the correlational analysis (Table 2-3 and Supplemental Figure 2-3), suggesting an increase in serine concentration in the placenta of mothers with higher depression symptoms. This elevation may potentially influence the NMDA receptor function, consequently impacting the developing fetal brain in the offspring of mothers with untreated MPD. During pregnancy, serine levels have been found to be lower in healthy pregnant women during late pregnancy compared to non-pregnant women (Kalhan et al., 2003). This difference is thought to be due to lower rates of branched-chain amino acid transamination, serving the purpose of nitrogen accretion and conservation. In the context of MPD, however, it is possible that increased levels of serine may

provide an adaptive response to the metabolic and psychological stress of MPD, thus affecting metabolite balance.

2.5.4 Placental Proline Homeostasis May Influence Fetal Development

Proline is a non-essential amino acid and, like lysine, a component of collagen (Karna et al., 2020). Proline also aids in protein synthesis and wound healing (Albaugh et al., 2017). During pregnancy, proline is actively transported across the placental barrier to the fetus, contributing to the production of subsequent polyamines, including glutamate (Wu et al., 2005). Changes in proline homeostasis, therefore, have potential ramifications for both maternal and fetal health. In the context of MPD, the elevated proline levels observed in the present study may indicate a metabolic adaptation to the physiological and psychological stress of MPD. This adaptation might alter intracellular protein turnover, synthesis, degradation, and placental transport. As a result, any disruptions to proline homeostasis could potentially contribute to birth complications, such as intrauterine growth restriction (Tan et al., 2022) which may predispose offspring to adult-onset diseases (Wu et al., 2008). Moreover, considering proline's role in wound healing and collagen formation, elevated proline levels might suggest increased demand for tissue repair and maintenance in the placenta under the stress of MPD. This could potentially influence the structural integrity of the placenta, thereby affecting its function and possibly the development of the fetus. However, it is important to note that studies on proline metabolism are largely limited to animal models, such as pigs and sheep, due to the invasive nature of biochemical research on placental and fetal growth (Wu et al., 2008).

2.5.5 D-Glutamine and D-Glutamate Pathway Regulation is Essential for Healthy Pregnancy Outcomes

The present study found noteworthy changes in the D-glutamine and D-glutamate metabolism pathway within the placentas of mothers experiencing untreated MPD compared to

the placentas of non-depressed controls. This pathway, responsible for the interconversion of glutamine and glutamate, regulates placental amino acid transport and umbilical amino acid uptake during late pregnancy (Wu et al., 2015), highlighting the vital role of the glutamate-glutamine cycle, metabolism, and exchange between the mother and fetus during fetal growth and development (Xie et al., 2014). The observed alterations in this pathway among mothers with untreated MPD may have potential implications for fetal development. Given that perturbations in D-glutamine and D-glutamate pathway signaling have been linked to a range of neurodevelopmental and psychiatric conditions, including Rett syndrome (Viola et al., 2007), Fragile X syndrome (Davidovic et al., 2011), Alzheimer's disease, and neuronal ceroid lipofuscinosis (Dumas & Davidovic, 2015), it is reasonable to hypothesize that such disruptions in mothers with untreated MPD might contribute to changes in the neurodevelopmental trajectory of the offspring. Moreover, disruptions in the glutamate-glutamine cycle have been associated with pregnancy complications, such as intrauterine growth restriction (Jozwik et al., 2009), low birth weight (Ivorra et al., 2012), and preeclampsia (Yao et al., 2022). The presence of such disruptions in the placenta obtained from depressed mothers may potentially heighten the risk of these complications, suggesting a need for targeted interventions. Glutamate has a wide array of functions, which include providing energy for rapidly dividing placental and fetal liver cells (Wu et al., 2015). Notably, glutamate has the highest fetal/maternal plasma ratio of all amino acids as it is transferred from the maternal to the fetal side (Yamauchi et al., 2002). Similarly, glutamine, although classified as a "nonessential" amino acid and a major precursor for the production of glutamate and GABA in the brain, plays a critical role in placental-fetal circulation (Wu et al., 2015). It is absorbed and converted into glutamate to facilitate nucleotide synthesis and nitrogen metabolism (Timmerman et al., 2000). Alterations to glutamate metabolism, as observed in the

MPD cohort, could therefore disrupt these processes, potentially affecting fetal outcomes. SSRIs have been shown to modulate glutamatergic neurotransmission (Pehrson & Sanchez, 2014), which could potentially extend to the placenta and influence the transport of amino acids, including glutamate, and explain the normalization effect of the SSRI treatment observed in this study.

2.5.6 Arginine and Proline Pathway Regulation May Directly Impact Placental Function

Potential changes in the arginine and proline metabolism pathway in the placentas of mothers with untreated MPD were observed when compared to those without depression. The arginine and proline metabolism pathway encompasses the synthesis and metabolism of several critical amino acids, including arginine, ornithine, proline, citrulline, and glutamate (Wu et al., 2009), which are essential for healthy placental development and functioning. Arginine, a semi-essential amino acid, is a precursor for the synthesis of nitric oxide, a potent vasodilator critical for regulating placental blood flow (Wu & Morris, 1998; Zullino et al., 2018). Thus, alterations in arginine metabolism, such as those indicated in the placenta of depressed mothers, might potentially affect nitric oxide production and influence placental perfusion and consequently nutrient supply to the fetus. This is a particularly relevant consideration given that prior studies have found associations between maternal stress and reduced placental blood flow (Mulder et al., 2002). Such disruptions in arginine metabolism in placenta could be one mechanism by which untreated MPD impacts fetal development. Proline, another key component of this pathway, is integral to the structural integrity of the placenta. Therefore, altered placental proline metabolism in the context of untreated MPD might potentially affect the structure and integrity of the placenta, which could have implications for maternal-fetal nutrient and oxygen exchange. Interestingly, disruptions in this pathway have also been linked to Fragile X syndrome (Davidovic et al., 2011), likely due to the pathway's role in glutamate synthesis. However, how alterations in arginine and

proline metabolism affect birth outcomes and placental functioning, especially in the context of untreated MPD, remains unclear.

2.5.7 Limitations

This pilot study has some limitations. The sample size does not allow for an assessment of the influence of several potential confounding factors such as maternal age, lifestyle (diet and activity levels), socioeconomic status (income and education), other health conditions, and the varied potencies and half-lives of different SSRI medications. Similarly, a larger cohort would allow for more reliable correlational analyses of birth outcomes, such as gestational length, birth weight, Apgar score, and mode of delivery. Moreover, the cohort in this study did not express a representative distribution of low, medium, and high HAM-D scores. Rather, most depressed participants were of low symptom severity (HAM-D = 7 to 17), with only one being moderately symptomatic (HAM-D = 18 to 24) and none being severe (HAM-D > 25). Future analyses would benefit from capturing the broader spectrum of depressive symptoms, with the inclusion of severe cases, as the placental metabolome may be influenced differentially across the continuum of depression symptoms. These limitations present avenues for future research to gain an understanding of the metabolomic changes associated with MPD and prenatal SSRI exposure.

2.6 Conclusion

The lack of conclusive evidence on the downstream effects of prenatal SSRI usage introduces a predicament, wherein either choice—to pursue pharmaceutical intervention or not—may carry risk to the developing fetus. This study provides preliminary evidence of a complex interplay between untreated MPD, SSRI treatment, and placental metabolism. It suggests alterations exist in key metabolic pathways in mothers with MPD, particularly D-glutamine and D-glutamate metabolism and arginine and proline metabolism pathways. These alterations potentially influence physiological processes vital to pregnancy, such as nutrient transport, blood

flow regulation and placental integrity, thereby impacting fetal development and birth outcomes. The impact of SSRI treatment on the placental metabolome observed in this study raises intriguing questions about whether SSRIs counterbalance or ‘correct’ the metabolomic disruptions associated with prenatal maternal depression. It would also be of interest to determine whether these metabolic changes persist postpartum or have long-term implications for the child's health. Ultimately, this study supports the potential utility of placental metabolomic analysis in both personalized risk assessment following pregnancy and as a mechanism to better understand the effects of MPD and SSRI treatment. The translational value of these findings in clinical practice, however, necessitates further robust investigation. Future work should utilize a larger sample size to further validate these results and attempt to correlate changes in the placental metabolome with developmental and health outcomes in prenatally exposed children. It will be critical to focus on elucidating the precise mechanisms underlying these metabolic changes and exploring their long-term implications for child health. Gaining a deeper understanding of how the placental metabolome responds to both untreated MPD and SSRI treatment is a critical step in refining risk assessments and optimizing clinical decision-making processes.

2.7 References

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2.8 TABLES AND FIGURES

2.8.1 TABLES

Table 2-1. Cohort Demographic Characteristics.

		Control HAM-D < 8	Depressive symptoms/ No-SSRI treatment HAM-D ≥ 8	Depressive symptoms/ SSRI- treatment HAM-D ≥ 8	Test statistics (p-value)
n		23	21	14	
Mean HAM-D. mean (SD)		4.89 (2.03)	10.40 (2.21)	12.96 (2.93)	p<0.05
SSRI Type (n)	citalopram	-	-	5	
	escitalopram	-	-	4	
	fluoxetine	-	-	1	
	sertraline	-	-	3	
	paroxetine			1	
Length of SSRI exposure (days, mean (SD))				268.21 (19.52)	
Delivery Mode (n)	cesarean	18	11	5	
	vaginal	5	10	9	p<0.05
Maternal Ethnicity (n (%))	Black	-	1 (4.8)	-	
	Polynesian	2 (8.7)	-	-	
	Oriental	2 (8.7)	3 (14.3)	1 (7.1)	
	Indian	-	1 (4.8)	-	
	White	17 (73.9)	14 (66.7)	11 (78.6)	
	Other	2 (8.7)	2 (9.5)	2 (14.3)	
Fetal Sex	male:female	13: 10	12: 9	6: 8	ns
Apgar Score at 5 min (>7) n (%)		23 (100)	19 (90.5)	12 (85.7)	ns
Maternal age at birth mean (SD)		32.27 (2.89)	33.55 (1.89)	36.00 (4.19)	p < 0.01
Gravidity median (IQR)		1 (1)	2 (1.5)	2 (2)	ns
Parity median (IQR)		0 (1)	0 (1)	1 (1)	ns
Gestational age at birth (weeks) mean (SD)		39.63 (1.25)	39.47 (1.46)	39.07 (1.35)	ns
Birth weight (g) mean (SD)		3456.52 (404.69)	3495.33 (411.09)	3367.00 (544.50)	ns
Trimmed placental weight (g) mean (SD)		536.59 (127.86)	516.12 (85.38)	517.67 (131.91)	ns

Sample processing time (h)	53.80	60.87		
mean (range)	(65.34)	(67.01)	56.88 (51.27)	ns

Table 2-2. Statistically significant placental metabolites for the comparisons of mothers with depression and no SSRI treatment versus control group (mothers with no depression based on a HAM-D score < 5 and no SSRI treatment). The HAM-D score utilized for the depression cut-off in each comparison is indicated in the first column. In all cases, the cutoff was a score greater than the number indicated. Significance was determined by variable importance analysis based on random variable commination (VIAVC) and/or Mann-Whitney U-test. For metabolites that had more than one significant bin in each comparison the highest p-value is reported.

