Nutrient Intake and Lifestyle Patterns of Pregnant Indigenous Women Residing in Northern Manitoban Communities: A pilot Study for Implications for Fetal Alcohol Spectrum Disorder

by

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Abstract

Planning maternal health programs requires a comprehensive understanding of maternal behaviors that are associated with fetal alcohol spectrum disorder (FASD), as alcohol consumption is not the sole contributor. Compromised maternal nutrition is identified as one of the major factors contributing to FASD due to alcohol's abilities of displacing nutrients and interfering with metabolism. However, the information on nutrition status of women at-risk of having children with FASD, is scarce, especially in Indigenous women. This study aimed to identify dietary intake of nutrients important for fetal central nervous system development and examine if alcohol consumption influenced dietary intake in pregnant women. Through the community engagement and partnerships with two northern Manitoba First Nation communities (Opaskwayak Cree Nation and Chemawawin Cree Nation), 59 pregnant women, ages 14-42 years, were recruited to participate in the in-person survey using Nutrition for Two instrument. Information was obtained on participant demographics, dietary intake, substance use, pregnancy outcomes and maternal health. Additionally, biological samples, urine and plasma, were collected for measuring metabolic parameters, fatty acids, cytokines, and mineral profiles. The preliminary findings of this study demonstrated that all participants were below the serving size in all food groups recommended by the former Health Canada CFG. Higher prevalence of inadequacy was observed for three key nutrients: folate, iron, and DHA for both communities. Maternal self-reported alcohol consumption was associated with increased intake of fat macronutrient ($p<0.05$) and decreased intakes of niacin, folate, choline, and calcium ($p<0.05$). No relationship existed between dietary intake and other risk factors. With respect to biological data, alcohol consumption during pregnancy presented a significant positive relationship with plasma glucose ($p<0.05$) and negative relationship with anti-inflammatory cytokine, erythrocyte C18:2n6 and C20:4n6 ($p<0.05$). This pilot study contributes to the field of maternal health and nutrition by identifying the influence of alcohol consumption on the intakes of macro- and micro-nutrients in pregnant First Nations women residing in remote communities in Manitoba. The results of the present study could be utilized for maternal grass roots programming and future planning of community nutrition research.

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Foreword

This thesis is written in manuscript style and is composed of seven chapters, which is an original work to fulfill my PhD thesis. *Chapter I* consists of the introduction to the topic and literature review, which critically assesses the state of knowledge surrounding the FASD and nutrition. *Chapter II* presents study plans including the rationale, objectives and hypotheses. *Chapters III and IV* present the results of verbal and clinical data collected from Chemawawin Cree Nation. *Chapter V* presents the results of the Opaskwayak Cree Nation community. *Chapter VI* combines the findings from both Opaskwayak Cree Nation and Chemawawin Cree Nation communities. The last - *Chapter VII* encloses the overall summary and discussion of the thesis with concluding remarks of the work and proposed future research directions.

Up to date, this thesis produced 3 manuscripts and 1 book chapter published.. The manuscripts are specified at the end of the relevant chapters.

1) Kloss O., Eskin NAM., & Suh M. (2018). Thiamin deficiency on fetal brain development with and without prenatal alcohol exposure. Biochem Cell Biol. 96(2):169-177.
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3) Kloss O., Sharova L., & Suh M. (2022). Nutrition intervention as a preventative approach to Fetal Alcohol Spectrum Disorder. Advances in Fetal Alcohol Spectrum

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Kloss O, conceptualization and writing of the original manuscript; Sharova L, provision of information and guiding table development; Suh M, review & editing overall supervision.

4) Kloss O., Jebb M., Chartrand L., Chudley A., Eskin NAM., & Suh M. (2022). Dietary intake patters and lifestyle behaviors of pregnant women living in a Manitoba First Nations community: implications for fetal alcohol spectrum disorder. Nutrients, 14 (15), 3233:1-16. Kloss O, performed all aspects of research activities from the community engagement, data collection and analysis, and writing manuscript; Jebb M and Chartrand L, supported the research, data collection, review & editing; Chudley A and Eskin NAM, review & editing; Suh M, developed and designed the original study, community engagement writing, review & editing, funding acquisition, and overall supervision.

Alcohol and ethanol are used in the following context: Alcohol is used to describe consumption, as it is a common terminology utilized, and ethanol (EtOH) when referred to the physiology and metabolism, as this is the form in which consumed alcohol is being metabolized in. The definitions of Indigenous groups are used in alignment with the Constitution Act 1983, Section 35 definitions.

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List of Abbreviations

ADH	Alcohol Dehydrogenase
AI	Adequate Intake
ALDH	Acetaldehyde Dehydrogenase
ALT	Alanine Aminotransferase
AMC	Assembly of Manitoba Chiefs
APA	American Psychiatric Association
apo-CRBP1	Hepatic Cellular Retinol-binding Protein 1
ARBD	Alcohol Related Birth Defects
ARND	Alcohol Related Neurodevelopmental Disorders
AST	Aspartate Aminotransferase
BAC	Blood Alcohol Concentration
BCR	Band Council Resolution
BMI	Body Mass Index
BW	Beatrice Wilson Health Centre
CAMH	Center for Addictions and Mental Health
CCHS	Canadian Community Health Survey
CCN	Chemawawin Cree Nation
CFG	Canada's Food Guide
CHMS	Canadian Health Measures Survey
CIHR	Canadian Institutes of Health Research
CNF	Canadian Nutrient File
CNS	Central Nervous System
DHA	Docosahexaenoic Acid
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
EPA	Eicosapentaenoic acid
EtOH	Ethanol
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorder
FFQ	Food Frequency Questionnaire
FNFNES	First Nations Food, Nutrition & Environment Study
FNIGC	First Nations Information Governance Centre
GD	Gestational Day
GDM	Gestational Diabetes Mellitus
GGT	Gamma-glutamyl Transferase
HFI	Household Food Insecurity
HIRGC	Health Information Research Governance Committee
HDL-C	High-density Lipoprotein Cholesterol
HR-ICP-MS	High Resolution-Inductively Coupled Plasma-Mass Spectrometry
hs-CRP	High-sensitivity C-reactive Protein
IGF	Insulin Growth Factor
IOM	Institute of Medicine
IQR	Interquartile Range
IUGR	Intrauterine Growth Restrictions
LDL-C	Low-density Lipoprotein Cholesterol

MCP	Monocyte Chemoattractant Protein
MEOS	Microsomal Ethanol Oxidizing System
MHSAL	Manitoba, Health, Seniors and Active Living
MS	Methionine Synthase
MTHFR	Methylene Tetrahydrofolate Reductase
NAD	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NIH	National Institutes of Health
NPHS	National Population Health Survey
NTD	Neural Tube Defects
OCN	Opaskwayak Cree Nation
PAE	Prenatal Alcohol Exposure
PC	Phosphatidylcholine
PD	Postnatal Day
PHAC	Public Health Agency of Canada
PLD	Phospholipase D
POMC	Proopiomelanocortin
PPP	Pentose Phosphate Pathway
PUFA	Polyunsaturated Fatty Acid
RA	Retinoic Acid
RAR	Retinoic Acid Receptors
RDA	Recommended Dietary Allowance
RDH	Retinol Dehydrogenase
REH	Retinol Ester Hydrolase
RHS	Regional Longitudinal Health Survey
RLDH	Retinaldehyde Dehydrogenase
RM	Rural Municipality
ROS	Reactive Oxygen Species
RXR	Retinoid X receptors
SAH	S-adenosyl Homocysteine
SCTC	Swampy Cree Tribal Council
SD	Standard Deviation
SES	Socioeconomic Status
SVCT2	Sodium-dependent Vitamin C Co-transporter 2
TAG	Triacylglycerol
TC	Total Cholesterol
TCA	Tricarboxylic Acid
TNF	Tumor Necrosis Factor
TPP	Thiamin Pyrophosphate
TRC	Truth and Reconciliation Commission
TCP	Tri-Council Policy
UCN	University College of the North
US	United States
USDA	United States Department of Agriculture
95% CI	95% Confidence Interval

CHAPTER I: INTRODUCTION

First Nations communities in Canada suffer from a poorer quality of life, which is measured by higher mortality and morbidity. Maternal and child health indicators for First Nations women are routinely documented to be less favorable than for non-Indigenous women. Infant mortality rate among First Nations from all causes is reported to be 9.2 per 1,000 live births (95% Confidence Interval (CI) 7.2-11.3) compared to 4.4 per 1,000 births (95% CI 4.2-4.7) in the general Canadian population (Shepard et al., 2017). The rates of preterm labor (8.7%; 95% CI 8.1-9.3), small and large for gestational age babies (6.6%; 95% CI 6.0-7.1 and 18.8%; 95% CI 18.1-19.6, respectively) are notably higher for First Nations women compared to their non-Indigenous counterparts (6.7%; 95% CI 6.6-6.9%; 8.6%; 95% CI 8.5-8.6%; and 10.6%; 95% 10.5-10.8%, respectively) (Shepard et al., 2017).

Epidemiological studies indicate that the prevalence of fetal alcohol spectrum disorder (FASD), a continuum of conditions that result from prenatal alcohol exposure (PAE), among First Nations communities may be higher compared to general Canadians. Although there are no exact statistics, an estimated FASD prevalence in select First Nations communities is as high as 190 cases per 1,000 births (Robinson et al., 1987) compared to 9 in 1,000 births - the general Canadian average (Government of Canada, 2017).

The historical legacy of colonization policies has engendered multiple disadvantages for First Nations women. Appropriation of land and forced resettlements, eradication of language and culture, destruction of a family system through residential schools and assimilation policies, and multiple forms of discrimination resulted in intergenerational trauma to Indigenous communities (Regan, 2010). These events eradicated Indigenous matriarchal societies replacing them with emerging patriarchal ideologies, shifting Indigenous women's identities, autonomy, feminine capacity, and agency exposing women to a higher level of adverse consequences (Heart, 2003; Kloss et al., 2021). Studies indicate that First Nations women experience increasing levels of poverty, social marginalization, food insecurity, psychological distress, lower education levels, and exposure to partner's violence, thus making them at higher risk of alcohol consumption during pregnancy (Heaman et al., 2012; Tait, 2003).

Currently, major FASD prevention efforts targeting women, including Indigenous women, have typically been focused on the promotion of alcohol abstinence during pregnancy (CanFASD,

2013; Poole et al., 2016). However, emerging research and the findings of the Truth and Reconciliation Commission (TRC) make it clear that the focus on abstinence alone does not reduce the rates of FASD and in some instances may promote colonial practices (TRC, 2015; Wilkinson & Room, 2009). This necessitates a delineation of novel prevention strategies, which would effectively address an epidemic of FASD (TRC, 2015).

A novel preventative approach, which has promising prospects of mitigating the developmental dysfunctions associated with FASD, is food and nutrition intervention. While prenatal alcohol consumption is a number one risk factor for FASD, alcohol consumption alone cannot completely explain the spectrum of anomalies seen in persons with fetal alcohol syndrome (FAS), the most severe form of FASD (Abel & Hannigan, 1995; Drabble et al., 2011; Tait, 2003; Jacobson et al., 1996). Growing evidence reveals that factors such as poverty, poor nutrition, genetic predisposition, psychological health, and other conditions contribute to the risk of FASD (May & Gossage, 2011; Jacobson et al., 1996). Multitude of these factors are static and require insurmountable efforts from various sectors (political, social, economic, and clinical) to attenuate, this delineates nutrition as one of the most manageable and modifiable risk factors.

Adequate maternal nutritional status during pregnancy is key for the development of a healthy infant. The physiological changes during pregnancy lead to an increased need for a variety of macro- and micro-nutrients. Inadequate intake of these nutrients not only contributes to negative birth outcomes but exacerbates FASD outcomes. A wide body of research shows that adequate dietary intake and/or prenatal nutrient supplementation alleviates alcohol-induced damage in animal models and pre-clinical studies, reducing the severity of alcohol-related birth deficits and partially restoring cognitive function following PAE (Young et al., 2014; Ballard et al., 2012). Ballard and co-workers (2012), hypothesized that alcohol-related birth outcomes may be generated by the maternal nutritional deficiencies that are exacerbated by alcohol, rather than by direct alcoholic toxicity. Considering alcohol hinders the absorption and metabolism of nutrients at multiple levels, affecting cellular function, this hypothesis may be valid and needs to be tested (Ballard et al., 2012). Therefore, it is imperative to evaluate the current nutritional status of women who are at higher risk of bearing a child with FASD.