HAM-D Cut-off Value	Metabolite	p-value VIAVC	p-value Mann Whitney	Regulation
≥ 8	Ascorbate	6.92E-07	ns	Up
	Aspartate	ns	0.016	Up
	Diethylthiophosphate	4.96E-10	ns	Up
	Dihydrouracil	4.08E-06	ns	Down
	Ethanol	8.35E-06	ns	Down
	Fumarate	0.00780	ns	Down
	Glutamate	ns	0.038	Up
	Isoleucine	0.00872	ns	Up
	Lactate	7.85E-22	ns	Down
	Lysine	0.00478	ns	Down
	Proline	1.12E-07	ns	Down
	Serine	2.79E-09	0.043	Up
≥ 9	Betaine	ns	0.035	Up
	Choline	4.55E-22	ns	Up
	Citric acid	6.97E-10	ns	Up
	Glutamate	7.88E-09	ns	Up
	Serine	4.98E-17	0.035	Up
	Taurine	5.18E-40	ns	Up
≥ 10	2-Amino-3-phosphonopropionic acid	ns	0.043	Up
	Betaine	ns	0.043	Up
	Dimethylsulfide	ns	0.043	Up
	Glutamate	ns	0.034	Up
	Lysine	5.30E-13	ns	Up
	Proline	1.50E-15	0.009	Up
	Serine	1.44E-13	0.027	Up

Table 2-3. Comparison of the correlation results between serine and HAM-D scores when using different cut-offs for the control and depression HAM-D scores. Cell values represent Spearman's rank correlation coefficient with p values in parentheses. The arrow next to the Spearman rank correlation coefficient indicates an improved or reduced coefficient with respect to the central correlation in the table (Control HAM-D < 6 and Depression HAM-D ≥ 9).

Correlation Comparison Matrix of HAM-D Cut-off Scores: Serine

		Depression HAM-D Score		
		≥8	≥9	≥10
Control HAM-D Score	<5	0.551 (0.002) ↑	0.659 (0.001) ↑	0.658 (0.003) ↑
	<6	0.420 (0.010) ↓	0.486 (0.005) ↔	0.551 (0.004) ↑
	<8	0.395 (0.013) ↓	0.460 (0.006) ↓	0.545 (0.003) ↑

2.8.2 FIGURES

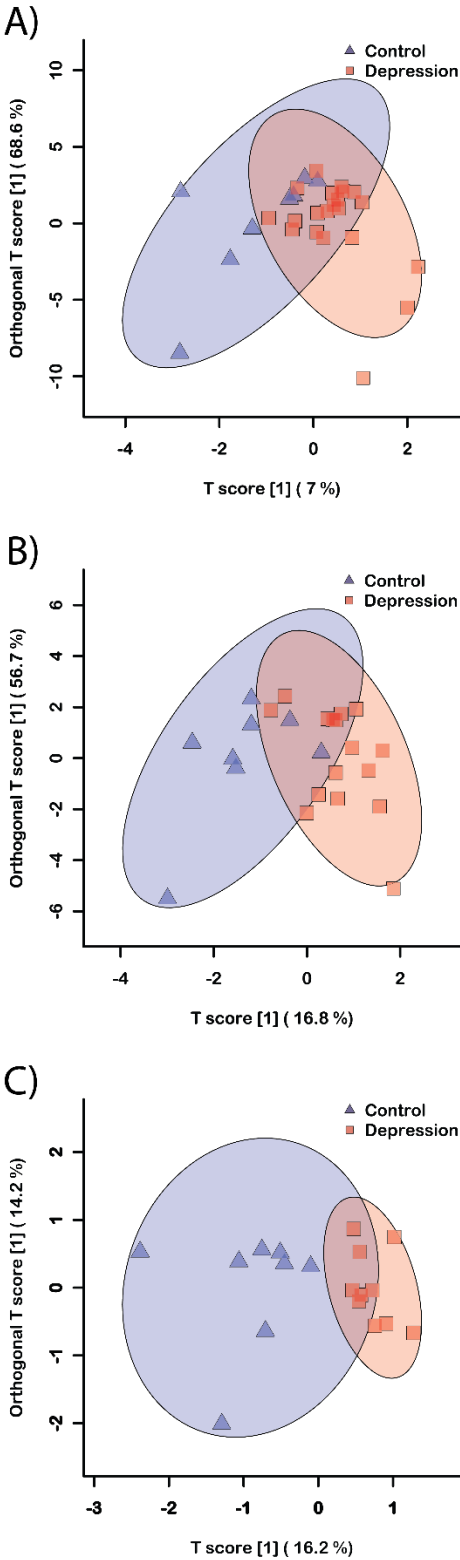
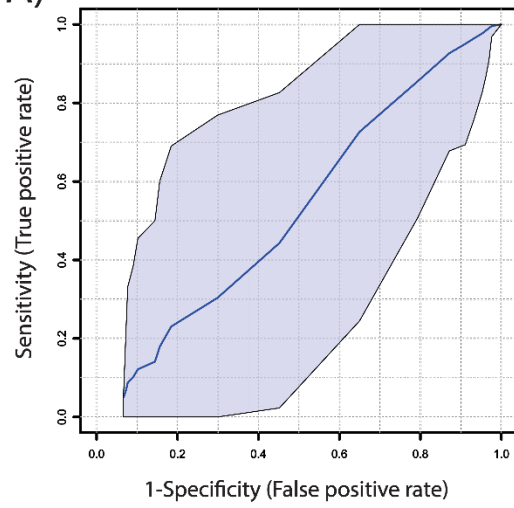
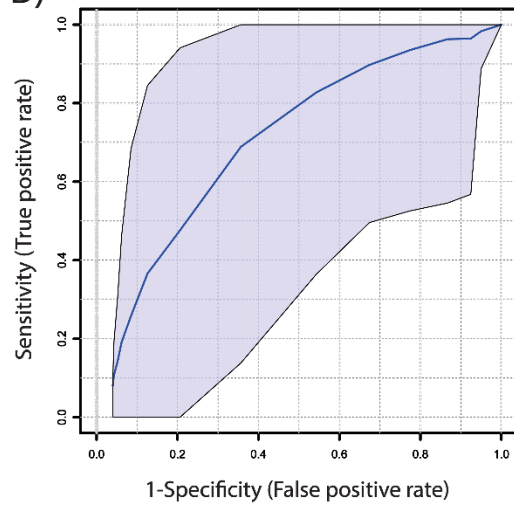


Figure 2-1. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) score plots for placental tissues comparing placental metabolomic signatures in mothers with depression and no SSRI treatment to the control group (mothers with no depression based on a HAM-D score < 5 and no SSRI treatment). The following HAM-D cut-off scores were used to determine depression: (A) ≥ 8 , $n = 21$ ($Q^2 = 0.328$, $p = 0.008$ $R^2 = 0.442$, $p = 0.0335$); (B) ≥ 9 , $n = 16$ ($Q^2 = 0.415$, $p = 0.01$; $R^2 = 0.592$, $p = 0.0215$) and (C) ≥ 10 , $n = 10$ ($Q^2 = 0.577$, $p = 0.001$; $R^2 = 0.745$, $p = 0.005$). The shaded areas represent the 95% confidence interval, while the x-axis and y-axis represent the between- and within-group variation, respectively. Note the significant separation between placental metabolomes of mothers with depression versus those without.

A)



B)



C)

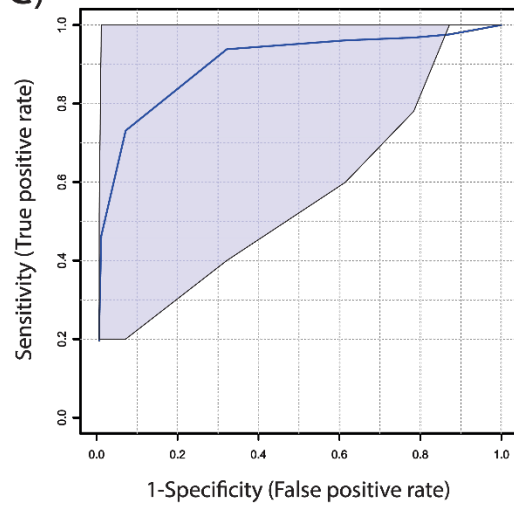


Figure 2-2. Receiver Operating Characteristic (ROC) curves for placental tissues comparing the metabolomic signatures from mothers with depression and no SSRI treatment to the control group (mothers with no depression based on a HAM-D score < 5 and no SSRI treatment). The following HAM-D cut-off score was used to determine depression: (A) ≥ 8 (AUC: 0.536, CI: 0.093-0.958, Predictive accuracy: 54.7%); (B) ≥ 9 (AUC: 0.759, CI: 0.007-1, Predictive accuracy: 63.7%) and (C) ≥ 10 (AUC: 0.964, CI: 0.645-1, Predictive accuracy: 89.5%). The shaded area represents the 95% confidence interval. Each figure was created using the metabolites identified as significant based on the VIAVC best subset.

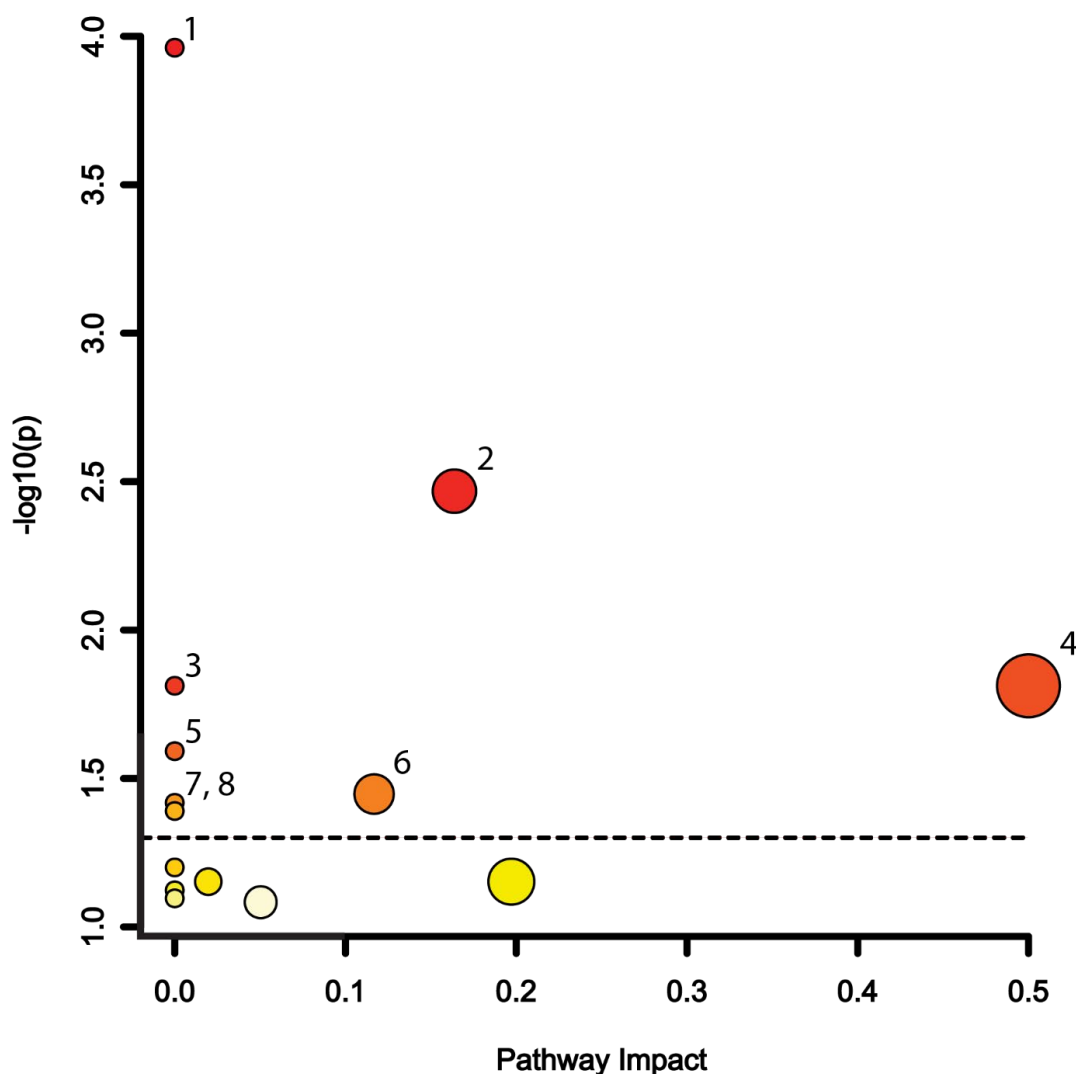
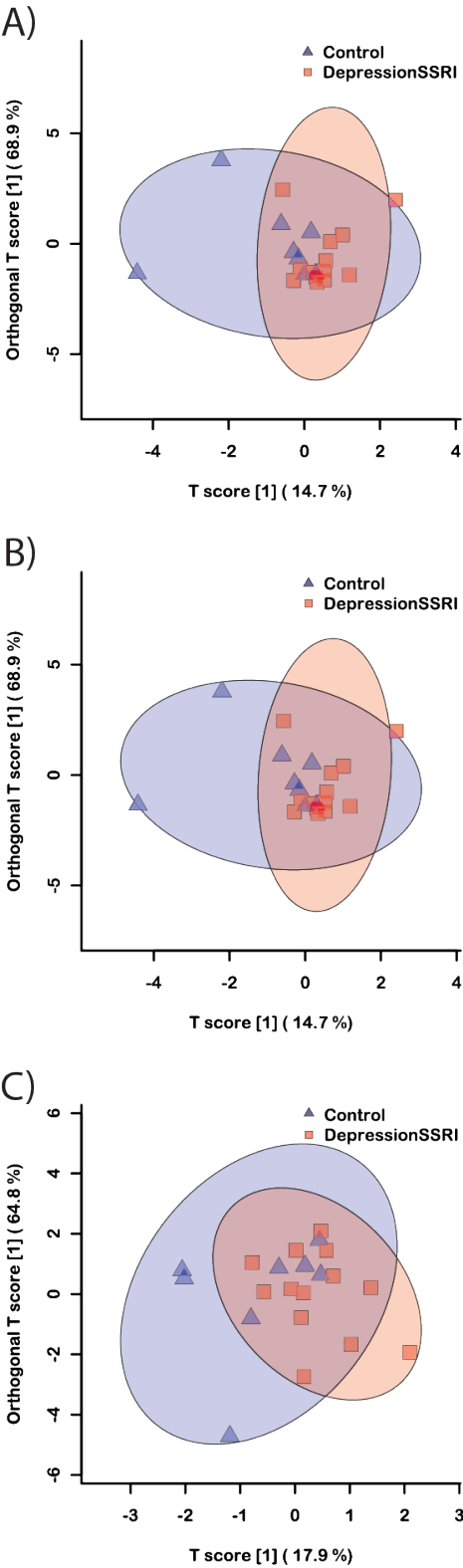


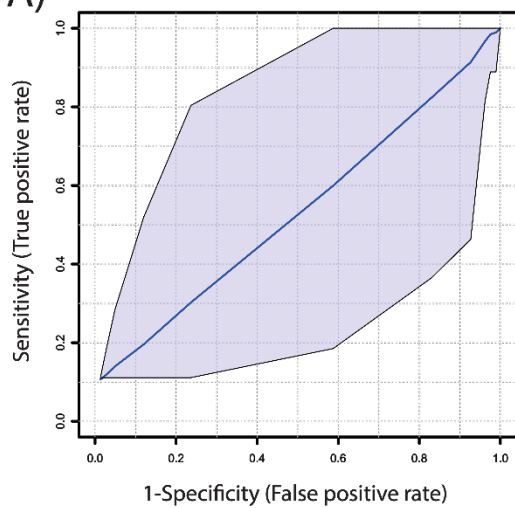
Figure 2-3. Pathway topology analysis for the metabolites determined to be significant by variable importance analysis based on random variable combination (VIABC) and/or Mann-Whitney (MW) U-test for depressed and non-treated mothers (HAM-D score ≥ 10) versus control mothers (HAM-D score < 5). The y-axis represents the p-value with a higher value on the axis corresponding to a lower p-value. A value of 1.3 on the y-axis corresponds to a p-value of 0.05 and only pathways with a p-value less 0.05 were labelled. The x-axis represents the predicted impact on the pathway due to the significantly altered metabolites. In addition, the colour and size of each circle also correspond to the p-value and pathway impact, respectively, with a darker colour and larger circle size corresponding to a lower p-value and higher pathway impact, respectively. Only the pathways with a significant p-value (less than or equal to 0.05) were numbered, which refer to the following pathways: (1) aminoacyl-tRNA biosynthesis; (2) arginine and proline metabolism; (3) nitrogen metabolism; (4) D-glutamine and D-glutamate metabolism; (5) biotin metabolism; (6) arginine biosynthesis; (7) butanoate metabolism; (8) histidine metabolism.

2.8.3 SUPPLEMENTAL MATERIALS

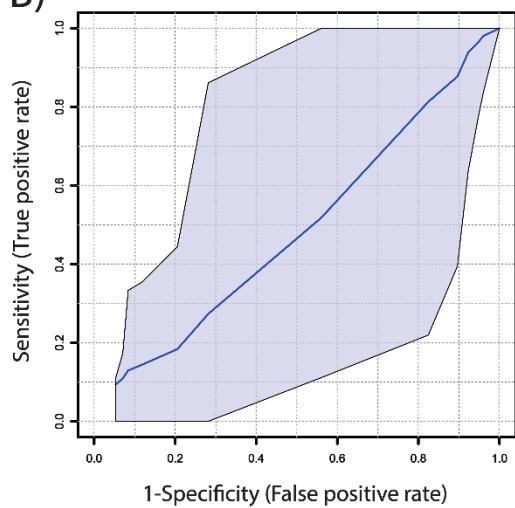


Supplemental Figure 2-1. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) score plots for placental tissues comparing the metabolomic signatures from mothers with depression and SSRI treatment to those of the control group (mothers with no depression based on a HAM-D score < 5 and no SSRI treatment). The following HAM-D cut-off score was used to determine depression: (A) ≥ 8 , $n = 14$ ($Q^2 = 0.112$, $p = 0.092$; $R^2 = 0.282$, $p = 0.092$); (B) ≥ 9 , $n = 14$ ($Q^2 = 0.112$, $p = 0.090$; $R^2 = 0.282$, $p = 0.099$) and (C) ≥ 10 , $n = 13$ ($Q^2 = -0.097$, $p = 0.339$; $R^2 = 0.362$, $p = 0.176$). The shaded areas represent the 95% confidence interval, while the x-axis and y-axis represent the between and within group variation, respectively.

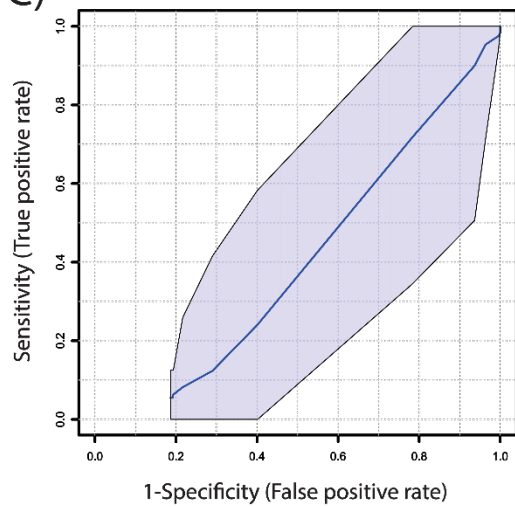
A)



B)

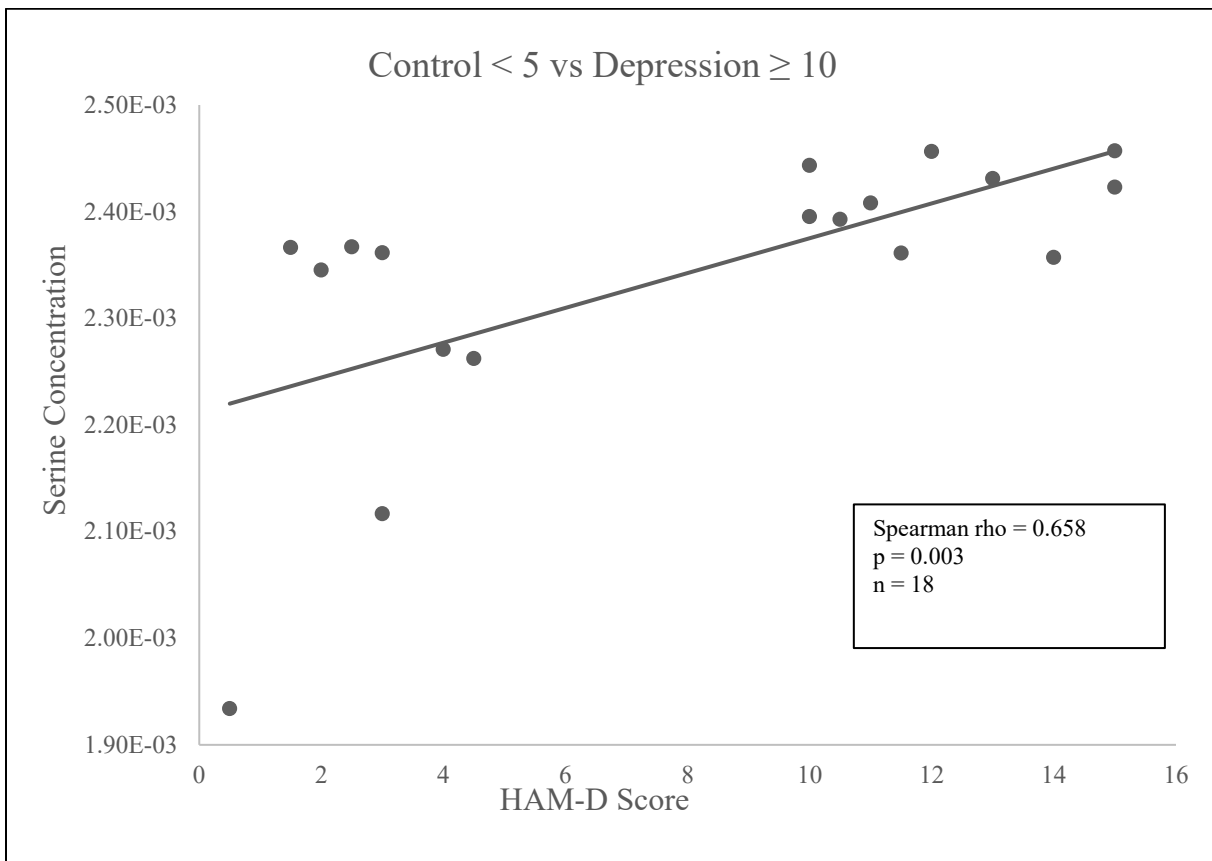


C)



Supplemental Figure 2-2. Receiver Operating Characteristic (ROC) curves for placental tissues comparing the metabolomic signatures from mothers with depression and SSRI treatment to those of the control group (mothers with no depression based on a HAM-D score < 5 and no SSRI treatment). The following HAM-D cut-off score was used to determine depression: (A) ≥ 8 (AUC: 0.49, CI: 0.222-0.844, Predictive accuracy: 49.7%); (B) ≥ 9 (AUC: 0.433, CI: 0.119-0.775, Predictive accuracy: 46.7%) and (C) ≥ 10 (AUC: 0.378, CI: 0.093-0.708, Predictive accuracy: 44.7%). The shaded area represents the 95% confidence interval. Each figure was created using the bins identified as significant based on the VIAVC best subset.