Despite the existence of data on the feasibility of nutrient supplementation as a potential FASD intervention, a formidable factor-the lack of information on the baseline maternal nutrition status,

-needs to be addressed, by determining food intake patterns, food choices, and nutrition status of pregnant First Nations women. This is of particular importance to First Nations communities as major national health surveys (Canadian Community Health Survey (CCHS), Canadian Health Measures Survey (CHMS), National Population Health Survey (NPHS)) exclude First Nations groups residing in reserves (First Nations Information Governance Centre (FNIGC), 2015).

Definition of FASD

Fetal Alcohol Spectrum Disorder is a non-diagnostic, umbrella terminology created to describe an array of anomalies that occurs in a fetus during prenatal alcohol consumption (CanFASD, 2013). The condition was first reported by French researchers in 1968; and the term FAS was coined by Jones and Smith in 1973 (Jones & Smith, 1973). It was described as a pattern of abnormalities observed in children born to alcoholic mothers. It was originally suggested that alcohol-related birth outcomes might be a result of malnutrition, exacerbated by alcohol (Jones & Smith, 1973).

Diagnosis and Classification of FASD

Presently, a few diagnostic guidelines for FASD exists. Most notably, a set of guidelines proposed by the Institute of Medicine (IOM, 1996), Diagnostic and Statistical Manual-5 (DSM-5) (American Psychiatric Association (APA, 2015)), and the Four-Digit Diagnostic Code introduced by Astley and Clarren in 1997(Astley & Clarren, 1997). The IOM-1996 guidelines, the most prevalently utilized guidelines, have been applied in many studies and clinical practice in North America. The guidelines include five categories: i) FAS with confirmed maternal alcohol exposure, including evidence of facial dysmorphology, growth retardation, and central nervous system (CNS) dysfunction; ii) FAS without confirmed maternal alcohol exposure, including evidence of facial dysmorphology, growth retardation, and CNS dysfunction; iii) partial FAS (pFAS) with confirmed maternal alcohol exposure, facial dysmorphology, and either growth retardation or CNS abnormalities; iv) alcohol-related neuro-developmental disorders (ARND) - confirmed maternal alcohol exposure and evidence of CNS abnormalities; iv) and alcohol-related birth defects (ARBD) - the presence of congenital anomalies (e.g., cardiac, skeletal, renal, ocular, auditory) known to be associated with a history of PAE (IOM, 1996; Chudley et al., 2005). FAS is referred to as the most severe form of FASD, whereas ARND-the least severe form of FASD. Due to the lack of specificity in the IOM-1996 guidelines, Astley and

Clarren (1997) formulated the four-digit diagnostic code (from 1 to 4) that aimed to address some limitations of the FASD diagnosis. This number system conveys the magnitude of expression in each of the four key diagnostic domains: growth deficiency, FAS facial phenotype, brain damage, and PAE. A score of 4 signifies the definitive presence of the criteria, while a score of 1 is given when the criteria are absent (Astley & Clarren, 1997). This approach is more precise in its evaluation of diagnostic categories and better characterizes the full spectrum of disabilities. Although the IOM-1996 and 4-Digit Diagnostic Code are different approaches, the underlying, fundamental criteria of the two approaches are similar (Chudley et al., 2005). It is suggested that clinicians use both approaches to strengthen the specificity of the diagnosis.

A more recent guidelines for the diagnosis of FASD was published by Canadian scientist and medical professionals, which proffer the recommendations on the screening, referral, medical assessment, physical examination, and differential diagnosis (Cook et al., 2016). The guidelines minimize the diagnostic categories down to two: FASD with sentinel facial features and FASD without sentinel facial features (Cook et al., 2016; Chudley, 2018). Presently, the Cook et al., 2016 guidelines are being used in several Canadian clinics.

Epidemiology of FASD in Canada

The most cited estimation of the prevalence of FASD in Canada is based on United States data, and it is reported to be 1–3 FAS cases per 1000 live births and about 9 FASD cases per 1000 births (Government of Canada, 2017). A recent Canadian study suggests that the population-based prevalence of FASD among elementary school children is about 2-3% (Popova et al., 2019). The prevalence among the total Canadian population is about 4% (CanFASD, 2020). However, the true prevalence of FASD is unknown due to underreporting and inconsistencies in diagnosis, and limitations of diagnostic guidelines and standardization of thereof worldwide.

The FASD rates in Indigenous communities vary. A few available studies indicate that FASD affects the Indigenous population disproportionately. Alarming FASD statistics have been reported in the Indigenous communities of 190 per 1,000 live births in British Columbia (Robinson et al., 1987); and in northern Manitoba communities of 55–101 cases per 1,000 live births (Square, 1997). A more recent Canadian report on the estimates of FASD among individuals 1-17 years old revealed a substantially higher prevalence of FASD among individuals with Indigenous identity (1.2% (95% CI 0.4–1.9) compared to 0.1% (95% CI 0.1–0.1))

(Government of Canada, 2021). An updated national FASD prevalence in the overall Canadian population and Canadian Indigenous populations is required.

Risk Factors for FASD

According to a body of literature on FASD, there is a multitude of factors contributing to the development of FASD. These include social and demographic factors, psychiatric and neuropsychological factors, concomitant substance use, the pattern of alcohol consumption, malnutrition and lower body mass index (BMI), and genetics (Esper & Furtado, 2014; May & Gossage, 2011, Young et al., 2014). These factors are further divided into a multitude of sub-factors, as displayed in **Table 1-1.**

Table 1-1: Risk factors for FASD

Risk Factors	
Social and demographic factors	**Pattern of alcohol consumption**
Lower income	Higher frequency of consumption
Lower educational attainment	Higher quantity of alcohol
Unemployment	Timing of alcohol consumption during gestation
Marital status: single during pregnancy	
Rural residence	
Psychiatric and neuropsychological factors	**Nutrition**
Mood disorders	Malnutrition
Cognitive disorders	Lower body mass index (BMI)
Behavioral disorders	
Substance use	**Genetics**
Use of tobacco during pregnancy	Protective ADH variants (ADH1B, ADH1C) and ALDH variant ($ALDH_2$)
Use of illegal drugs	
Maternal factors	
Gravidity	
Parity	
Older maternal age	
Gestational complications	
Chronic conditions during gestation	

Social and demographic factors; low SES

Although mothers of any socioeconomic status (SES) can have children with FASD, more severe cases of FASD are occurring at higher rates in the lower SES categories (May & Gossage, 2011). Bignol and colleagues (1987) reported a 15.8 times higher FAS incidence among individuals with lower SES compared to the individuals with greater SES with similar alcohol consumption.

A Manitoba-based study on the service needs of pregnant addicted women found that women exposed to substance use during pregnancy lived in extreme poverty with annual incomes below $10,000 (Tait, 2000). Population studies in South Africa, Italy and the United States of America (USA) have shown higher estimates of FASD cases among women with lower educational attainment. A study by May and co-workers (2006), revealed that only 37.5% of women had senior high school or higher education completed in the FASD group compared to 71.1% in the control group ($p<0.05$) (May et al., 2005). Considerably dramatic findings were observed for the US-based population (63.6% vs 100%, $p<0.001$) (May & Gossage, 2011).

Although there are generally no known incidence rates of FASD, individual community reports demonstrate that First Nations communities may be at a higher risk of FASD. First Nations women experience higher levels of poverty, social marginalization, psychological distress, food insecurity, and exposure to partner's violence, this might be at an elevated risk of alcohol consumption during pregnancy.

Psychiatric and neuropsychological factors

The comorbidity of psychiatric conditions and alcohol use disorder has been extensively documented in the literature (Jane-Llopis & Matytsina, 2006; Singal et al., 2017). While FASD specific research is scarce, studies have consistently demonstrated an association between alcohol consumption in women and anxiety disorders, depression and depressive symptoms, bipolar disorder, and psychotic disorders such as schizophrenia (Jane-Llopis & Matytsina, 2006; Cormier et al., 2004). A single Canadian, FASD-specific, population-based study reported substantially higher rates of personality disorder (RR 12.93; 95% CI 4.88-34.22), and mood and anxiety disorders (RR 1.75; 95% CI 1.49-2.07) among women who gave birth to children with FASD. Women at-risk also experienced higher rates of postpartum psychological distress (RR, 1.71; 95% CI 1.53-1.90), which suggests the need for support and alcohol consumption monitoring during lactation (Singal et al., 2017).

Substance use

Drug use and smoking are shown to be risk factors for FASD (Abel, 1995). Nicotine and carbon monoxide in cigarette smoking directly reduce blood flow and oxygen concentration, resulting in ischemia and tissue hypoxia in a fetus. Smoking during pregnancy is positively correlated with placental abruption, preterm delivery, premature rupture of membranes, fetal growth restriction,

and low birth weight (Stein et al., 1999; Castles et al., 1999). Several studies show higher smoking prevalence in at-risk women. Popova and colleagues (2019), reported the use of tobacco in 57.9% of women who had children diagnosed with FASD compared to 8.1% of women who had healthy infants. An FASD scholar May and his co-workers, consistently identify higher tobacco use among women with FASD diagnosed children compared to women with healthy infants (May et al., 2005; 2006; 2008; May & Gossage, 2011). In their South-African studies of maternal risk factors for FASD, May and colleagues reported a smoking prevalence of 77% among women who had children with FASD, as opposed to 34.8% among healthy controls (May et al., 2005; 2006; 2008; May & Gossage, 2011).

Similarly to cigarette smoking, drug use has been reported to be higher in women who are at risk of carrying a child with FASD (Popova et al., 2020; May et al., 2020). A Canadian, cross-sectional study reported more than double the rate of marijuana use among women who had children diagnosed with FASD compared to the control group (68.4% vs. 27.0%) (Popova et al., 2019). A large, US-based, case-control study (n=4,047) reported a significantly higher proportion of women with FASD diagnosed children using any drug during a studied pregnancy compared to the controls (15.6% vs 4.3%). The main drug reported were marijuana and cocaine (May et al., 2020).

A combination of alcohol, smoking, and drug use aggravates the teratogenicity of each of these substances on the fetus, increasing its malformations and poor development (Floyd et al., 2008). Therefore, identifying women that may be at risk of aggravated substance abuse is critical for developing prevention strategies for FASD.

Maternal factors

Epidemiological data on maternal factors indicate that age (chronological), gravidity (total number of pregnancies), and parity (total number of births) contribute to the severity of FASD (May & Gossage, 2011). Evidence indicates that older alcohol-consuming women with more pregnancies and births have a higher likelihood of having offspring with FASD (May & Gossage, 2011; Jacobson et al., 1996). Jacobson and co-workers (1996) identified a strong negative relationship between higher maternal age of drinking women and birth weight (β=-0.22), head circumference (β= -0.24), mental and psychomotor development (β= -0.21 and β= -0.17, respectively), and elicited play (β= - 0.31). The authors speculate that the reason behind

this phenomenon may be that older women have more years of cumulative alcohol consumption, thus alcohol tolerance may increase, leading to the fetus being exposed to higher levels of blood alcohol concentration (BAC) throughout the perinatal period (Jacobson et al., 1996).

Gravidity and parity also are risk factors for FASD (May & Gossage, 2011; Jacobson et al., 1996). Population studies report higher mean gravidity among women with children with FASD compared to women with children without FASD diagnosis (3.6 ± 1.6 vs 2.9 ± 1.3; 5.2 ± 1.8 vs 3.7 ± 1.5) (May et al., 2005; 2006; 2008; 2020). Similar findings are observed for the parity factor (May et al., 2005; 2008; 2020). Researchers speculate that greater gravida increases fetal hypoxia due to increased levels of structural proteins-collagen and elastin in the uterus and placenta, consequently exacerbating ethanol-related impacts (Jacobson et al., 1996).