Supplemental Figure 2-3. Serine concentration correlation with HAM-D scores plot for control < 5 vs depression ≥ 10 cut-off demonstrating the strongest correlation (0.658, $p = 0.003$).



Supplemental Table 2-1. Statistical data for the pathway topology analysis presented in Figure 2-3.

Metabolic Pathway	Total	Hits	Raw p	-log10(p)	Impact
Aminoacyl-tRNA biosynthesis	48	3	1.09E-04	3.96E+00	0
Arginine and proline metabolism	38	2	3.41E-03	2.47E+00	0.16
Nitrogen metabolism	6	1	1.54E-02	1.81E+00	0
D-Glutamine and D-glutamate metabolism	6	1	1.54E-02	1.81E+00	0.5
Biotin metabolism	10	1	2.56E-02	1.59E+00	0
Arginine biosynthesis	14	1	3.57E-02	1.45E+00	0.12
Butanoate metabolism	15	1	3.82E-02	1.42E+00	0
Histidine metabolism	16	1	4.07E-02	1.39E+00	0
Lysine degradation	25	1	6.30E-02	1.20E+00	0
Glutathione metabolism	28	1	7.04E-02	1.15E+00	0.02
Alanine, aspartate, and glutamate metabolism	28	1	7.04E-02	1.15E+00	0.2
Porphyrin and chlorophyll metabolism	30	1	7.53E-02	1.12E+00	0
Glyoxylate and dicarboxylate metabolism	32	1	8.01E-02	1.10E+00	0
Glycine, serine, and threonine metabolism	33	1	8.26E-02	1.08E+00	0.05

Supplemental Table 2-2. The results from VIAVC and Mann Whitney U Test statistical analysis for comparisons of the placental profile of control mothers (HAM-D cutoff of <5) against mother with depression with and without SSRI treatment. The HAM-D cut-off scores utilized for the depression groups were the following: ≥ 8 , ≥ 9 , and ≥ 10 . Also reported for each comparison are the OPLS-DA model's quality (Q^2), amount of variation explained by the model (R^2), the p-values associated with both the Q^2 and R^2 values, area under the receiver operating characteristic curve, and predictive accuracy. The bins found to be significant by either the VIAVC best subset or Mann-Whitney univariate analysis were utilized for all comparisons and modelling, except for the control versus depression 8 with SSRI treatment and 9 with SSRI treatment. In these cases, the VIAVC F-ranked variables were utilized because the total number of bins that were found to be significant by either the VIAVC best subset or Mann-Whitney analysis was too small to allow for multivariate modelling.

Comparison	Number of MW Bins	Number of VIAVC Best Subset Bins	MW + VIAVC OPLS-DA Q^2	MW + VIAVC OPLS-DA Q^2 P value	MW + VIAVC OPLS-DA R^2	MW + VIAVC OPLS-DA R^2 P value	VIAVC Best Subset AUC	VIAVC Best Subset Predictive Accuracy
Control vs. Depression 8	11	20	0.238	0.008	0.423	0.0335	0.536	54.7%
Control vs. Depression 9	6	10	0.285	0.01	0.444	0.0215	0.759	63.7%
Control vs. Depression 10	12	6	0.507	0.001	0.575	0.005	0.964	89.5%
Control vs. Depression 8 with SSRI treatment	1	3	0.112	0.092	0.282	0.092	0.490	49.7%
Control vs. Depression 9 with SSRI treatment	1	3	0.112	0.09	0.282	0.099	0.433	46.7%
Control vs. Depression 10 with SSRI treatment	2	7	0.0974	0.339	0.362	0.176	0.378	44.7%

CHAPTER 3: NATURAL DISASTER IMPACTS ON THE PLACENTAL METABOLOME: THE QF2011 QUEENSLAND FLOOD STUDY

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Declarations of Conflict of Interest: None

Keywords: Nuclear magnetic resonance spectroscopy, ¹H NMR, metabolites, prenatal maternal stress, natural disaster, placenta, metabolomics, midwifery care, fetal development, developmental programming, developmental origins of health and disease (DOHaD).

3.1 Abstract

Environmental disasters such as floods are significant prenatal maternal stress (PNMS) factors that may be detrimental to placental function and fetal programming. This study investigated PNMS induced by the 2011 Queensland flood (QF2011 Study) and the human placental metabolome, and whether midwifery-led care modulated its impact. Placental tissues (n = 88) were collected from flood-exposed mothers and analyzed using proton nuclear magnetic resonance (^1H NMR) spectroscopy. Objective (QFOSS) and subjective (COSMOSS) measurements of maternal stress were used to classify participants into low and high stress groups, and subsequent analyses examined the impact of midwifery program participation (M@NGO). Contrary to our hypotheses, no significant group separation or metabolite differences were observed between high and low stress groups, nor between participants who did or did not participate in midwifery care. These null results either suggest that PNMS associated with QF2011 did not yield detectable metabolic changes in the placenta, or that compensatory physiological mechanisms limited its impact. In absence of differential metabolites, this work emphasizes the complexity of PNMS, the resiliency of the placental metabolome, and the necessity for larger longitudinal cohorts in future related studies.

3.2 Introduction

Extreme weather events, such as wildfires, hurricanes, and floods, accounted for roughly \$320 billion US dollars in economic losses worldwide in 2024, increasing by 30% from \$268 billion in the previous year (Financial Times, 2025). Alarming, environmental catastrophes have increased five-fold globally over the past five decades and will likely continue increasing in the future (Douris & Kim, 2021). This trend is not only concerning socioeconomically, but also for the impacts on human health and well-being. For example, pregnant mothers face unique challenges during natural disasters, as these events pose threats to both the mother and the developing fetus. Aside from potential environmental toxicants, mothers and their unborn babies may face loss and trauma during a natural disaster, as well as elevated physiological stress due to these experiences. Severe distress during pregnancy, or prenatal maternal stress (PNMS), has been associated with numerous adverse outcomes, including preeclampsia (Khalida et al., 2023), gestational diabetes (OuYang et al., 2021), and preterm birth and low birth weight (Ding et al., 2021). These effects may be mediated through the placenta, the fetal organ that regulates nutrient and waste transport between the mother and fetus but also provides a protective barrier against environmental toxins during pregnancy (Cindrova-Davies & Sferruzzi-Perri, 2022). Recent studies suggest that PNMS experienced during natural disaster may impact placental functioning, with potential risks for fetal programming and adverse childhood health outcomes (St-Pierre et al., 2018).

PNMS initiates a cascade of physiological responses that may adversely affect fetal development. A central mechanism involved in this cascade is the hypothalamic-pituitary-adrenal (HPA) axis, which is a main neurological structure of the body's stress response system (McGowan & Matthews, 2018). When an individual experiences stress, the hypothalamus releases corticotropin-releasing hormone (CRH), which instructs the secretion of

adrenocorticotropin hormone (ACTH) from the pituitary gland. ACTH stimulates the production of cortisol by the adrenal glands, which is the primary glucocorticoid involved in the stress response (Stevens & White, 2010). Under typical conditions, the placenta functions as a semi-permeable barrier, expressing the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) which deactivates cortisol into cortisone, thus protecting the fetus from excessive glucocorticoid exposure (Kajantie et al., 2003). However, long-lasting elevations in cortisol levels by chronic PNMS may downregulate the expression of 11 β -HSD2, thereby compromising the placenta's ability to inactivate cortisol (Stevens & White, 2010). Therefore, higher concentrations of active glucocorticoids can pass into the fetal environment. Heightened fetal exposure to maternal cortisol is concerning because excessive glucocorticoid levels can disrupt neurodevelopmental processes and exert neurotoxic effects. As such, excessive glucocorticoid exposure during critical fetal development stages has been associated with abnormal neuronal structure and function, thereby increasing risks for neurodevelopmental and psychiatric disorders after birth and into childhood (Graham et al., 2019; Davis et al., 2017). Hence, understanding the mechanisms by which PNMS affects fetal development underscores the importance of managing stress during pregnancy to protect both fetal and maternal health.

The placenta functions as a dynamic mediator of the maternal-fetal interface and modulates the intrauterine environment (Cindrova-Davies & Sferruzzi-Perri, 2022). Previous metabolomic studies have linked PNMS to alterations in the placental metabolome, particularly in pathways related to amino acid processing, energy metabolism, and oxidative stress regulation (Baker et al., 2024; Kramer et al., 2023). However, findings across studies remain inconsistent, likely reflecting differences in the duration and severity of stress exposure, the timing of stress during gestation, and maternal coping or buffering strategies (Glover, 2015). For background, a

metabolome is defined by the complement of small molecules, called metabolites, in a biological sample (Heynen et al., 2022). Metabolite profiles serve as lenses into physiological functioning and gene-environment reactions to diet, toxins, and pathology (Monni et al., 2021). Given that the placenta is instrumental in optimizing the fetal environment via nutrient transfer and waste product removal, the placental metabolome potentially provides insight into how PNMS may impact fetal development. Proton nuclear magnetic resonance (^1H -NMR) spectroscopy represents a powerful way to identify and quantify the placental metabolome (Scott et al., 2021; Karaer et al., 2022). This technique reveals metabolite changes that can be linked to alterations in cellular functioning in a relatively fast, simple, and inexpensive manner, including placental tissue.

Objective stress measures, such as financial loss or other quantifiable stressors, provide a tangible framework for assessing stress levels. However, subjective measures, including maternal perceptions of stress and psychological resilience, are more nuanced and may provide context in ways objective tools cannot (King et al., 2012). Objective and subjective measures are therefore complementary approaches that can tackle the multifaceted nature of stress and its potential impacts on placental metabolism, but they may also complicate comparing findings across studies. Supportive prenatal interventions, such as midwifery care, may provide substantial protective effects against PNMS. Midwifery-led models emphasize emotional support, continuity of care, culturally responsive practice, and holistic approaches to maternal health. These features have been associated with reduced maternal anxiety, improved birth outcomes, and greater maternal sense of control (Kildea et al., 2018). However, research directly evaluating how midwifery or other supportive prenatal interventions modulate placental function, metabolomic pathways, and fetal neurodevelopment under conditions of PNMS remains limited. Addressing

this gap is a promising direction for developing targeted, evidence-based strategies to improve maternal and offspring health outcomes, particularly in populations exposed to chronic or acute stressors.

Research regarding the impact of PNMS on the placental metabolome must consider potential effects of stress measurement methodologies, environmental context variability, and the impacts of protective factors (Goyal et al., 2016; Dorsey et al., 2022; Nolvi et al., 2023). The present study investigated the relationship between PNMS resulting from the 2011 Queensland flood (QF2011 Study) and alterations in the placental metabolome. We recruited a cohort of pregnant individuals exposed to this natural disaster and examined both objective and subjective measures of stress and their associations with placental metabolic profiles using ¹H-NMR spectroscopy. Additionally, this study explored whether participation in a midwifery care trial (M@NGO) before or during the flood may serve as a protective factor against potential stress-related changes in placental metabolites. Our findings aim to contribute to the growing body of literature on PNMS, fetal programming, and the role of interventions in promoting maternal and fetal health in the wake of environmental disaster.

3.3 Materials and Methods

3.3.1 Participants

The initial cohort included 230 English-speaking pregnant women carrying singletons and residing in the Brisbane area of Australia during a significant flood event in mid-January 2011. At the time, a randomized clinical trial of midwifery care, known as the M@NGO study, was underway (Tracy et al., 2011). Participants in the M@NGO trial who met the inclusion criteria were invited to join the current study, alongside additional pregnant women from the flood-affected region who were recruited via various media outlets. A comprehensive explanation of the study protocol is available in King et al. (2015). Ethical approval for the study

was granted by the ethical review board of the primary study site (#1844 M), the affiliated university ethics committee (#2013001236), and the University of Lethbridge ethics committee (#2021–061). All participants provided written informed consent at each stage of the study.

Participants were assessed for prenatal maternal stress experienced during the flood based on composite stress questionnaires issued upon M@NGO recruitment, 36 weeks pregnancy, and at 6-12 weeks and 6 months postpartum. Of these questionnaires, composite scales for objective stress (Queensland Flood Objective Stress Scale, QFOSS; King et al., 2015) and subjective stress (COMposite Score for Mothers' Subjective Stress, COSMOSS; McLean et al., 2018) were computed to simplify the numerous questionnaires issued. In brief, mothers' objective stress (QFOSS) was assessed based on four dimensions of flood-related hardship: threat (e.g., "Were you physically hurt because of the flood?"), loss (e.g., "... please estimate the total value of all material loss and damage experienced because of the flood."), scope: (e.g., "To what extent was your immediate community changed because of the flooding?"), and change (e.g., "How many times were you required to change homes because of the flood?"). The responses to these questions were weighed equally and scored for a maximum of 200 points indicating the greatest degree of objective hardship. For subjective stress (COSMOSS), mothers were assessed on three measures: the Impact of Event Scale-Revised (IES-R; Weiss, 2007), the peritraumatic distress inventory (PDI-Q; Brunet et al., 2001), and the Peritraumatic Dissociative Experiences Questionnaire (PDEQ; Marmar et al., 1997). The total scores from all three subjective stress assessments were compiled and reduced to minimize the number of variables in the regression analyses using a principal component analysis (PCA)-derived algorithm.

3.3.2 Sample Collection

Placenta samples (155 mg) were collected from 105 mothers within 20 minutes after expulsion; however, only 88 samples had sufficient material for NMR spectroscopic analysis.

For each placenta, parameters such as weight, morphology, cord position, calcification, etc. were recorded before processing. Biopsies of chorionic villi were collected in accordance with the International Federation of Placenta Associations (Lash et al., 2009). Eight 1-cm³ parts were selected from the fetal side of each placenta using multistage, unbiased random sampling, ensuring equal representation of all pieces. This approach minimized the risk of contamination from maternal decidual tissue and fetal amnion/chorion membranes (Lash et al., 2009; Wyatt et al., 2005). The collected placental villi samples were snap-frozen and preserved at -80° C. Additionally, fetal chorion/amnion membranes, decidual tissue, and chorionic villi were obtained from two distinct regions of the placenta (central and peripheral), thoroughly rinsed to remove blood, snap-frozen, and sent to the University of Lethbridge for further analysis where they were stored at -80° C.

3.3.3 Metabolite Extraction

Placental tissue was removed from -80°C storage and ground while frozen in a mortar and pestle that was pre-chilled with liquid nitrogen. 150 mg of ground placental tissue was then mixed with 375 µL of buffer (0.5M 4:1 KH₂PO₄:K₂HPO₄, 3.75 mM NaN₃, and 0.375M KF; pH 7.4) and 150 mg of glass beads in a 2 mm centrifuge tube with a snap-tight lid for each sample. This mixture was then homogenized for one minute in a bullet blender homogenizer at an intensity setting of 8. After samples were homogenized, a 365 µL aliquot of the homogenized mixture and 135 µL of buffer top-up was transferred into a pre-rinsed Amicon 3 kDa Centrifugal Filter (Sigma-Aldrich, ON, Canada). The filters were pre-rinsed with milliQ water (Sigma-Aldrich, ON, Canada) ten times to remove residual glycerol from the filter (Tiziani et al., 2008). Each filter was then centrifuged at 14,000 x gravity for 30 minutes at 4°C and the resulting filtrate was used for NMR sample preparation.

3.3.4 NMR Sample Preparation and Data Acquisition

A total of 350 μL of filtrate, 280 μL of buffer, and 70 μL of D_2O containing 0.03% w/v trimethylsilyl propanoic acid (TSP) were combined in a 2 mL centrifuge tube. The mixture was centrifuged at $12,000 \times g$ for five minutes at 4°C . From the resulting supernatant, 550 μL was carefully transferred to a 7-inch glass NMR tube for data collection. NMR spectra were acquired using a Bruker 700 MHz Avance III HD spectrometer (Bruker, ON, Canada) fitted with a TBO-Z probe, employing the noesygppr1d pulse sequence. Each sample underwent 512 scans with a total acquisition size of 128 k. Spectral data were processed in TopSpin 4.1, applying zero-filling to 256 k points, a line broadening of 0.3 Hz, and automatic phase- and baseline-correction. The spectra were referenced to TSP at zero ppm before being exported to MATLAB (The MathWorks, MA, USA) for statistical analysis.

3.3.5 Statistical Methods

Statistical analysis was conducted using MATLAB (The MathWorks, MA, USA) and MetaboAnalystR version 3.0 (Pang et al., 2020) within R version 3.5.3. In MATLAB, spectra were binned using dynamic adaptive binning (Anderson et al., 2011), followed by manual inspection and correction. A total of 404 bins were generated for the analysis of placental samples. Each binned spectrum was normalized to the total unit area (excluding the water region) and subsequently pareto scaled and log-transformed (Craig et al., 2006). Differences in placental metabolite profiles were assessed through both univariate and multivariate statistical tests. For univariate analysis, the Shapiro-Wilk test determined that all data sets were non-parametric. Consequently, the Wilcoxon-Mann-Whitney U (MW) test was applied, with significance set at $p < 0.05$, and multiple comparisons were corrected using the Bonferroni-Holm method. Multivariate analysis employed Variable Importance Analysis based on random Variable Combination (VIAVC; Yun et al., 2015), a machine learning algorithm implemented in

MATLAB. VIAVC identified significant metabolites and synergistic changes in the ^1H NMR spectra through Receiver Operating Characteristic (ROC) analysis and calculation of the Area Under the Curve (AUC; Fawcett, 2006). Binary matrix resampling of spectral bins provided a robust framework for random data sampling. MetaboAnalystR was used to perform unsupervised principal component analysis (PCA), supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA), and ROC modeling. PCA and OPLS-DA models were created using both all spectral bins and subsets identified as significant by either VIAVC or univariate MW analysis. ROC models were developed using bins deemed significant via VIAVC ($p < 0.05$). All supervised models underwent double ten-fold cross-validation (Wiklund et al., 2008; Szymanska et al., 2012) to ensure their robustness and validity.

3.3.6 Group Creation for Analysis

Metabolomic profiles of 88 placental tissues were compared within stress categories (QFOSS and COSMOSS) based on a 2-level (high and low) tiering of raw scores (Table 3-1). For the QFOSS comparison, raw scores ranged from 2 to 62, with scores of 14 and below being categorized as low ($n = 55$), and scores of 15 and above being categorized as high ($n = 33$). In the COSMOSS comparison, raw scores ranged from -1.078 to 3.076, with scores of -0.31 and below being labelled as low ($n = 51$), and scores of -0.30 and higher being labelled as high ($n = 37$). An additional subset of groups was created for participants who were part of the M@NGO midwifery trial and those who were not. Of the 87 participants (one was omitted due to unknown midwifery status), 59 mothers were not part of the program and 28 were (Table 3-1). For those included in the midwifery program, 13 were in the low stress group and 15 were in the high stress group for both the QFOSS and COSMOSS categories. For those not included in the midwifery program, 41 were in the low QFOSS stress group and 18 were in the high QFOSS stress group, and 38 were in the low COSMOSS stress group and 21 were in the high COSMOSS stress group.

3.3.7 Metabolite Identification and Pathway Topology Analysis

Significantly altered metabolites corresponding to the identified bins were determined using the Chenomx Chemical Profiler software (Chenomx, AB, Canada) in conjunction with two libraries: the 700 MHz version 11 metabolite library from Chenomx and the Human Metabolome Database (HMDB_700) metabolite library (Wishart et al., 2022). After identification, the complete set of significantly altered metabolites for each comparison was subjected to metabolomic pathway topology analysis in MetaboAnalystR (Xia & Wishart, 2010). Pathway analysis utilized the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway library (Kanehisa et al., 2023), applying the hypergeometric test for over-representation analysis and relative-betweenness centrality for pathway topology analysis.

3.4 Results

Univariate and multivariate statistical analysis was utilized to investigate the placental metabolic profiles between high and low stress groups in both general QFOSS and COSMOSS categories as well as subgroupings based on midwifery status. For both general 2 level stress parameters, no unsupervised group separation in the PCA scores plot were revealed and failed both cross validation and permutation testing for supervised OPLS-DA modelling. Furthermore, midwifery subgroupings for QFOSS and COSMOSS categories revealed similar insignificant results. Despite the null findings, Table 3-2 summarizes which OPLS-DA models could and could not be generated with quality and significance values for the general and midwifery 2 level QFOSS and COSMOSS comparisons. In addition, Figure 3-1 illustrates the OPLS-DA score plots and Figure 3-2 illustrates the associated ROC curves based on significant bins identified through VIAVC best subset. A subsequent 3-level test was utilized by recategorizing the raw scores from QFOSS and COSMOSS scales into high, medium, and low groups. QFOSS low scores ranged from 2 to 10 ($n = 45$), medium scores ranged from 11 to 24 ($n = 24$), and high

ranged from 25 to 62 (n = 19). COSMOSS low scores ranged from -1.078 to -0.65 (n = 32), medium scores ranged from -0.615 to -0.0014 (n = 35), and high scores ranged from 0 to 2.623 (n = 21). Medium group scores from both stress categories were then omitted to enhance group separation of high and low values. Similarly, midwifery status subgroupings were created with the omission of medium stress participants. A breakdown of sample distribution statistics for the subsequent 3 level stress analysis is summarized in Supplemental Table 3-1. Similar to the initial 2 level stress analysis, the 3-level unsupervised PCA and supervised OPLS-DA modelling for both the general and midwifery groupings also revealed no group separation and failed cross-validation and permutation testing for QFOSS and COSMOSS categories. The models that did and did not fail data generation are compiled in supplemental Table 3-2. Moreover, the OPLS-DA and ROC curve plots are illustrated in Supplemental Figures 1 and 2, respectively. Finally, due to insufficient significant bins, no metabolites could be identified as being altered in any of the comparisons ran, and therefore pathway topology analysis could not be performed.