Pattern of alcohol consumption

A pattern of alcohol consumption is found to be one of the most important contributing factors to FASD development. Particularly, binge drinking has been demonstrated as the most damaging alcohol consumption pattern for fetal development (Abel & Hannigan,1995). The National Institute on Alcohol Abuse and Alcoholism (NIAAA, 2015) defines binge drinking as a pattern of drinking that increases BAC to 0.08% per occasion, which is equivalent to 4 or more standard drinks. Binge drinking pattern rapidly elevates BAC in a short period creating negative effects on the developing fetus. Population studies found that communities that had high rates of binge drinking, generally, had more FASD cases, whereas communities, where alcohol is moderately consumed, have fewer FASD cases (May et al., 2013).

The quantity of alcohol consumed is also a factor that contributes to FASD outcomes. Studies demonstrate higher rates of FASD diagnosis among women with higher alcohol consumption. A report on the maternal pattern of alcohol consumption and FASD diagnosis by May and colleagues (2013), revealed a higher weekly number of drinks consumed in women with FAS and pFAS-diagnosed children compared to the women exposed to a lesser amount ($F=16.23$, $p<0.001$). Although not FASD-focused, longitudinal reports by Streissguth and colleagues (1990, 1994), identified a strong relationship between the number of drinks and cognitive and behavioral development of children born to women who reported moderate alcohol consumption (more than 3-5 standard drinks per day across 7 days) during pregnancy.

Another contributing factor to FASD is the frequency of alcohol consumption. Abel (1998), an expert in the field of FASD research, argues that for FASD to occur a mother must consume alcohol regularly over the course of pregnancy. Frequent alcohol consumption leads to elevated BAC that is high enough and regular enough to disturb the normal developmental processes of a fetus (May & Gossage, 2011). South African population studies concluded that FASD children were born to mothers who drank on average 6.6 drinks per evening for 2 days every weekend (May et al., 2013).

Timing of alcohol consumption during gestation plays a key role in the FASD outcomes, as distinct developmental and neurodevelopmental processes are occurring throughout the 40-week period. Feldman and colleagues (2012) found a higher association between the PAE during the 7-12 gestational week (1st trimester) and dysmorphological characteristics (RR 1.25; 95% CI 1.14-1.36). These findings are consistent with the early FASD studies (Abel & Hunnigan, 1995) and the latter findings of May and his team (May et al., 2013).

Nutrition and BMI

It is hypothesized that malnutrition may be more pronounced in pregnant women who consume alcohol, which increases fetus vulnerability to the teratogenic effects of alcohol (Ballard et al., 2012). A study in three North West Territories communities revealed that although dietary intakes were similar, women consuming alcohol had lower intakes of total folate and thiamin compared to women who did not consume alcohol (Rittmueller et al., 2012). One of the South African population studies demonstrated significantly lower nutrient intakes in women who had children diagnosed with FASD for riboflavin, choline, and calcium (1.09 ± 0.55 vs 1.16 ± 0.63 mg; 255.4 ± 115.5 vs 271.1 ± 140.7 mg; 362 ± 165 vs 392 ± 187 mg respectively) compared to healthy controls (May et al., 2014).

BMI is another nutrition-related risk factor for FASD. May and colleagues (2005, 2008) found that children with a more severe form of FASD were born to mothers who had lower weight, height, and BMI. The mean BMI of the women who had children with FASD was 24.4 ± 5.9, compared to the control group with a mean BMI of 27 ± 6.5. These findings are consistent with May's other findings in Italy and USA (May et al., 2006, May et al., 2020; May et al., 2011). These studies showed the teratogenic action of alcohol is exacerbated by low BMI and micronutrient deficiencies as it increases maternal BAC levels in women with lower BMI and

precludes proper metabolism, antioxidant support and homeostasis, aggravating ethanol-induced damage.

Genetics

Alcohol metabolism varies widely between individuals due to multiple genetic variants of genes encoding for alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) enzymes. The most common examples of protective variants include *ADH1B* and *ADH1C* alleles which encode ADH with higher oxidizing capacity. These alleles result in the swift conversion of alcohol to acetaldehyde, consequently leading to higher acetaldehyde accumulation in a shorter time, which deters consumers from over-consumption. Another protective allele is *ALDH2*2* - a genetic variation in ALDH enzyme encoding. This allele is more common in the Asian population. Research suggests that women who have protective genetic variants have less negative alcohol metabolism-related consequences than those who do not, therefore are less likely to have children with FASD (Gemma et al., 2007). Genetic variants that are responsible for such outcomes will be discussed in the next section.

FASD-associated Health and Social Cost

The total costs associated with FASD range from $1.3 billion to $2.3 billion per year for all services required in Canada (Popova et al., 2016). The highest contributor to the overall FASD costs is the cost of productivity losses due to morbidity and premature mortality, which accounts for 41% ($532-$1.2 billion) of the overall cost (Popova et al., 2016). The second highest contributor is the cost of corrections services, accounting for 29% ($378.3 million). The alleviation of this burden has been targeted in Manitoba through a new pilot program that sets out to improve access to FASD assessment, for individuals involved with the criminal justice system. The third highest contributor is the cost of health care at 10% ($128.5-$226.3 million) (Popova et al., 2016). Considering that there might be an increase in FASD rates, the lifelong support, required for people with FASD, is projected to increase (Flannigan et al., 2018)

Prevention, Intervention, and Programming for FASD

Prevention and programming in Canada

In Canada, there are ample programs and organizations dedicated to FASD prevention and treatment (CanFASD, 2013). An overview of FASD prevention approaches in Canada, published by the Public Health Agency of Canada (PHAC), grouped all FASD prevention into four major

streams (Poole et al., 2016): i) the first is raising public awareness through campaigns, public policy initiatives, and health promotion activities supportive of women's health. Examples of this prevention stream include labels on alcoholic drink containers, large-scale distributions of posters, and flyers, media campaigns, and the international campaigns taking place annually on September 9, International FASDay (Poole et al., 2016; Wilkinson & Room, 2009; Center for Addictions and Mental Health (CAMH), 2018); ii) the second stream is enhancing access to contraception services to prevent unplanned pregnancies. This stream of prevention occurs through primary care services, maternal and child health programs, and community services (Poole et al., 2016); iii) the third stream of prevention includes maternal programs that provide supportive and educational services. The maternal programs are often trauma-informed harm-reduction oriented, which considers the unique vulnerabilities of women attending the programs and promotes their recovery (CanFASD, 2013); iv) the fourth stream is focused on the support for women after childbirth rather than prenatally. Women are encouraged to maintain healthy changes that they have been able to make during pregnancy (Poole et al., 2016, CanFASD, 2013).

Nationally, the major action on FASD prevention was initiated by PHAC in 2003 (Government of Canada, 2012). PHAC has developed a "Framework for Action" which includes five broad goals: increase awareness, develop and increase capacity, create effective national screening, diagnostic, and data reporting tools, expand the knowledge base, and increase commitment and support for FASD (Government of Canada, 2012). This framework guides all action on FASD prevention work in Canada until the present.

Provincial and territorial prevention initiatives have been taking place predominantly in British Columbian, Saskatchewan, Manitoba, and Nunavut. British Columbia is an international leader in FASD initiatives, a 10-year Provincial Plan– FASD: Building on Strengths had been developed to outline FASD priorities in British Columbia (Ministry of Children & Family Development, 2008). The document emphasized prevention priorities across individual, professional, policy, and research levels. The Government of Saskatchewan developed the FASD Prevention Framework 2014 (Government of Saskatchewan, 2014). The framework aimed to guide the development and implementation of FASD prevention initiatives across the service sectors throughout the province. Similarly, the Government of Alberta developed the FASD 10 -

Year Strategic Plan, which aimed to address timely diagnosis and prevention at all levels (Government of Alberta, 2008). Alberta's Strategic Plan also emphasized the importance of new FASD research, which was not as strongly outlined in the afore-stated provincial plans. The government of Nunavut created the FASD Prevention and Awareness Project (Government of Nunavut, 2016). This work included the creation of an Inuktitut language FASD awareness training program and community surveys (Government of Nunavut, 2016). While the provincial approaches set out critical initiatives and goals, they are out-of-date, which necessitates re-appraisal of the approaches to reformulate the strategic FASD plans within the current context.

The most recent FASD Action Plan is released by Yukon (Government of Yukon, 2019). The plan is built upon TRC's #33 and #34 Calls to Action and Whitehorse Correctional Centre Inspection Report, Recommendations #4 and #5 (Government of Yukon, 2019; McLaughlin, 2017). The Calls to Action and the Recommendations collectively call all governments to address an over-representation of FASD among Indigenous incarcerated individuals (TRC, 2015; McLaughlin, 2017). The FASD Action Plan established 7 priority areas which include support for people with FASD and caregivers, awareness, prevention, diagnosis, knowledge exchange, and research and evaluation (Government of Yukon, 2019).

While Ontario has committed $26 million to combat the staggering rates of FASD, no official FASD-specific strategy has been released by provincial authorities (Burns et al., 2020; FASD Ontario Network of Expertise, 2018). Atlantic provinces and Quebec do not have an FASD strategy, however, the mental health strategies developed by these provinces encompass all mental disabilities in their respective priorities (Atlantic Intergovernmental FASD Partnership, 2013; Burns et al., 2020). Although the strategies contain a mental health focus, an explicit FASD action plan is required. FASD is a multidimensional disability, and it is important to ensure that the needs of all FASD-affected populations are not overlooked.

Prevention and programming in Manitoba

Manitoba government and Healthy Child Manitoba have developed a strategy named "Together We are Stronger" (Manitoba Government, 2012). This strategy has more specific FASD prevention goals such as ensuring that all Manitobans know the outcomes of drinking during pregnancy, that information, support, and services are provided to women of childbearing age, that assessments and diagnoses are available to anyone who might have FASD, that supports and

services are evidenced-based, and that service providers know the impact of FASD (Manitoba Government, 2012). Additional programs offered through Healthy Child Manitoba include Choices where women are educated on birth control, alcohol consumption, and sexual behaviors; InSight Mentoring Program where support and advocacy are provided to women who are pregnant and use substances; Manito Ikwe Kagiikwe (The Mothering Project)-a program operating out of Mount Carmel Clinic which offers outreach services, one on one support, access to prenatal care, medical referrals, advocacy, and access to traditional ceremonies and teachings (Government of Manitoba, 2021). The Province also has a Manitoba FASD Centre. The center is involved in the province-wide multidisciplinary team diagnosis, treatment, and prevention of FASD (Manitoba FASD Network, 2021).

Limitations of FASD prevention strategies

While great social and political efforts have been directed at raising public awareness of FASD through campaigns, public health initiatives, and health promotion activities in Canada, 10.5% of post-partum women reported alcohol consumption at some time point during pregnancy (PHAC, 2013). The rate of prenatal alcohol consumption on First Nations reservations is reported to be higher than non-Indigenous Canadians (Assembly of Manitoba Chiefs (AMC) Health Information Research and Governance Committee (HIRGC), 2006).

Research on the efficacy of warning labels and awareness building on drinking during pregnancy indicated that very little change in the drinking behavior of women during pregnancy (Wilkinson & Room, 2009; Hankin, 1998). A Detroit study of drinking behaviors of women of childbearing age found that 52% of the women who had seen a warning label in the past 12 months continued consuming alcohol regularly (Hankin, 1998). Although oral contraception has been identified as an effective method of pre-conceptional prevention of alcohol-exposed pregnancy, an intervention evaluation revealed that women do not use contraception consistently and often feel uneasy discussing contraception with their doctors (Hanson, et al., 2017, 2013). Additionally, women who are residing in remote communities have limited access to contraception services. Therefore, delivering FASD prevention through existing prevention policies alone may not be sufficient and other strategies need to be explored.