3.5 Discussion

Understanding how prenatal maternal stress is biologically reflected in the placenta is a key objective in developmental programming research, yet emerging evidence suggests that these effects may be more context-dependent than initially assumed. This study aimed to investigate the effects of prenatal maternal stress (PNMS) induced by the 2011 Queensland flood on the placental metabolome, while also evaluating whether participation in a midwifery program (M@NGO) was a mediating factor. Contrary to our hypotheses, we found no significant differences in placental metabolomic profiles between high and low stress groups, nor did we observe differences between participants in midwifery and non-midwifery care.

3.5.1 PNMS and the Placental Metabolome

Previous studies argued that elevated maternal cortisol caused by PNMS alters placental metabolic functioning by disrupting nutrient and oxygen transport, amino acid metabolism, energy production and oxidative stress (Joseph et al., 2020; Bronson & Bale, 2016; Cowell et al., 2020). Consequently, these alterations have been linked to numerous post-natal complications, including intrauterine growth restriction (IUGR; Fowden, 2017), low birth weight, and premature delivery (Rondó et al., 2003). The present metabolomics results, however, do not replicate the link between PNMS and altered placental metabolism, possibly due to several methodological factors. Other studies report variability in outcomes due to differences in how stress is measured, environmental conditions, and population-specific factors (Goyal et al., 2016; Dorsey et al., 2022; Nolvi et al., 2023). While the use of objective (QFOSS) and subjective (COSMOSS) stress measures provided complementary perspectives, the lack of significant findings may draw attention to the challenges inherent to the physiological and psychological dimensions of stress.

3.5.2 Timing and Severity of Stress Exposure

The timing and severity of stress exposure are important considerations while investigating PNMS effects. Distress endured during early pregnancy has been shown to have a greater impact on placental function and fetal programming compared to stress later in pregnancy (Glover, 2015). Stratifying timing of flood exposure could have improved the ability to detect subtle, timing-dependent effects on the metabolome. Future studies should elect to stratify participants by gestational age at the time of stress exposure to better understand how timing could influence metabolic outcomes.

3.5.3 Midwifery Care as a Protective Factor

Although midwifery care has previously been recognized as a protective factor for healthy pregnancies, the present study did not identify substantial metabolomic differences between

participants that were and were not part of the M@NGO trial. While midwifery programs provide emotional and psychological support, they may not directly influence placental metabolic pathways in the context of an extreme stressor such as a flood. Future research should explore whether personalised interventions, such as trauma-focused prenatal care or integrated stress-reduction programs, could mediate the effects of disasters on placental function.

3.5.4 Future Directions

While the present findings did not reveal significant differences in the placental metabolome with PNMS or midwifery care, they highlight the complexity of maternal stress and its impacts on placental function. Future research should incorporate larger, more diverse cohorts to increase statistical power and stress response variability. Longitudinal studies examining maternal and child outcomes may also uncover the long-term implications of PNMS.

3.6 Conclusion

This study examined the effects of prenatal maternal stress (PNMS) induced by the 2011 Queensland flood on the placental metabolome and tested the potential protective role of midwifery care. While no significant differences were found in metabolomic profiles between stress or care groups, the present findings emphasize the importance of expanding on the methodologies adopted in maternal stress research, particularly by developing universal and consistent stress measures, considering timing of exposure, and adapting factors of individual resilience. It is also important to consider the possibility that PNMS from the 2011 Queensland flood may not have produced measurable metabolic changes in placental tissue. Stress caused by environmental disaster may exert a more limited or transient impact on placental biochemistry than what is previously shown in other types of exposure. The placenta is a resilient and compensatory organ, which lends plausibility that these mechanisms were sufficient at maintaining metabolic equilibrium under the levels and types of stress experienced by the

present cohort. Likewise, midwifery care may not have directly altered placental metabolic pathways in ways detectable by ^1H -NMR. Therefore, the present null findings possibly indicate a true absence of metabolic disruption rather than methodological problems alone. Nonetheless, by addressing the methodological gaps, future studies can home in on effective strategies to limit the impact of stress and ultimately improve maternal and fetal health.

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3.8 TABLES AND FIGURES

3.8.1 TABLES

Table 3-1. Sample distribution statistics based on 2 level QFOSS (QFOSS 2) and COSMOSS (COSMOSS 2) stress classification and with (+) or without (-) midwifery program participation.

	QFOSS 2 Low Stress	QFOSS 2 High Stress	COSMOSS 2 Low Stress	COSMOSS 2 High Stress
Total (n)	55	33	51	37
(+) Midwifery (n)	13	15	13	15
(-) Midwifery (n)	41	18	38	21

Table 3-2. Summarized results of 2 level QFOSS (QFOSS 2) and COSMOSS (COSMOSS 2) high vs low stress comparisons based on Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) Q2 and R2 values (with coinciding p-values) and Receiver Operating Characteristic (ROC) curve area under the curve (AUC) and 95% confidence interval (CI) values. Midwifery status is defined as with (+MW) or without (-MW) program participation. Table entries showing N/A are representative of comparisons that could not be completed due to a lack of significant metabolites.

	QFOSS 2	COSMOSS 2	QFOSS 2 (+MW)	QFOSS 2 (-MW)	COSMOSS 2 (+MW)	COSMOSS 2 (-MW)
OPLS-DA						
Q2 (p-value)	N/A	0.0262 (0.0655)	N/A	N/A	-0.135 (0.545)	0.0292 (0.0835)
OPLS-DA						
R2 (p-value)	N/A	0.1 (0.049)	N/A	N/A	0.156 (0.188)	0.0977 (0.2)
ROC						
AUC (CI)	N/A	0.534 (0.221-0.704)	N/A	N/A	0.359 (0.06-0.699)	0.483 (0.198-0.746)

Supplemental Table 3-1. Sample distribution statistics based on supplemental analysis with 3 level QFOSS (QFOSS 3) and COSMOSS (COSMOSS 3) stress classification and with (+) or without (-) midwifery program participation.

	QFOSS 3 Low Stress	QFOSS 3 High Stress	COSMOSS 3 Low Stress	COSMOSS 3 High Stress
Total (n)	45	19	32	21
(+) Midwifery (n)	12	9	7	7
(-) Midwifery (n)	32	10	25	13

Supplemental Table 3-2. Summarized results of supplemental 3 level QFOSS (QFOSS 3) and COSMOSS (COSMOSS 3) high vs low stress comparisons based on Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) Q2 and R2 values (with coinciding p-values) and Receiver Operating Characteristic (ROC) curve area under the curve (AUC) and 95% confidence interval (CI) values. Midwifery status is defined as with (+MW) or without (-MW) program participation. Table entries showing N/A are representative of comparisons that could not be completed due to a lack of significant metabolites.

	QFOSS 3	COSMOSS 3	QFOSS 3 (+MW)	QFOSS 3 (-MW)	COSMOSS 3 (+MW)	COSMOSS 3 (-MW)
OPLS-DA Q2 (p-value)	N/A	N/A	-0.324 (0.69)	N/A	-0.161 (0.218)	N/A
OPLS-DA R2 (p-value)	N/A	N/A	0.088 (0.764)	N/A	0.294 (0.432)	N/A
ROC AUC (CI)	0.478 (0.282-0.696)	0.414 (0.146-0.673)	0.621 (0.29-0.876)	0.457 (0.165-0.797)	0.7 (0.06-1.0)	N/A

3.8.2 FIGURES

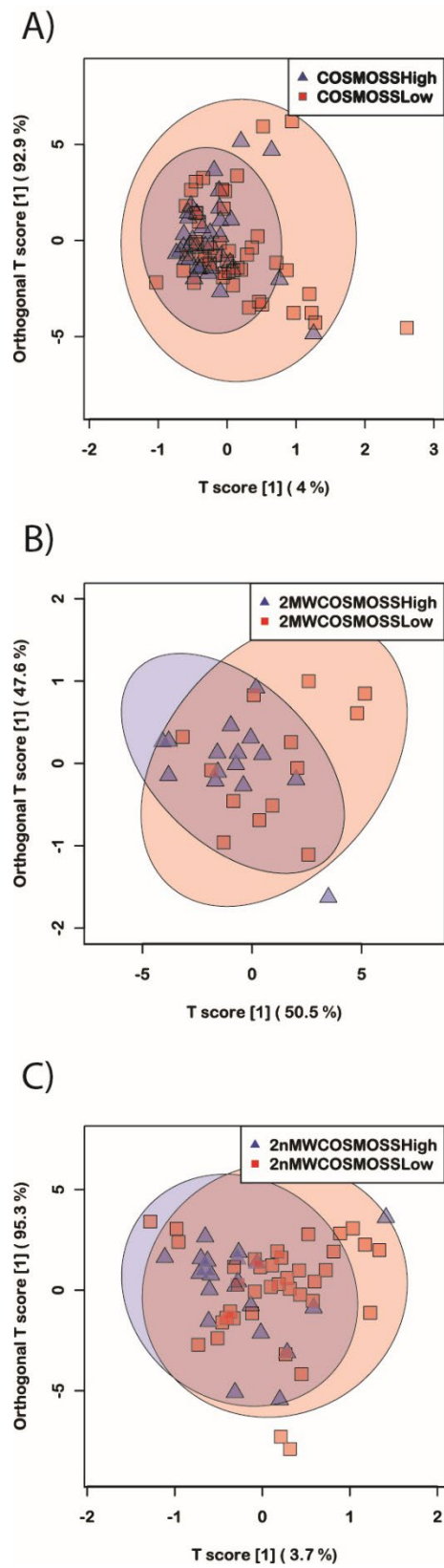
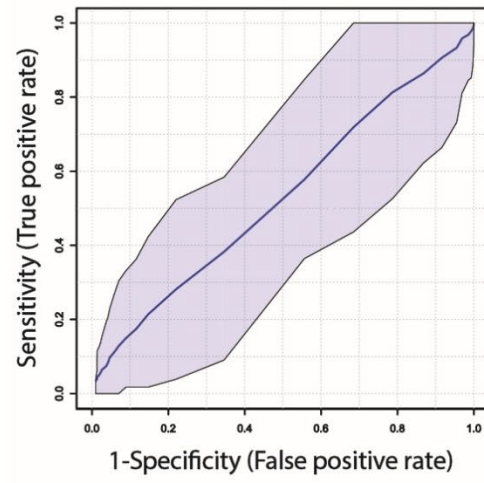
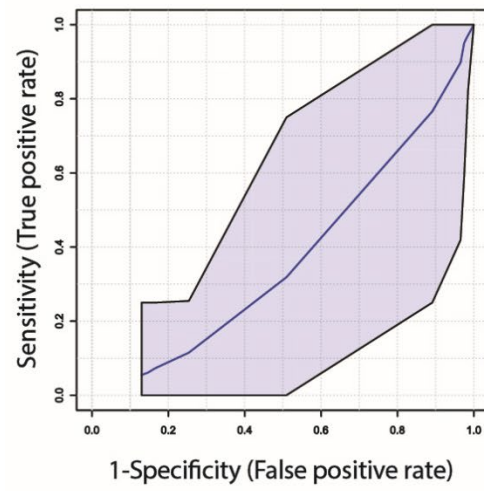


Figure 3-1. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) score plots for placental tissues that passed modeling from all comparisons ran in the 2 level QFOSS and COSMOSS high vs low stress tests. These comparisons include: (A) COSMOSS general 2 level ($Q^2 = 0.027$, $p = 0.07$; $R^2 = 0.1$, $p = 0.05$); (B) COSMOSS 2 level with midwifery participation ($Q^2 = -0.135$, $p = 0.55$; $R^2 = 0.156$, $p = 0.188$); and (C) COSMOSS 2 level without midwifery participation ($Q^2 = 0.029$, $p = 0.08$; $R^2 = 0.098$, $p = 0.02$). The shaded areas represent the 95% confidence interval, while the x-axis and y-axis represent the between- and within-group variation, respectively. Note that none of the models demonstrated group separation and were below null thresholds for significance.