Alcohol Metabolism

Alcohol (in the form of ethanol, EtOH) is an organic two-carbon compound with a hydroxyl group, which is soluble in both aqueous and lipid environments (Horton et al., 2006). These properties allow EtOH to pass freely through all tissues and fluids. The metabolism of EtOH involves at least three distinct enzymatic pathways: ADH and ALDH, microsomal ethanol oxidizing system (MEOS) which involves the cytochrome P450 enzyme, and catalase pathway in peroxisomes (Horton et al., 2006; Young et al., 2014).

ADH and ALDH

Cytosolic ADH and mitochondrial ALDH are the major pathways of oxidative EtOH metabolism contributing to the rate of EtOH elimination from the blood. They are expressed at the greatest levels in the liver and to a lesser extent in the gastrointestinal tract, kidneys, nasal mucosa, testes, and uterus (Horton et al., 2012). In the first step of the reaction, with the presence of an intermediate electron carrier nicotinamide adenine dinucleotide (NAD), cytosolic ADH metabolizes EtOH to acetaldehyde, a highly reactive compound that contributes to tissue damage and possibly addictive process (**Figure 1**) (Horton et al., 2006). As a result of this reaction, a reduced cytosolic environment is generated in liver cells. In the second step, mitochondrial ALDH metabolizes acetaldehyde to acetate. The ALDH reaction is essentially irreversible. The resulting acetate produced departs from the liver and circulates to peripheral tissues where it is metabolized to acetyl-CoA and its carbon atoms are utilized for energy production.

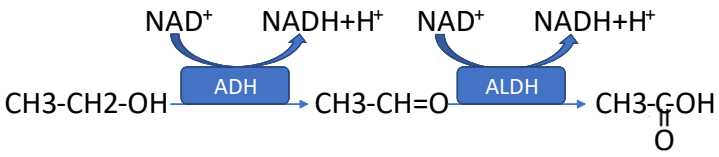


Figure 1-1. Ethanol metabolism involving ADH and ALDH in the liver. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase

There are multiple ADH and ALDH enzymes that are encoded by different genes, which exist in different variants as stated in an earlier section. These variations are responsible for the kinetic and structural properties of the ADH and ALDH complexes, thus affecting the rate of

acetaldehyde production and detoxification, thereby influencing a person's drinking limit and, consequently, the risk of developing alcohol abuse or dependence (Edenberg, 2007). Studies demonstrate that people carrying certain *ADH* alleles (*ADH1B*2* and *ADH1B*3*) and *ALDH* alleles (*ALDH2*2*) are at a lower risk of becoming alcohol dependent (Edenberg, 2007). The variant alleles *ADH1B*2* and *ADH1B*3* which produce more active ADH enzymes, and *ALDH2*2* which encodes for low active ALDH enzymes, have been shown to have a protective effect due to an increased production of acetaldehyde (Dickson et al., 2006). High acetaldehyde production causes adverse reactions, including facial flushing, nausea, and rapid heartbeat, which in its turn reduces alcohol consumption (Dickson et a., 2006; Edenberg, 2000). Therefore ADH/ALDH variations that encode less active ADH and more active ALDH enzymes may contribute to the development of alcohol dependence, which in its turn may increase the risk of having a child with FASD.

Cytochrome p450

Cytochrome p450, including CYP2E 1A2, and 3A4 isozymes, which are present mostly in the microsomal membranes within the endoplasmic reticulum, also contribute to EtOH oxidation in the liver. CYP2E1 is activated by continuously elevated EtOH concentrations and assumes an important role in metabolizing EtOH to acetaldehyde. In addition, CYP2E1 dependent EtOH oxidation may occur in other tissues, such as the brain, where ADH activity is low. It also produces reactive oxygen species (ROS), including hydroxyethyl, superoxide anion, and hydroxyl radicals which are responsible for oxidative damage to the tissues (Zakhari, 2006).

Catalase

Catalase, located in peroxisomes, is capable of oxidizing EtOH in vitro in the presence of a hydrogen peroxide-generating system, such as the enzyme complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or xanthine oxidase (Zakhari et al., 2006). Quantitatively, however, this is considered a minor pathway of EtOH oxidation, except in the fasted state (Zakhari et al., 2006).

Ethanol-induced damage

The metabolism of EtOH results in the production of acetaldehyde and ROS which impede normal physiological processes and damage organ tissues (Zakhari et al., 2006). The severity of tissue damage inflicted by EtOH varies, depending on the degree of oxidative stress,

inflammatory state, and local immune function (Jung et al., 2010). The primary source of oxidative stress results from EtOH, acetaldehyde, and its by-products (Jung et al., 2010). Secondary sources of oxidative stress and inflammation result from increased levels of endotoxins and pro-inflammatory cytokines, which are elevated due to immune response to EtOH (Zakhari, 2006; Jung et al., 2010). Oxidative stress and inflammatory process directly affects nutrient metabolism. It inhibits glucose and lipid metabolism, decreases protein synthesis, and hinders the metabolism of micronutrients, further perpetuating an-already existing damage (Jung et al., 2006). The EtOH-associated nutrition aberrations will be discussed in subsequent sections.

Fetal Development

Gastrulation and neurulation

The gestational period is divided into pre-embryonic, blastogenic, embryonic, and fetal stages (Niakan et al., 2012). Pre-embryonic stage involves fertilization, cleavage of the zygote (rapid mitotic cell division), and the development of a blastocyst. Within the structure of blastocyst, a group of cells, inner cell mass, form into an epiblast and hypoblast destined to become an embryo and a yolk sac, which consequently develops into chorion and placenta (Carlson & Kantaputra, 2019) (**Figure 2**). During the blastogenic stage, a blastocyst is transported from the ovary into the uterus where the trophoblast layer excretes enzymes that disintegrate the zona pellucida and implantation takes place (Carlson & Kantaputra, 2019). In the course of implantation, the blastocyst adheres to the superficial cells of the endometrium with the assistance of the syncytiotrophoblast – a multinucleated cell that digests endometrial cells (**Figure 2**). In response, the uterine mucosa rebuilds itself and engulfs the blastocyst. Implantation is complete by the middle of the second week of gestation (Carlson & Kantaputra, 2019). At the implantation, the trophoblast secretes human chorionic gonadotropin (hCG) for an at-home urine pregnancy test to give a positive result (Carlson & Kantaputra, 2019).

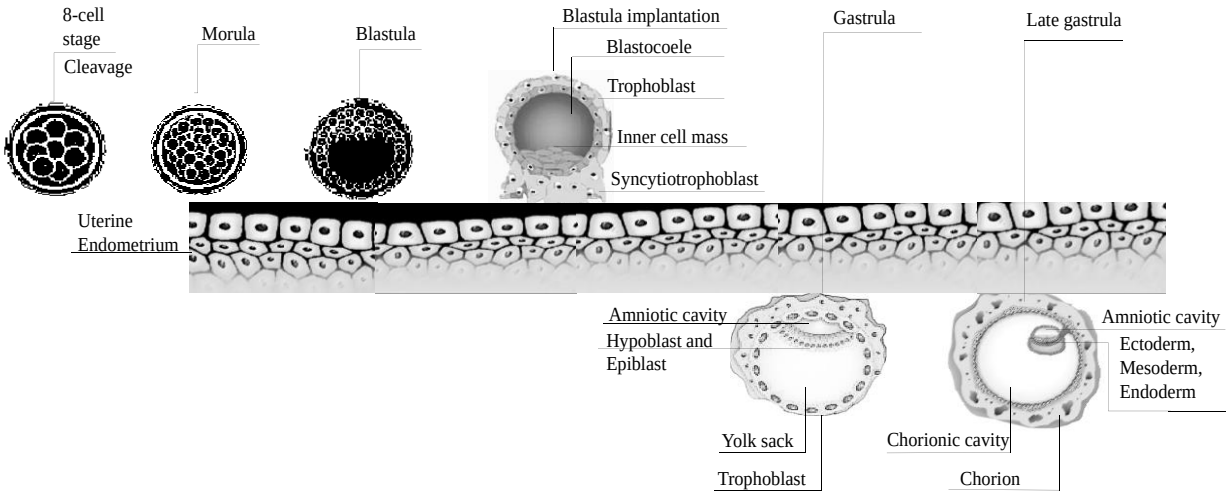


Figure 1-2. Formation of germ layers, gastrulation

The embryonic stage takes place at implantation, which falls at about the 5th week of pregnancy and lasts till the 10th week of pregnancy (Carlson & Kantaputra, 2019). At this stage, the inner mass of the blastocyst called epiblast differentiates into three germ layers, by the process known as gastrulation (Moore et al., 2019). The epiblast in the blastocyst takes the shape of an oval-shaped disc and the formation of the notochord, a distinct cylinder of cells that extends along the midline of the embryo from anterior to posterior starts to take place. The notochord forms from an aggregation of mesodermal cells that invaginate and create an indent referred to as the primitive streak along the dorsal surface of the epiblast (Moore et al., 2019). A notochord exudes growth factors that induce cell division and migration. Cells migrate toward and under the primitive streak and then move laterally to create two new layers of cells. The resulting germ layers are ectoderm - external, mesoderm - middle, and endoderm - internal, layers of embryonic cells (Moore et al., 2019). During this stage, embryonic cells transition from totipotency to multipotency, and organogenesis begins to take place (Carlson & Kantaputra, 2019). The germ layers are differentiated into CNS, ears, eyes, heart, kidneys, gastrointestinal tract, and lungs are being formed in this stage. This stage is identified as a critical period of formation when exposure to teratogenic substances such as alcohol use, smoking, and illicit drugs could result in serious birth defects (Moore et al., 2019).

The fetal stage is the longest stage of the gestational period. It starts at about the 11th week of pregnancy and lasts till birth (Carlson & Kantaputra, 2019). This stage is characterized by rapid fetal growth. Fetal milestones in this period include the formation of red blood cells from the

liver (weeks 11-14), production of liver and pancreatic secretions (weeks 15-18), first movement (weeks 19-21), development of the meconium (week 22), rapid brain growth (weeks 27-30), development of rhythmic breathing (weeks 31-34), and fully developed muscles and bones (weeks 35-37) (Carlson & Kantaputra, 2019; Moore et al., 2012).

CNS development

The nervous system develops from the neural plate, a thickened area of the embryonic ectoderm (Carlson & Kantaputra, 2019). The embryonic mesoderm also plays an important role in nervous system development. As the layer develops between the endoderm and ectoderm, it is separated into lateral halves by the neural tube and notochord. The notochord is a long cylinder of cells along the longitudinal axis of the body whose formation is completed by the 4^{th} week of gestation (Carlson & Kantaputra, 2019). Notochord helps to induce the differentiation of the surrounding tissues and influences the ectoderm to thicken and form the neural plate. The formation of neural plates marks the beginning of the process called neurulation. Neurulation begins during the early stage of the 4^{th} week and marks the beginning of the nervous system formation (Carlson & Kantaputra, 2019). During neurulation, the formed neural plate folds to form the neural groove. These folds fuse together to form the neural tube-the precursor to the CNS. The next step of neurulation is the formation of a neural crest. During the closure of the neural tube, cells on the crests of the neural folds detach forming a new cell population, called the neural crest. The neural crest contributes to the formation of the peripheral nervous system. Once the neural tube has completely fused the process of neurulation is complete. By the end of this period, brain structures are beginning to form. As the walls of the neural tube thicken they form the brain and spinal cord (Carlson & Kantaputra, 2019).

Maternal Nutrition Status During Pregnancy

Optimal maternal nutrient intake is vital during pregnancy as it plays a crucial role in fetal growth and development and thus an outcome of the pregnancy. A significant body of studies indicates that compromises in maternal nutrition status led to developmental adaptations that may permanently alter the physiology, structure, and metabolism of a fetus, predisposing an offspring to endocrine, metabolic, and chronic illnesses in adult life (Wu et al., 2004). Retrospective cohort, cross-sectional, case-control, experimental animal, and clinical studies reveal that compromised prenatal nutrition is predictive of intrauterine growth restriction (IUGR)

(Kaestel et al., 2005), high and low birth weight (Campbell et al., 2012; Agarwal et al., 2018), pre-term birth (Lu et al., 2018), obesity and type 2 diabetes (Barker et al., 1992), and cardiovascular disease (Leon et al., 1998). Historical famine studies revealed that offspring exposed to famine had a higher predisposition to chronic illness in adulthood such as hypertension, and type 2 diabetes (Barker, 1992); and restricted CNS development, which was manifested as schizophrenia later on in life (Hulshoff et al., 2000). A cohort study of 15,000 Swedish men and women born 1915-1929 revealed an inverse association between an increased size at birth and mortality from ischemic heart and cerebrovascular disease in men (RR 0.77; 95% CI 0.67-0.90) (Leon et al., 1998). Several experimental animal studies support the afore-stated findings, identifying mechanisms responsible for these evolutionary adaptations (Sudgen et al., 2002; Connor et al., 2019; Palladino et al., 2021). Such findings demonstrate that implications of maternal nutrition status and intrauterine environment extend past pre- and peri-natal periods and are critical for all stages of human life. This section will cover nutritional requirements during pregnancy for the mother and the infants.

Energy requirements

Maternal weight gain is one of the most commonly used indicators of good maternal health and proper fetal development. An increase in maternal size and weight is generally associated with an increase in fetal size and weight (Wu et al., 2004). According to the Health Canada's prenatal dietary recommendations (2010), the caloric increase is not required in the first trimester, and an additional 340-360 kcal/day and 450 kcal/day are recommended in the second and third trimester, respectively, to compensate for an increase in maternal metabolic rate. To fulfill these requirements women are recommended to consume an additional 2-3 servings from any food group in former Canada's Food Guide (CFG) (2007) during the second and third trimesters (Health Canada, 2010). This is to help gain a healthy amount of weight during pregnancy and provide additional nutrients.

Protein

Protein requirements increase significantly during gestation, as protein is required for the products of conception and the growth of maternal and fetal tissues. Protein recommendations are provided by the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) with an estimated intake of 0.88 and 1.1 g /kg of body weight/day,

respectively, during all stages of pregnancy (**Table 1-2**) (Health Canada, 2010).

Carbohydrate

The RDA for carbohydrates during gestation is 175 g/day, which is an increase compared to non-pregnant women (130g/day) (**Table 1-2**) (Health Canada, 2010). Fruits, vegetables, and whole grains are the recommended sources of carbohydrates to sustain healthy weight gain and fetal growth.

Fat

There is no distinct RDA/Dietary Reference Intake (DRI) recommendation for dietary fat intake during gestation. The recommendation remains 20%-35% of total calories, which is the same as for the general population. However, the recommendations emphasize an increase in essential fatty acids-linoleic acid (omega-6) and linolenic acid (omega-3) (**Table 1-2**) (Health Canada, 2010).

Micronutrients

Due to the rapid growth and tissue development of the mother and the fetus the requirements for vitamins and minerals increase. The most significant increase in recommendations is seen in folate (50%), vitamin B6 (45%), iodine (45%), iron (50%), and zinc (40%) (IOM, 2002, 2009, 2014). **Table 1-2** summarizes the macro- and micro-nutrient requirements for pregnant and non-pregnant women.

Table 1-2. Macro and Micronutrient Recommendations Dietary Allowance During Pregnancy

Nutrients	Age (years)	non-pregnancy	pregnancy
Macronutrients			
Carbohydrate (g/day)	14-50	130.0	175.0
Protein (g/kg/day)	14-50	0.8	1.1
Linoleic acid (n-6) (g/day)	14-50	12.0	13.0
Linolenic acid (n-3) (g/day)	14-50	1.1	1.4
Micronutrient			
Biotin (AI) (mcg/day)	14-50	30.0	30.0
Folate (mcg/day)	14-50	400.0	600.0
Niacin (mcg/day)	14-50	14.0	18.0
Pantothenic Acid (AI) (mg/day)	14-50	5.0	6.0
Riboflavin (mg/day)	14-50	1.1	1.4
Thiamin (mg/day)	14-50	1.1	1.4
Vitamin A (mcg/day)	14-18	700.0	750.0
	19-50	700.0	770.0
Choline (AI) (mg/day)	14-50	425.0	450.0
Vitamin B6 (mg/day)	14-50	1.3	1.9
Cobalamin (mcg/day)	14-50	2.4	2.6
Vitamin C (mg/day)	14-18	65.0	80.0
	19-50	85.0	75.0
Vitamin D (mcg/day)	14-50	5.0	5.0
Vitamin E (mg/day)	14-50	15.0	15.0
Vitamin K (AI) (mcg/day)	14-18	75.0	75.0
	19-50	90.0	90.0
Calcium (mg/day)	14-18	1,300.0	1,300.0
	19-50	1,000.0	1,000.0
Chromium (AI) (mcg/day)	14-18	24.0	29.0
	19-50	25.0	30.0
Copper (mg/day)	14-50	0.9	1.0
Fluoride (AI) (mg/day)	14-50	3.0	3.0
Iodine (mcg/day)	14-50	150.0	220.0
Iron (mg/day)	14-50	18.0	27.0
Magnesium (mg/day)	14-18	360.0	400.0
	19-30	310.0	350.0
	31-50	320.0	360.0
Manganese (AI) (mg/day)	14-50	1.8	2.0
Molybdenum (mcg/day)	14-50	45.0	50.0
Potassium (AI) (mg/day)	14-50	4,700.0	4,700.0
Selenium (mcg/day)	14-50	55.0	60.0
Sodium (AI) (mg/day)	14-50	1,500.0	1,500.0
Zinc (mg/day)	14-18	9.0	12.0

Source: Adapted from IOM, 2002; 2009; 2014; AI, Adequate Intake

Nutrients Affected by Alcohol

Continuous alcohol use leads to malnutrition by impairing the proper metabolism of certain nutrients. The nutrients which interact with alcohol have been delineated in the literature include vitamins A, C, E, and B12, thiamin, folic acid, choline, selenium, zinc, and iron (Young et al., 2014). In the presence of alcohol, these nutrients are not properly absorbed and utilized leading to metabolic inefficiencies and compromised homeostasis (Young et al., 2014). Evidence in experimental animal models reveals that nutrient intervention through supplementation or diet may mitigate the effects of alcohol on the fetus (Young et al., 2014). This section summarizes the available evidence on EtOH-nutrient interaction and the effect of nutrient intervention on the restoration of cognitive and developmental function in the presence of PAE.

Vitamin A

Retinoic acid, a biologically active metabolite of vitamin A, is an essential lipophilic nutrient that regulates over 3 -5% of genes in the mammalian genome (Petrelli, 2017). Retinoic acid is required for proper signaling during fetal development, specifically for the migration and differentiation of the neural crest and mesoderm cell lineages during gastrulation, craniofacial development, and organogenesis of multiple organs (Petrelli, 2017). Similarly to EtOH, retinol is metabolized to retinaldehyde via cytosolic ADH (subtype IV) and several types of microsomal and cytosolic retinol dehydrogenases (RDH) (Deltour et al., 1996); resulting in retinaldehyde is oxidized to retinoic acid via retinaldehyde dehydrogenase (RLDH) and ALDH subtypes (Duester, 1998). Consequently, retinoic acid binds to retinoic acid receptors (RAR) and retinoid X receptors (RXR), initiating intracellular signal transduction leading to a cascade of cellular events (Clugston & Blander, 2012; Petrelli, 2017).

Chronic alcohol consumption decreases concentrations of active and storage forms of the retinol, which results in downregulation of RARs and RXR, through several proposed mechanisms: First, higher plasma EtOH and subsequent acetaldehyde concentrations act as competitive inhibitors of ADH and ALDH enzymes respectively, leading to reduced retinoic acid synthesis and successive deficiency of the active metabolite-retinoic acid (Duester, 1998); Second, EtOH elevates retinol transport protein – hepatic retinol-binding protein (apo-CRBP1), which promotes hydrolysis of retinol and inhibits esterification reducing hepatic retinyl ester levels (Boerman & Napoli, 1991): Third, elevated BAC activates cytochrome P450 enzymes (CYP2E1), which increases retinoic acid catabolism and thus makes it unavailable for its cellular function (Sato &

Lieber, 1981). Some less established mechanisms of EtOH-retinol interferences, also include alterations in hepatic and intestinal retinol ester hydrolase (REH) levels upon ethanol consumption, which result in impaired retinol mobilization and transport to tissues (Clugston & Blander, 2012). However, more experimental studies are required to support and solidify this evidence.

FASD literature suggests that 'The Vitamin A Hypothesis' is one of the several theories of FASD etiology. Vitamin A deficiency has been known to cause similar developmental malformations as PAE (Marrs et al., 2010; Petrelli, 2017; Ribes et al., 2006). Retinoic acid deficiency has also been studied extensively in animal models, showing similar CNS anomalies, including microcephaly, mental retardation, decreased learning ability and plasticity as well as ventricular septal defects, atrial septal defects, and conotruncal anomalies (Marrs et al., 2010; Petrelli, 2017; Cartwright & Smith, 1995). Studies indicate that RAR and RXR knock-out mice models have similar phenotypes to PAE phenotypes. These phenotypes are derived from mesodermal and neural crest cell lineages which form during gastrulation and early neurulation and are controlled by retinoic acid (Petrelli, 2017). These findings serve as a basis for the 'Vitamin A Hypothesis' and why it is stipulated that retinoic acid insufficiency and metabolic obstructions are one of the causes of FASD (Cartwright & Smith, 1995; Petrelli, 2017).

Animal studies display that supplementation with vitamin A in alcohol-exposed embryos can correct dysmorphic features caused by alcohol consumption (Yelin et al., 2005; Marrs et al., 2010). A study done by Mars and colleagues (2010), showed that retinoic acid (RA) supplementation (medium containing 10^{-9} M of RA) alongside 100 mM of ethanol in zebrafish, decreased craniofacial cartilage formation and neural axis patterning, normally observed in the EtOH group. Retinoic acid supplements did not exhibit significant improvement in the retinoic acid plus EtOH group (Mars et al., 2010). Another study in zebrafish looked at the effect of retinoic acid supplementation on congenital heart defects in ethanol-affected animals (Sarmah et al., 2013). The authors found embryos that were supplemented with retinoic acid, had reduced presence of most cardiac defects with the exception of endocardial cushions (Sarmah & Marrs, 2013).

Choline

Choline is a water-soluble nutrient, usually grouped with B-vitamins. It performs three main cellular functions: it is a precursor for neurotransmitter - acetylcholine, it is a structural part of cell plasma membranes and cell signaling, and it is a methyl-group donor in the betaine-homocysteine methyltransferase pathway (folate-independent pathway) (Ziesel, 2011). Adequate choline intake is required during pregnancy due to its role in fetal growth and nervous system development. Choline deficiency during critical periods of embryonic development adversely affects fetal neurogenesis resulting in neural tube defects (NTD), poor neuronal migration; it also modulates DNA methylation and fetal gene expression in the brain, leading to poor differentiation (Ballard et al., 2013; Bekdash et al., 2013; Thomas et al., 2004)
After absorption, choline is converted to phosphatidylcholine (PC), which is subsequently converted to phosphatidic acid, a compound required for intracellular signaling (Ziesel, 2011). Upon consumption of alcohol, it competes with the water required for the phospholipase D (PLD) catalysis of PC, which in turn, diminishes phosphatidic acid and obstructs cell signaling pathways and protein expression (Ziesel, 2011; Klein, 2005). In addition, EtOH directly inhibits G-coupled muscarinic receptors and impairs acetylcholine synthesis due to a decrease in available choline (Ballard et al., 2012).