A)



B)



C)

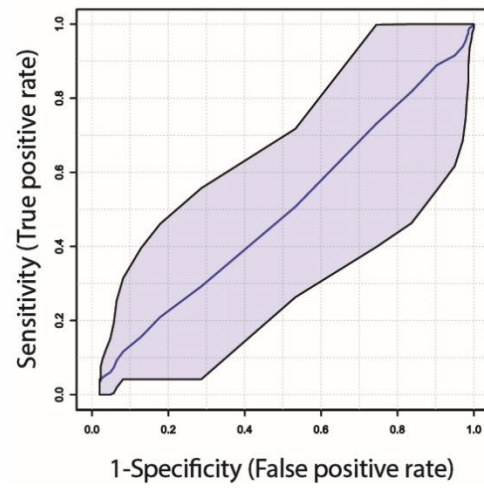
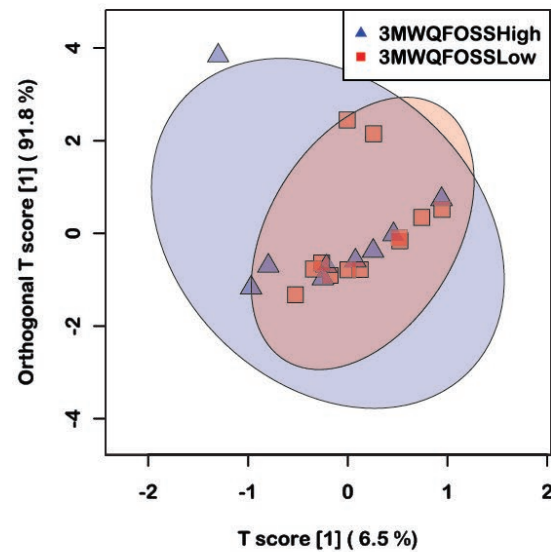


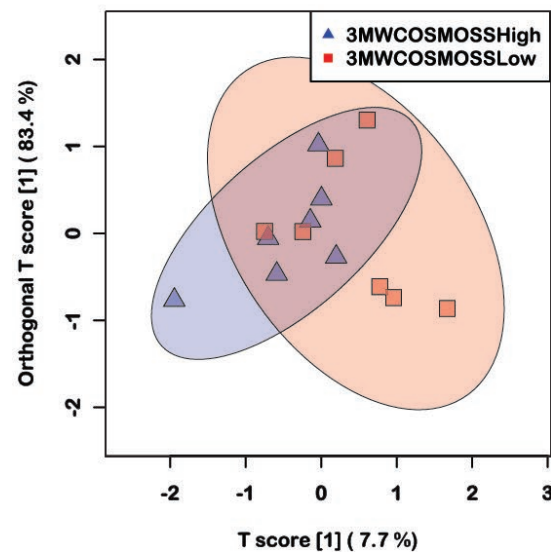
Figure 3-2. Receiver Operating Characteristic (ROC) curves for placental tissues that passed modeling from all comparisons ran in the 2 level QFOSS and COSMOSS high vs low stress tests. These comparisons include: (A) COSMOSS general 2 level (AUC: 0.534, CI: 0.221-0.704, Predictive accuracy: 54.1%); (B) COSMOSS 2 level with midwifery participation (AUC: 0.359, CI: 0.06-0.699, Predictive accuracy: 45.6%); and (C) COSMOSS 2 level without midwifery participation (AUC: 0.483, CI: 0.198-0.746, Predictive accuracy: 50.7%). The shaded area represents the 95% confidence interval. Each figure was created using the metabolites identified as significant based on the VIAVC best subset.

3.8.3 SUPPLEMENTAL MATERIALS

A)

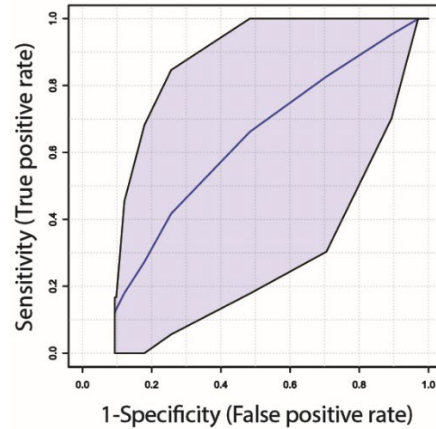


B)

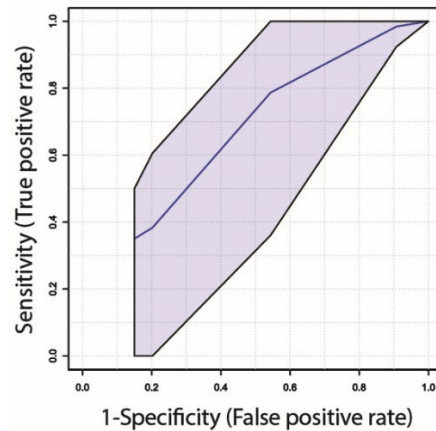


Supplemental figure 3-1. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) score plots for placental tissues that passed modeling from all comparisons ran in the subsequent 3 level QFOSS and COSMOSS high vs low stress tests. These comparisons include: (A) QFOSS 3 level with midwifery participation ($Q^2 = -0.324$, $p = 0.69$; $R^2 = 0.088$, $p = 0.764$); (B) COSMOSS 3 level with midwifery participation ($Q^2 = -0.161$, $p = 0.218$; $R^2 = 0.294$, $p = 0.432$). The shaded areas represent the 95% confidence interval, while the x-axis and y-axis represent the between- and within-group variation, respectively. Note that none of the models demonstrated group separation and were below null thresholds for significance.

A)



B)



Supplemental figure 3-2. Receiver Operating Characteristic (ROC) curves for placental tissues that passed modeling from all comparisons ran in the 3 level QFOSS and COSMOSS high vs low stress tests. These comparisons include: (A) QFOSS 3 level with midwifery participation (AUC: 0.621, CI: 0.29-0.877, Predictive accuracy: 56.7%); (B) COSMOSS 3 level with midwifery participation (AUC: 0.7, CI: 0.06-1.0, Predictive accuracy: 63.0%). The shaded area represents the 95% confidence interval. Each figure was created using the metabolites identified as significant based on the VIAVC best subset.

CHAPTER 4: CONCLUSION

4.1 Hypotheses and Results

The objective of this thesis was to investigate the impacts of maternal stress on the placental metabolome caused by clinical and environmental exposures. Two distinct stress paradigms (maternal prenatal depression with or without SSRI treatment and prenatal maternal stress following natural disaster) were evaluated to demonstrate how different forms of adversity influence the intrauterine environment and potentially program fetal outcomes.

The first study (VSSRI) examined placental metabolomic profiles in pregnant women with or without symptoms of depression, contrasting those who were or were not prescribed SSRI medication. The findings demonstrated that depression without SSRI treatment was associated with distinct metabolic alterations in placental tissue, including changes in glutamate, lysine, proline, and serine signatures. These metabolites are involved in pathways related to amino acid metabolism, oxidative regulation, and neurotransmitter precursors, all of which play vital roles in healthy fetal growth and neurodevelopment (Bonnin et al., 2011; Burton & Fowden, 2016; Rosenfeld, 2021; Sferruzzi-Perri & Camm, 2016; Vaughan & Fowden, 2016). Notably, placentas from depressed mothers who took SSRI medication did not exhibit discernable metabolic differentiation from non-depressed/non-SSRI treated controls, therefore suggesting that SSRI treatment may minimize or negate metabolic disruptions otherwise associated with maternal depression. This observation matches the hypothesis that untreated depression symptoms impart detectable metabolic alterations in the placenta and provides evidence that SSRIs may have a stabilizing effect at the maternal-placental fetal interface (Bondarenko et al., 2024; Heikkinen et al., 2002; Hendrick et al., 2003; Rao, 2007; Rosenfeld, 2020).

The second study (QF2011) analyzed placental tissue from mothers exposed to the 2011 Queensland flood, which quantified stress by objective hardship and subjective distress. In addition, this study considered whether midwifery care through the M@NGO trial offered resilience against the stress-related metabolic impacts. Contrary to the findings of the VSSRI study, no consistent or reproducible separation was measured between high and low objective or subjective stress groups, nor were there differences between participants who did or did not participate in midwifery care. While these results do not support the original hypothesis that prenatal maternal stress would alter placental metabolic signatures, they are nonetheless informative. They suggest that the placental metabolome may be resilient to certain types or timings of stress. Alternatively, the heterogeneity of stress experiences following a natural disaster may mask subtle biochemical disruptions, thus obscuring potential metabolomic differences. The null findings may also indicate methodological challenges in stress assessment, due to objective and subjective measures sometimes yielding different results and possibly capturing different aspects of maternal experience (King et al., 2015; King & Laplante, 2005; O'Donohue et al., 2021). Finally, the absence of a true “control” group of mothers that were not exposed to the flood or did not experience any level of stress also complicates the interpretation of the results.

In combination, the results of these two studies highlight the potential and the challenges of placental metabolomics in stress research. On one hand, the VSSRI findings show that placental tissue may provide a source for biomarkers of untreated maternal depression and that therapeutic intervention may mitigate the alteration of those biomarkers. On the other hand, the QF2011 study emphasizes the complexity of environmental exposures and the difficulty of

interpreting psychosocial stress in metabolic fingerprints. Taken together, these studies demonstrate the duality of the placenta as being both a sensitive and resilient organ.

4.2 Limitations

While the findings presented in this thesis contribute to the scientific understanding of maternal stress and placental function, various limitations must be noted. Above all, small sample sizes limited the predictive power for both studies. For the VSSRI analysis, only 58 placentas divided into subgroups of increasingly discriminant criteria restricted the capability to identify subtle or heterogeneous effects. Meanwhile, the QF2011 study started with a substantial cohort of recruited participants, yet only 88 placentas were supplied that provided sufficient material for metabolomic analysis. Further division of this sample group into stress-based subgroupings and midwifery status reduced statistical capability, conversely raising the probability that real but nominal metabolic differences may have gone undetected.