There are several studies on the effect of choline supplementation during pregnancy in animals exposed to EtOH. A study by Bekdash and colleagues (2013), investigated the effect of prenatal choline supplementation (642 mg/L of liquid diet) in conjunction with EtOH exposure on the expression of the hypothalamic proopiomelanocortin (POMC) gene, aberrations in which are implicated in anxiety, hyperactivity, and a reduced ability to cope with stress. The supplementation demonstrated a corrective effect on the methylation and expression of the gene, leading to normalized numbers of POMC neurons (Bekdash et al., 2013). A series of experiments by Thomas and colleagues (2004, 2009), revealed positive influences of choline supplementation (subcutaneous injections of 0.1 ml of choline chloride solution) (Thomas et al., 2004); 250 mg/kg maternal body weight/day on working memory, hyperactivity, and learning in EtOH exposed mice in utero (Thomas et al., 2009). Furthermore, the restorative effects of choline (100 mg/kg of body weight; 25 mg choline chloride/ml saline) persisted after completion of the supplementation therapy in the immediate postnatal period (Thomas et al., 2007). Another experimental study investigated the effects of choline supplementation in conjunction with EtOH

exposure on the hippocampal M1 and M2/4 muscarinic receptors during the periods of active CNS development (Postnatal Day (PD) 4-9), which is equivalent to the third trimester in humans (Monk et al., 2012). The results revealed that choline supplementation (100 mg/kg of body weight/day) ameliorated the M2/4 receptor density rendering it no different from that of the controls (no EtOH group) (Monk et al., 2012). The authors concluded that choline supplementation could potentially reduce the alcohol-related behavioral deficits associated with long-lasting changes in the hippocampal cholinergic system (Monk et al., 2012).

Up to date, only one study has investigated choline supplementation during PAE in human subjects. An exploratory study performed in South Africa reported an improved eyeblink conditioning reflex, postnatal growth, and cognition in infants with the prenatal supplementation (2 g of choline per day) from mid-pregnancy up until the delivery in alcohol-consuming women (Jacobson et al., 2018).

Thiamin

Thiamin or vitamin B1 is an essential water-soluble micronutrient, required for the metabolism of fats, proteins, and carbohydrates (Carpenter, 2000). Thiamin pyrophosphate (TPP) is an active form of thiamin, which is found in high concentrations in the liver, kidney, myocardium of the heart, and throughout the CNS. It acts as a coenzyme in various metabolic pathways, most notably in the decarboxylation of α-ketoglutarate by α-ketoglutarate-dehydrogenase in the tricarboxylic acid (TCA) cycle, non-oxidative transfer of carbon groups by transketolase in pentose phosphate pathway (PPP), and decarboxylation of pyruvate by pyruvate dehydrogenase (Carpenter, 2000).

Thiamin deficiency is a common deficiency associated with alcohol consumption (Martin et al., 1993). Chronic alcohol use directly affects thiamin absorption in the gastrointestinal tract, which is well documented in alcoholic patients. It results in impaired phosphorylation /dephosphorylation mechanisms leading to severely diminished concentrations of thiamin in its active form (Martin, 1993). Significantly lower TPP levels are found in patients who chronically abuse alcohol (McLaren et al., 1981). Human and animal studies indicate that after three weeks of a thiamin deficient diet, the blood level of thiamin significantly decreases, leading to an impaired function of thiamin-dependent enzymes, which consequently affects the functions of cardiovascular, central, and peripheral nervous systems (Baker & Frank, 1968).

The experimental studies demonstrated that alcohol toxicity in conjunction with thiamin deficiency alters neuronal environment and morphology, decreases cell differentiation, and impairs membrane developmental processes during the initial and late stages of pregnancy. An animal study performed by Ba (2009) had shown that PAE and a thiamin-deficient diet are significant risk factors for fetal death, small litter size, and spontaneous abortion. Significant increases in fetal death were observed in thiamin-deficient and EtOH-exposed groups (48.3% and 84.5%, respectively) compared to the control dams. Substantial reductions in litter size were observed for the thiamin deficient group and the EtOH exposed group (3.0 ± 0.25 pups/dam and 6.1 ± 0.52 pups/dam, respectively) compared to the control rats (11.0 ± 0.42 pups/dam). A reduction in birthweight was also detected in the thiamin-deficient and EtOH groups (3.7 ± 0.20 g and 2.9 ± 0.16 g, respectively compared to 5.5 ± 0.22 g for controls).

Thiamin supplementation had attenuated the effects of EtOH-induced low birth weight. The authors speculate that deficiency of thiamin during early gestation interferes with insulin growth factors (IGF)-I and IGF-II, which promote CNS ontogenesis, cell proliferation, and differentiation, leading to restricted fetal growth, and impaired cellular maturation, neuronal function, and viability (Ba, 2009). This hypothesis is partially substantiated by Ba's consecutive work (Ba, 2011). The authors observed peaks of the vulnerability of the fetus to thiamin deficiency and PAE (Ba, 2011). These peaks of vulnerability occurred during cellular differentiation and membrane developmental processes. Collectively, the findings from Ba's studies suggest that thiamin supplementation can act as a potential mediator in rectifying EtOH-induced insults during the most critical periods of development (Ba, 2011).

Although there is a scarcity of data on thiamin deficiency and EtOH interactions and outcomes in human subjects, one report revealed a link between IUGR and thiamin deficiency. Women whose pregnancies were complicated by the IUGR had significantly lower thiamin in the erythrocytes (130 vs 170 nmol/L, IUGR-pregnancy vs normal pregnancy, respectively) at the end of gestation (39^{th} week) (Heinze & Weber,1990). Furthermore, the IUGR-pregnancy patients had a higher rate of preterm labor (37% vs 14%) and fetal acidosis (26.3% vs 2.3%). Authors advise that thiamin supplementation during gestation might mitigate IUGR outcomes (Heinze & Weber, 1990). Presently, data on women consuming alcohol in conjunction with a thiamin deficient diet is unavailable.

Folate

Folate and or folic acid intakes are critically important during pregnancy due to an increase in DNA synthesis, DNA methylation, and cell proliferation. During gestation, the placenta concentrates folates into the fetal circulation and as a result, fetal levels are 2- to 4- fold higher than maternal levels (from 400 to 600 μg/day) (IOM, 2014; Hutson et al., 2012). Inadequate intake of folate during pregnancy results in congenital abnormalities, including neural tube defects, heart defects, cleft lips, urinary tract abnormalities, and limb defects (Ballard et al., 2012; Ford et al., 2021).

Ethanol inhibits intestinal absorption, renal reabsorption, and cellular entry of folate, thus reducing maternal and fetal levels (Ballard et al., 2012). Ethanol reduces the level of RNA transcription of various enzymes such as methionine synthase (MS), S-adenosyl homocysteine (SAH) hydrolase, methionine adenosyltransferase III, and methylenetetrahydrofolate reductase (MTHFR), which are the enzymes important in the production of methyl group donors (Ballard et al., 2012). Furthermore, EtOH decreases the expression of folate transport proteins in a placenta, impairing the transport of folate across the placenta (Hutson et al., 2012). Studies demonstrate that there are similarities between deficits observed in the FASD and reduced folate status during pregnancy. These similarities include common physical malformations such as neural tube defects, congenital heart defects, and limb defects; as well as neuro-developmental behavioral consequences such as hyperactivity, peer problems, lower cognitive function, and hypersensitivity (Ballard et al., 2012; Hewitt et al., 2011).

Folic acid has been credited with preventative properties with respect to a range of neurological abnormalities associated with alcohol consumption. In animal studies, various amounts of folate supplementation during PAE mitigated the formation and progress of microcephaly (60 mg/kg maternal body weight/day) (Cano et al., 2001), restored hepatic stores (2 mg/kg maternal body weight/day) (Hewitt et al., 2011), reduced oxidative stress by-products (152 μg/d) (Cano et al., 2001), and prevented EtOH-induced IUGR (10.5 mg/kg maternal body weight/day) (Han et al., 2012). Several reports have repeatedly identified folate's potential to attenuate EtOH-induced damage to cardiac tissues and function (Ford et al., 2021; Linask & Han, 2016). A recent report by Ford and colleagues (2021) demonstrated that folic acid supplementation of 3.2 μg at gastrulation events significantly reduced regurgitant blood flow ($p = 0.001$) and enhanced endocardial cushion volumes ($p = 0.048$) of embryos in binge drinking animal model. Similarly,

in another binge-drinking animal study, a rich folate diet (10.5 mg/day) introduced concurrently with EtOH at gastrulation (embryonic day 6.75) attenuated abnormalities in cardiogenesis, improved myocardial wall and cardiac valve malfunctions associated with EtOH exposure with 51% of the embryos displaying normal cardiac development and function vs 32% in EtOH group (Serrano et al., 2010).

There are a limited number of reports on folate supplementation in alcohol-consuming subjects. A meta-analysis on orofacial clefts, an outcome associated with PAE, demonstrated that maternal folate dietary intake and supplementation reduced the risk of orofacial clefts (p=0.008, OR=0.70, 95% CI: 0.65–0.94; p=0.028, OR=0.80, 95% CI:0.66–0.98, respectively) (Butali et al., 2013). One report measured folate in maternal and umbilical cord blood at the time of delivery in women with and without chronic and heavy alcohol consumption during gestation (Hutson et al., 2012). Alcohol-exposed dyads had a maternal-fetal folate ratio below 1 compared to controls which had a maternal-fetal folate ratio above. Authors speculated that this is an indication of mobilization of folate at the placental level induced by EtOH exposure (Hutson et al., 2012). Although more research is required, the results stated in the above studies demonstrate great potential for folate supplementation to be utilized as one of the components of targeted nutrition intervention for FASD prevention in an at-risk population.

Zinc

Zinc is an essential micronutrient for human growth and development due to its function in regulatory, and structural biological pathways, most notably RNA polymerases, alcohol dehydrogenases, various hydrolases, lyases, and transferases (Cousins, 1996). Zinc deficiency during gestation results in congenital malformations in the fetus (Moghimi et al., 2017) growth restriction (Wang et al., 2015), and CNS malformations and dysfunction (Keen et al, 1995). Although no clear mechanisms have been elucidated, it is postulated that multiple antagonistic interactions between zinc deficiency and an alcohol insult in utero elicit FASD-associated outcomes (Keen et al., 2010). Miller and colleagues (1983) found a higher degree of fetal dysmorphogenesis in dams with lower zinc consumption (10 µg /g diet) compared with the control dams supplemented with (45 µg /g diet). The findings were corroborated by other animal studies (Ruth et al., 1981; Keppen et al., 1985). Mechanistically, these results were explicated by zinc's involvement in the oxidative defense systems, which functions sub-optimally in the

presence of zinc deficiency in conjunction with alcohol consumption (Miller et al., 1983; Keppen et al., 1985). A study by Carey and colleagues (2003) found that animals that were administered EtOH (2.9 g/kg) on gestation day (GD) 8 had smaller sized and lower weighted litters with malformations of the eye compared to zinc supplemented mice (250 μg/ml). These outcomes could be attributed to zinc involvement in the insulin-like growth factor axis, which regulates cell proliferation, differentiation, and survival during gestation, and may offer additional explanations for EtOH-induced zinc deficiency and FASD-related outcomes (Carey et al., 2003; McGough et al., 2009).

Two reports examined the effects of alcohol consumption on zinc prenatally. A report by Flynn and colleagues (1981) documented a substantially reduced maternal plasma (5.7 vs 7.2 μg/dl) and fetal cord plasma zinc concentrations (65.5 vs 81.3 μg/dl) in dyads exposed to alcohol compared to non-exposed. Moreover, the authors identified a correlation between frequency and severity of birth defects in the infants who had lower zinc concentration ($r = 0.37$, $p<0.05$) (Flynn et al., 1981). Consistent with these results, an international study done in Russia and Ukraine found lower plasma zinc concentrations in women who reported medium to high alcohol intakes (0.57 ± 0.02 μg/ml vs 0.64 ± 0.05 μg/ml; $p<0.022$) (Keen et al., 2010). Collectively, these findings strongly indicate zinc deficiency to be an etiologic factor in human FASD outcomes.

Cobalamin

Cobalamin is crucial for proper DNA methylation, which is an essential step in normal cell division, differentiation, myelination of neurons, and the functioning of genes. Methylation relies on the reserve of methyl groups supplied by methyl donors (i.e. methionine) in the diet and on B vitamins (i.e. cobalamin and folate) as cofactors and substrates of the methionine-homocysteine cycle (Yajnik et al., 2008). Animal studies indicate that low dietary intake of cobalamin, folate, and methionine around conception leads to offspring with altered DNA methylation and phenotype (Yajnik et al., 2008). The phenotype includes heavier, insulin-resistant, and higher blood pressure manifestations. Population studies reveal that women with low cobalamin status gave birth to children with the high levels of insulin resistance (Yajnik et al., 2008), neural tube defects (Kirke et al., 1993; Suarez et al., 2003), spontaneous abortions (Bennet, 2001), and anencephaly (Schorah et al., 1980).

Chronic alcohol consumption has been reported to interfere with one-carbon metabolism, for which cobalamin serves as a co-enzyme. While little information is available with regard to cobalamin and alcohol interaction, some reports indicate altered cobalamin plasma concentrations and cellular metabolism. Laufer et al., (2004) observed significantly lower serum cobalamin concentrations and significantly higher homocysteine levels (a metabolite of one carbon cycle)-a cardiovascular risk factor, in women consuming moderate amounts of alcohol (30g/day).

A limited number of studies that examine the effects of a methyl-supplemented diet presently exist. One study investigated the effects of methyl-cycle involved nutrients (cobalamin 1.5mg, choline 15g, betaine 15g, folic acid 15mg, L-methionine 7.5 g, zinc 150 mg) on the DNA methylation and growth outcomes in mice exposed to EtOH (5.8 g/kg on GD 9-18) (Downing et al., 2010). The mice on the methyl-supplemented diet had exhibited restored methylation concentrations, ameliorated vertebral malformations, decreased prenatal mortality, and improved prenatal growth after exposure to EtOH (Downing et al., 2010). While it is difficult to attribute this evidence to cobalamin alone, this data suggests great potential for the methylation associated co-factors in restoring teratogenic effects of EtOH in utero. More experimental-animal, supplementation, and population studies are required to elucidate interaction mechanisms and the effects of alcohol on cobalamin.

Docosahexaenoic Acid (DHA, 22:6n-3)

DHA is a major n-3 polyunsaturated fatty acid (PUFA) that is found in high concentrations in neuronal membranes. It comprises about 40% of the brain PUFAs and plays a major role in neuroprotection, memory, and vision (Wellman et al., 2015). In addition, DHA also activates energy-generating metabolic pathways that stimulate an increase in brain-derived neurotrophic factor and IGF-1, which subsequently activate signaling cascades and transcription of genes involved in neuronal development and growth (Patten et al., 2013; Wellman et al., 2015).
DHA is synthesized from α-linolenic acid (ALA) or it can be supplied by the diet of fish or algae sources (Wellman et al., 2015). It rapidly accrued in the brain during the 3rd trimester and primary years of life (Georgieff, 2007). Although breast milk naturally contains DHA, an inadequate maternal diet can negatively affect DHA levels (Wellman et al., 2015). Studies show that supplementation of infant formula or maternal diet with DHA results in improved cognitive

function and higher Mental Developmental Index score, a series of tests developed to assess motor and cognitive development in infants (Wellman et al., 2015). Conversely, DHA deficiency leads to the loss of DHA content in the brain and impaired learning abilities (Patten et al., 2013).

PAE has adverse effects on the DHA status of the developing fetus. Alcohol reduces the bioavailability of DHA in the mother and decreases the transfer of DHA to the fetus through the placenta, thus decreasing its potential for utilization. An animal study done by Patten and colleagues (2013) found that an immediate postnatal DHA supplementation (3.4% out of 10% fat concentration in dietary formulation), in EtOH exposed animals (35.5% EtOH-derived calories) during the prenatal period, significantly reversed reductions in glutathione levels in the dentate gyrus and cerebellum. These findings are corroborated by the recent findings of Feltham and colleagues (2019), who found that DHA supplemented diet (1.4%, w/w total fatty acids) normalized glutathione reductase and glutathione peroxidase mRNA expressions in the liver of fetuses exposed to moderate levels of EtOH during gestation (3 g/kg of maternal body weight). Another supplementation study revealed that an immediate postnatal DHA supplementation (10 g/kg weight per day) in EtOH exposed rodents improved somatosensory performance, reduced anxiety, and increased activity of the animals (Wellman et al., 2015).

While DHA supplementation human trials are scarce, a small cohort study in African American pregnant women reported altered DHA status in women at risk of carrying a child with FASD. A significant increase in DHA concentration was observed in the umbilical cords of moderate and heavy drinkers (10% and 14% respectively), compared to the non-alcohol group (Beblo et al., 2005). A case-control study also found alterations in the fatty acid profile of alcohol-exposed dyads. The authors observed a positive Spearman rank-order correlation coefficient between alcohol intake and plasma concentration for omega-3 fatty acids including α-linolenic acid (ALA) ($\rho=0.388$, $p<0.05$) (Sowell et al., 2020). Alcohol exposed women with FASD diagnosed children also had a significantly higher ratio of omega-6 to omega-3 fatty acids end products (22:5n-6/22:6n-3) compared to the control group, despite no differences in the plasma concentration for DHA (Sowell et al., 2020). Authors of both reports speculate that these observations might be due to aberrations in DHA metabolism, distribution, and tissue mobilization, as EtOH is a potent modulator of fatty acid metabolism (Beblo et al., 2005). Although these studies provide some insights into the DHA metabolism during EtOH exposure,

clinical and experimental supplementation trials are required to identify potential mechanisms of DHA role and efficacy in the amelioration of developmental abnormalities observed in FASD.

Vitamin C

Vitamin C, ascorbic acid, is essential for the normal metabolism and function of all mammalian cells due to its role as a cofactor for several important enzymes, collagen production, biosynthesis of hormones, and as an antioxidant. In addition, vitamin C plays a key role in the regulation of neuronal differentiation, maturation, and development and is a principal nutrient during CNS formation (May, 2012). Within the brain, the hippocampus has one of the highest ascorbic acid concentrations and a correspondingly high level of the vitamin C transporter–the sodium-dependent vitamin C co-transporter 2 (SVCT2) (May, 2012).

Consumption of alcohol obstructs the appropriate absorption, transport, and metabolism of vitamin C. This leads to reduced concentration of the nutrient in leukocytes and thus reduced clearance of EtOH from the blood (Fazio et al., 1981), the insufficient hepatic transformation of vitamin C to active ascorbic acid metabolites, and diminished pancreatic acinar stores (Susick et al., 1986; Seitz & Suter, 1994). Peng and colleagues (2005) demonstrated a reduction in EtOH-induced ROS damage and prevention of microcephaly and growth restriction in Xenopus Laevis (the African clawed frog) when treated with 100 μM of ascorbic acid during the neural plate developmental stage. A cell culture study with the cardiomyocytes from chicks found that vitamin C (100 μM), in combination with folic acid supplementation (1 mM) at day 3 protected the cells from the cytotoxic effects of EtOH and ameliorated the overall survival of embryos to the levels comparable of controls (Memon & Pratten, 2009).

A few intervention reports suggest that vitamin C is protective against the toxicity of acetaldehyde (Sprince et al., 1975), reduces hepatotoxicity (Majumdar et al., 1981), and improves EtOH clearance (Fazio et al., 1981). Research identifying EtOH and vitamin C interactions has been sporadic and inconsistent. The afore-stated reports are the only identified reports on vitamin C intervention prenatally, thus more animal experimental and clinical trial studies are required.

Iron

Iron is an essential element for cell proliferation, heme synthesis, mitochondrial electron transfer, and energy production. Iron is especially important for women of childbearing age, due to the iron losses from menstruation and childbirth (Guo et al., 2019). The outcomes of iron deficiency during pregnancy are well documented. These include high and low birth weight, premature delivery, spontaneous abortion, stillbirth, and neurodevelopmental impairments (Scholl, 2011; Sangkhae et al., 2020; Huebner et al., 2016).

Alcohol modifies iron utilization elevating serum ferritin, serum-free iron, and transferrin-iron saturation. Suggested mechanisms of elevated hepatic iron include EtOH downregulation of liver hepcidin expression resulting in limited ferroportin (iron-regulated transporter 1) degradation and increased dietary absorption, despite elevated hepatic iron stores (Harrison-Findik, 2009). Excess circulating and stored iron caused by alcohol consumption may be a critical step in alcoholic liver hypoxia and cirrhosis (Harrison-Findik, 2009).

Several animal studies have extensively demonstrated the effects of iron deficiency in conjunction with EtOH exposure on the growth and development of a fetus. A study by Miller and colleagues (1995), showed that PAE (2.2%-6.7% from GD 6-21) altered iron homeostasis in the cerebral cortex, subcortical forebrain, and brainstem of the pups, delaying normally occurring postnatal iron concentration dip from PD 21 to PD 60 (Miller et al., 1995). Diminished iron levels persisted into adulthood in EtOH treated group, suggesting long-lasting defects in iron regulation (Miller et al., 1995). Animal studies corroborate these findings (Huebner et al., 2016; Rufer et al., 2012). Rufer et al (2012) postulated that iron deficiency directly impacts FASD associated outcomes. The study found that iron deficiency during PAE (5 g/kg body weight) significantly reduced growth and body weight and negatively affected myelination within the granule cell layer of cerebellar lobules and impaired eyeblink classical conditioning learning (Rufer et al., 2012). Iron deficiency-EtOH interactions were also observed for a cue-induced conditioned response for males and contextual conditioned response for both males and females. Iron deficiency exacerbated the rate of cerebellar neuronal apoptosis in pups exposed to the same lower amount of EtOH (3.5 g/kg body weight) (Rufer et al., 2012). This data indicate that iron status has a strong modifying influence upon the physiological and cognitive EtOH -induced

damages and thus could be further investigated as a nutrition intervention target to ameliorate outcomes in FASD.

Reports on iron and PAE in humans present consistent findings of reduced iron levels in alcohol-consuming women. One of the earliest findings on alcohol and nutrition interactions presented by Jones and colleagues (1973) revealed that some alcohol-consuming women became anemic during pregnancy. Streissguth et al. (1981) found that pregnant women who drank heavily (eight or more drinks per day) had significantly lower transferrin saturation (16%) and ferritin levels (18 ng/mL) than women with lower levels of alcohol consumption (25-27% and 21-23 ng/mL for respective variables) (Streissguth et al., 1981). Unfortunately, this study did not measure fetal levels of iron status as one of the birth outcomes. A series of studies by Carter and colleagues (2007, 2012) directly associated PAE with infants' iron deficiency. Infants who were prenatally exposed to binge drinking (more than 4 drinks on occasion) were almost 4 times more likely to be diagnosed with iron-deficient anemia than infants who were not prenatally exposed (Carter et al., 2007). Iron-deficient anemia due to PAE was associated with a decrease in birth weight and head circumference at 6 months and a year (Carter et al., 2007). The consecutive study by the authors revealed that heavy PAE (more than 2 drinks/day or more than 4 drinks/occasion) exacerbated by iron deficiency was associated with reductions in weight, height, and head circumference from birth to 9 years of age (Carter et al., 2012). This association between maternal iron deficiency and compromised growth outcomes in FASD is unlikely to be unique to the afore-stated cohort, given the high incidence of marginal iron status among child-bearing age women across all socioeconomic groups and the prevalence of gestational drinking across populations.

Manuscripts published as a part of this chapter:

1. Kloss O., Sharova L., Suh M. (2022). Nutrition intervention as a preventative approach to Fetal Alcohol Spectrum Disorder (accepted to Advances in Fetal Alcohol Spectrum Disorder in Neuromethod 188, pp 189-212 (Editors, Chudley AE; Hicks G), Springer Nature.

2. Kloss O., Dyck K., Giesbrecht H., Eni R., Eskin NAM, Chudley A., & Suh M. (2021) A Scoping literature review of the nutrition status among Canadian First Nations women during pregnancy: What does the evidence reveal? *Fam Med Med Sci Res*. 10(7), 1-8.