The two studies presented here were also limited by the usage of placental tissue collected at term delivery, which is inherent to the natural birth processes but nonetheless can only permit cross-sectional analysis. While this offers valuable data about the accumulated effects of maternal stress, it cannot capture fluctuations of diet and circadian rhythm, nor at different stages of pregnancy (Palmeiro-Silva et al., 2018; Tadanki et al., 2025; Lammertink et al., 2021; Burton & Fowden, 2015; Weinisch et al., 2022; Hancox et al., 2021). In absence of periodical longitudinal sampling, it is unknown whether alterations observed in the VSSRI cohort began early and persisted throughout gestation, or if they appeared later because of prolonged depressive symptoms. Likewise, the null QF2011 results may not have been linked to the absence or weakness of stress impacts on physiology but may rather indicate compensatory adaptive mechanisms emerging earlier in pregnancy, leading to normalized metabolomes at term.

Methodological limitations of ^1H NMR spectroscopy are also worthy of consideration. Although NMR offers a robust, reproducible, and comprehensive overview of metabolic features, it is less sensitive than other mass spectrometry approaches in detecting metabolites at low concentrations (Emwas et al., 2019; Dunn et al., 2011; Gika et al., 2014; Markley et al., 2017). Therefore, potentially biologically relevant stress signatures in either study may have gone undetected.

Further limitations lie in the measurement of psychosocial stress and depression. The HAM-D scale, used in the VSSRI study, requires mindful interpretation, whereby even minor shifts in cut-off thresholds can alter group classification (Hamilton, 1960). The QF2011 Study relied on measurements of objective hardship and subjective distress as different constructs of stress assessment (King et al., 2015; King & Laplante, 2005). Although these measures capture different facets of the stress experience, neither construct is infallible. Both study metrics are influenced by recall bias, cultural context, and personal resilience among other internal and external factors, which complicate their direct alignment with the biological measurements of metabolite signatures (O'Donohue et al., 2021; King et al., 2015; Levinson et al., 2023). The lack of metabolic differences in QF2011 may therefore reflect challenges in measurement and classification of stress experiences rather than true biological null effects.

Another important limitation in interpreting the results of both studies is the absence of data regarding other potentially relevant symptoms of depression or stress factors. Specifically, the VSSRI study included participants who did not provide information indicating whether they had opted for mental health counseling at any point prior to or during pregnancy, have a history of depression prior to recruitment, or struggled with interpersonal or vocational challenges (Shishehgar et al., 2015; Lobel & Preis, 2025; Westdahl et al., 2007; Seely & Mickelson, 2019;

Carlson et al., 2025). These variables can potentially impact the effects of SSRI medication, depression, and stress on metabolomic signatures, either through added resilience or vulnerability.

The VSSRI study could not account for different types of SSRI treatments. This was largely due to the scale of total SSRI drugs being taken by the participants ($n = 14$), which, when divided by compound (citalopram = 5, escitalopram = 4, fluoxetine = 1, paroxetine = 1, sertraline = 3), could not meet sufficient group sizes for comparison. Pooling these drugs together was the only option for metabolomic analysis, despite these ingredients having different half-lives, potencies, and side effect profiles (Altamura et al., 1994; Hemeryck & Belpaire, 2002; Hendrick et al., 2003; Heikkinen et al., 2002; Rao, 2007). Consequently, any causal alterations to placental metabolites by any of these drugs cannot be distinguished. Moreover, it is possible that some drugs were more effective at managing symptoms of depression (and therefore may have different downstream metabolic impacts) than others. It may also be noteworthy that potential side effects unique to a particular drug may have contributed to mental and physical well-being. The lack of detailed dosing information, along with the absence of patient history regarding the reported medication and any previously used treatments, represents another important limitation. Without information on the dosage regimen, how doses were adjusted over time, the consistency of medication use, or whether the patient switched drugs during pregnancy, it becomes difficult to establish a coherent and comparable quasi-experimental group. Lastly, the timing of each drug is also very important and was not considered in this analysis. Each stage of pregnancy poses different risks for the mother and the developing fetus, but this analysis does not discriminate what stage of pregnancy a drug was initiated. Hence, it is possible that placental metabolic disruptions could be more sensitive at different stages of drug administration. Altogether, there

are numerous pharmacological specificities that cannot be accounted for because the sample sizes were too small for partitioning and several important considerations that were not incorporated in the analysis.

Without physiological, cognitive, and behavioural follow-up assessments for the VSSRI-exposed children, beyond term measures such as Apgar scores, birth weight, and gestational age that extend longitudinally into adolescence, it is not possible to link metabolomic alterations associated with untreated maternal depression to actual developmental abnormalities (Barker & Osmond, 1986; Roseboom et al., 2011; Lumey, 2016; Gu & Guan, 2021; Lamichhane et al., 2020). By contrast, the QF2011 study includes childhood follow-up assessments; however, the absence of significant group differences in metabolite concentrations prevents these developmental measures from being meaningfully incorporated into further analyses. Finally, concerns remain about generalizability. The VSSRI cohort was geographically and demographically narrow, thus limiting translation to other populations with different cultural, genetic, or healthcare conditions. The QF2011 cohort, meanwhile, also struggled with similar constraints and was specific to a single natural disaster event. Combined, these factors caution against overextending the findings and urge the need for replication in broader and more varied populations (McEwen et al., 2019; Sookoian et al., 2013; Yadav et al., 2022).

4.3 Future Directions

Following the findings of this thesis, future research should pursue several directions to strengthen and extend the contributions of this work. First, longitudinal studies that collect biomarkers demonstrating maternal and fetal status across gestation, such as blood, urine, and/or saliva, provide critical insight into the timing and progression of stress-related metabolic alterations (Barker & Osmond, 1986; Gluckman et al., 2008; Hayward & Lummaa, 2013;

Weinisch et al., 2022). Although it is neither ethical nor methodologically feasible to obtain repeated human placental samples during pregnancy, integrating these alternative biomarker sources would complement placental analyses and offer a more comprehensive picture of the maternal–fetal interface throughout gestation. Such an approach could help determine when metabolic changes first emerge and whether they represent transient fluctuations, compensatory adaptations, or persistent disruptions. Equally important is the need to increase sample sizes and include more diverse populations in future studies (McEwen et al., 2019; King et al., 2015). Larger cohorts improve statistical power and discrimination of more subtle metabolic effects, while greater demographic diversity will help distinguish whether findings from Canadian and Australian origins generalize across cultural and genetic conditions. Replicating the VSSRI findings in larger, more robust samples is especially crucial to ratify the apparent protective effect of SSRIs against depression related placental disruption. Similarly, expanding natural disaster cohorts beyond the QF2011 study could elucidate whether the lack of metabolic differences is because of resilience specific to that event or to a broader, more generalizable phenomenon.

Another promising research avenue lies in the integration of metabolomics with other omic approaches. Pairing metabolite analysis with genomic, transcriptomic, and proteomic methods could illuminate multi-level regulatory pathways that link maternal stress exposures to placental adaptation/disruption (Chuang et al., 2010; Yadav et al., 2022; Sookoian et al., 2013). For example, DNA methylation and metabolite signatures may provide a lens into how stress-responsive genes translate into altered biochemical pathways. These approaches may not just identify robust biomarkers across the omics but also can enhance the understanding of mechanisms linking them together.

Future research should also consider pairing stress measures with other quantitative variables. Combining subjective self-report with objective biological indicators, such as inflammatory markers or cortisol, may yield more precise and reliable assessments of maternal stress (King et al., 2015; Kotozaki & Kawashima, 2012; Gao et al., 2014). A multidimensional approach could strengthen correlations with metabolomic data and mitigate misclassification. Moreover, other clinically relevant data during pregnancy, such as mental health counseling participation, comorbid health conditions, interpersonal and vocational satisfaction, and so forth should be considered for collection to deconvolute confounding or external factors within the analysis.

Additional VSSRI or similarly designed studies should also include longitudinal developmental assessments for pre-exposed and control offspring to determine whether any placental metabolic associations can be linked to risks of childhood, teenage, or adult cognitive, motor, behavioral, and/or physiological outcomes (Grizenko et al., 2008; Ronald et al., 2011; Sandman et al., 2011; Korja et al., 2017; Babenko et al., 2015). It is fundamental to these biomarker studies to pair quantitative biological data with measurable real-world consequences. If clinicians can collect early indicators of later life complications, such as obesity, cardiovascular disease, ASD, ADHD, etc., then they can provide intervention strategies to caregivers that may prevent those adversities from progressing or even manifesting altogether.

Finally, more insight into clinical interventions is needed to test potential protective factors (Metz et al., 2025; Bleker et al., 2020; Siebach et al., 2024; Karamoozian & Askarizadeh, 2015; Shimada et al., 2021; Kildea et al., 2018; Hartz et al., 2013; Simcock et al., 2018). The apparent normalization of placental profiles in SSRI-treated mothers observed in the VSSRI study demands further investigation into the underlying mechanisms. A better understanding of

whether all SSRIs confer similar effects or whether certain drugs differentially stabilize placental metabolism will be crucial for clinical perspectives. What is more, the personalized nature of efficacy for each drug needs to be accounted for when pooling results on a larger scale. It may be required to balance subgroups based on an equal representation of drug brands, dosages, durations, and depression symptoms at different time periods. Similarly, non-pharmacological interventions such as midwifery care should be evaluated with equally powered and controlled designs to determine their influence on resiliency. Other non-pharmacological interventions should be explored to provide a clinically proven pool of options for mothers to choose from, such as psychotherapy (Metz et al., 2025), cognitive behavioural therapy (CBT), meditation, or music therapy. Given that some strategies work better for some more than others, the more options that are available means more mothers will find a strategy suitable for their needs.

4.4 Overall Contributions

In conclusion, this thesis contributes to the growing body of knowledge regarding placental metabolomics in response to maternal stress factors. It first provides evidence that maternal depression without SSRI treatment imparts a detectable imprint on the placental metabolome, an effect that may be attenuated with SSRI treatment. It also illustrates the complexity of environmental stress exposure, which, despite comprehensive measurement and design, yielded no consistent metabolic differentiation. Together, the findings of this thesis reinforce the vital role of the placenta as a biosensor and an adaptive organ, demonstrating its ability to adapt to certain maternal stressors while resisting others (Burton & Fowden, 2015; Sferruzzi-Perri & Camm, 2016; Walejko et al., 2018).

Situated within the DOHaD framework, the present body of work underscores the value of maternal psychology and environmental adversity as determinants of child health (Barker &

Osmond, 1986; Faa et al., 2024; Roseboom et al., 2011; Lumey, 2016; Gluckman et al., 2008; Hayward & Lummaa, 2013). It highlights the advantages and challenges of the placental metabolomics approach, advancing the knowledge of fetal programming while also acknowledging methodological and conceptual barriers that remain. Ultimately, this thesis encourages future research to continue developing more integrative and clinically relevant strategies. Through the continued pursuit of biomarkers and interventions, there is potential to develop individualized prenatal care and contribute to healthier beginnings for future generations.

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