3. Kloss O., Eskin NAM, & Suh M. (2018). Thiamin deficiency on fetal brain development with and without prenatal alcohol exposure. *Biochem Cell Biol*. 96(2):169-177. *Part of the manuscript was based on the received Dr. Feniak Award for Excellence in Technical Writing 2015 in the graduate category, Canadian Home Economics Foundation.

CHAPTER II: RESEARCH PLAN

Rationale

FASD is a complex, multifactorial disorder that is public health concern around the world. Although there are no exact statistics on the incidence rate of FASD in Canada, an estimated 9 in 1,000 infants are born with FASD (Government of Canada, 2017). According to a few available reports, the prevalence of FASD in the First Nations communities varies from 7.2 to 190 cases per 1,000 births (Robinson et al., 1987; Square et al., 1997). Due to the lifelong physical disabilities and social deficits of individuals diagnosed with FASD, the economic impact is significant. A Canadian study by Stade and colleagues (2009), measured total federal expenditures for all FASD, which summed up to be about $5.3 billion for individuals 0-53 years of age. The lifelong support, required for people with FASD, will continue to increase as FASD increases 4.8 times last 15 years. Therefore, preventing and reducing the severity of FASD is a top priority for individuals and the healthcare system.

While social and political efforts have been directed at promoting the negative effects of alcohol consumption during pregnancy in Canada, 10.7% (95% CI: 9.5–11.8%) of post-partum women admitted to drinking alcohol at some time point during pregnancy in National Longitudinal Survey of Children and Youth (PHAC, 2013). Evidence indicating the presence of alcohol-induced teratogenic effects are dependent on the alcohol exposure pattern (quantity, frequency, and timing), the developmental stage of the fetus, as well as the social factors such as income, level of food security, access to services, and other determinants of health (May and Gossage, 2011). Nutrition is another strong predictor of FASD, as it has a direct influence on the growth, development, and physiological alterations during gestation for the mother and the fetus, as described in Chapter I in detail.

The physiological demands during the prenatal period lead to an increased requirement for macro- and micronurients. Poor maternal nutrition is a pre-determinant of premature birth and low birth weight babies, congenital malformations, poor development and other negative outcomes (Ballard et al., 2012; Bennett, 2001; Esper & Furtado, 2014). Ethanol compromises nutritional status. It decreases nutrient absorption, alters nutrient digestion, and inhibits cellular entry of nutrients, thus leading to secondary malnutrition (Carter et al., 2007; Downing et al., 2011; Fazio et al., 1981). The most debilitating effect of PAE is on fetal development. It affects

virtually every physiological system with the highest impact on CNS (Ba, 2009; Floyd et al., 2009; Hulshoff et al., 2000). PAE induces microcephaly (Miller et al., 1996), alters neuronal proliferation and differentiation mechanisms (Monk et al., 2012; Peng et al., 2005), obstructs normal oxidation protection, disturbing homeostatic processes during fetal development (Patten et al., 2013). Experimental data in animal models show that prenatal vitamin and mineral supplementation, particularly supplementation with several nutrients (vitamin A, C, thiamin, folate, choline, iron, zinc, and DHA) alleviates EtOH-induced teratogenicity reducing the severity of physiological dysfunctions and other related outcomes. Such evidence demonstrates that nutrition intervention during PAE may be one of the strongest preventative approaches for FASD, however, this topic is largely understudied in humans due to a lack of evidence on baseline nutrition status in prenatal populations.

FASD has especially disproportionate effects on Indigenous communities. Due to higher levels of poverty and food insecurity, social marginalization, psychological distress, lower education levels, and exposure to partner violence, First Nations women are at higher risk of alcohol consumption during pregnancy (TRC, 2015). This places First Nations women at risk of compromised nutrition, obstructing the appropriate nutrition status of a fetus and increasing the risk for FASD. In 2015 TRC called on all governments and organizations to take preventative action on FASD. FASD prevention necessitates evidence-based approaches which can mobilize available findings and knowledge into feasible and deliverable interventions and programming (TRC, 2015). Once diagnosed, several support systems are available, but there are no treatment options to reverse FASD. Therefore, it is necessary to investigate nutrition intervention during pregnancy with PAE. However, the baseline nutrition status and/or dietary intake information is limited, especially for pregnant Indigenous women, who are at high risk of consuming alcohol during pregnancy, which is of a high priority.

Research Objectives and Hypotheses

The overall objective of this project is to explore the relationship between macro- and micro-nutrient intake and lifestyle factors, specifically alcohol consumption, of pregnant First Nations women residing in Manitoba Northern First Nation communities.

The specific objectives are:

1. To identify and compare macro- and micro-nutrient intake of pregnant women with and without PAE. With PAE is defined as at-risk of carrying a child with FASD; without PAE is defined as not-at-risk.
2. To explore the relationship between alcohol consumption and the intakes of macro- and micronutrients;
3. To identify and compare maternal health status (chronic illness, pregnancy outcomes) of the pregnant women with and without PAE.
4. To identify and compare the status of nutritional and inflammatory biomarkers of pregnant women with and without PAE.

The driving hypotheses of the study are:

1. Pregnant women with PAE have a lower intake of nutrients identified for FASD protection; and their nutrient intake are affected by alcohol consumption
2. Pregnant women with PAE have a lower intake of all four food groups, Vegetable and Fruit, Meat and Alternatives, Milk and Alternative, and Grain Products (based on 2007 CFG);
3. Pregnant women with PAE have compromised health status and a higher degree of lifestyle and pregnancy risk factors such as smoking, drug use; and higher numbers of miscarriages, stillbirths, and chronic illness.
4. Pregnant women with PAE have higher levels of inflammatory markers and lower levels of nutrients and their metabolites in the blood.

The findings demonstrated in the following chapters provide a comprehensive picture of maternal adverse exposures, dietary intake, and health status, which can inform health care assessments, interventions, and programming approaches to improve maternal health outcomes for First Nations women.

CHAPTER III: A PILOT STUDY ON THE ASSOCIATIONS BETWEEN NUTRIENT INTAKE AND RISK EXPOSURES OF FIRST NATIONS PREGNANT WOMEN RESIDING IN REMOTE MANITOBAN COMMUNITY

Abstract

Background: While nutrition is implicated in fetal alcohol spectrum disorder (FASD), information is limited on the nutrition status of First Nations women who are identified as at-risk of carrying a child with FASD. ***Objective:*** The purpose of this study was twofold: i) to examine the adequacy of the maternal macro- and micro-nutrient intakes during gestation; and ii) to identify the associations between the alcohol consumption and intakes of macro- and micro-nutrients of First Nations women residing in a Manitoban community. ***Methods:*** Partnership was established with a Cree Northern community in Manitoba, where 22 pregnant women (aged 14-39 years) were recruited to participate in the study. Data were collected through an in-person, multi-section questionnaire, which collected information on demographics, anthropometrics, dietary intake, risk exposures, and maternal health. Multiple linear regression models were employed to assess the relationship between nutrient intakes and maternal risk, demographic, and health variables. ***Results:*** A high proportion of participants were not meeting Health Canada's food group recommendations for Vegetables and Fruit and Milk and Alternatives food groups (95%, 95%CI =77-100) and Grain Products (86%, 95%CI = 65-97). Majority of the participants' micronutrients intake was within or above the recommended reference DRIs (%), however high prevalence of inadequacy was noted for folate, iron, and DHA (73%, 95%CI = 50-89; 73%, 95%CI = 50-89; and 91%, 95%CI = 71-99, respectively). The results of regression models, with the trimester and body mass index (BMI) covariates, revealed significant positive association between alcohol consumption and total energy intake (β= 0.525, $p < 0.05$) and the energy from fat (%) ($\beta = 0.443$, $p < 0.05$). Drug use showed significant positive association with fat (%, energy) ($\beta = 0.607$, $p < 0.01$). With respect to micronutrients, statistically significant inverse associations were detected for alcohol consumption and thiamin (%DRI) ($\beta = -0.606$, $p < 0.01$), niacin ($\beta = -0.528$, $p < 0.05$), folate ($\beta = -0.551$, $p < 0.05$), choline ($\beta = -0.530$, $p < 0.05$), calcium ($\beta = -0.506$, $p < 0.05$), iron ($\beta = -0.636$, $p<0.01$), and zinc ($\beta = -0.565$, $p < 0.01$). ***Conclusion:*** These preliminary findings indicate that risk exposures are inter-related with nutrition intake during pregnancy. Large prospective data are required to further solidify the findings of this pilot, as this information is critical for maternal health programming and policy.

Keywords: Maternal nutrition; First Nations; FASD; Risk factors; Lifestyle factors.

Introduction

Alcohol consumption during pregnancy presents a significant burden on maternal and child health. Prenatal alcohol exposure (PAE) may result in FASD - a spectrum of physical and cognitive abnormalities. FASD is characterized by structural and biochemical central nervous system (CNS) abnormalities, as well as cognitive, developmental, and behavioral delays and deficits (Chudley et al., 2005). Additionally, physical manifestations of severe forms of FASD may include musculo-skeletal, cardiovascular, and urogenital deficits (Karunamuni et al., 2013).

Maternal alcohol consumption is the sole necessary agent required for the diagnosis of FASD, however research has not yet delineated consumption patterns (timing, frequency, amount, and critical periods) for causation. Additionally, much of the FASD literature points toward a multifactorial origin of the outcome (Chudley et al., 2018; May & Gossage, 2011; Abel & Hunnigan, 1995). Maternal nutritional intake, environment, genetics, timely recognition of pregnancy, concomitant substance use, socio-demographics, maternal health status, pregnancy history, and lifestyle factors all have profound impacts on the identification and the course of the diagnosis (Chudley et al., 2005; May & Gossage, 2011; Young et al., 2014). Thus, various international and national health governing bodies set prenatal standards to abstain from alcohol and substance use and advise women to maintain a healthy lifestyle and dietary intake during preconception and prenatal periods (Health Canada, 1996; Government of Canada, 2022; the Royal Australian and New Zealand College of Obstetricians and Gynaecologists, 2022).

Compromised maternal nutrition is the major contributing factor to the severity of FASD (Ballard et al., 2012; Young et al., 2014; May & Gossage, 2011; Jones, 1973; Abel & Hunnigan, 1995). Alcohol greatly impacts nutrient absorption, transport, metabolism, and utilization, consequently compromising placental development, fetal growth, neuroendocrine-metabolic functioning, and intrauterine environment (Ballard et al., 2012). Prominent works on the topic postulated that PAE in conjunction with compromised nutrition status lies at the foundation of the disorder (Ballard et al., 2012; May and Gossage, 2011; Jones, 1973). A number of international population-based, cross-sectional, and exploratory studies reported inadequate nutrient intakes, smaller body weight and body mass index (BMI) (May et al., 2014, 2016), and reduced plasma vitamin and mineral levels (Flynn et al, 1981; Keen et al., 1995; 2010;2013; Hutson et al., 2012; Laufer et al., 2004) in women consuming alcohol during pregnancy. Thus,

optimizing dietary intakes is critical where prenatal risk behaviours are present and/or abstinence may not be possible. However, prior to the development of a prescriptive clinical dietary guideline for maternal populations of various backgrounds, detailed information on baseline nutrition intake and the interplay between dietary and risk factors needs to be established.

Nutritional inadequacies, food insecurity, and risk exposures have been previously reported to be higher among Indigenous populations. Although available data are limited, several studies reported that pregnant and lactating women and women of child-bearing age in First Nations communities fall below the recommendations for vegetable and fruit, including fiber (Back et al., 2012), and dairy intakes (Johnson-Down & Egeland, 2012); studies also report the lower status of vitamins A, C, D, E, folate (Berti et al., 2008; Waiters et al., 1998), and minerals, such as calcium, magnesium, and iron (Berti et al., 2008; Chan, 2012). First Nations populations have been exposed to socioeconomic and health disparities, which contribute to high rates of substance use prenatally. According to the Manitoba First Nations Regional Longitudinal Health Survey, 15% of First Nations women consumed alcohol during their pregnancy. However, these findings are dated (Assembly of Manitoba Chiefs (AMC), Health Information Research Governance Committee (HRIGC), 2006) and an updated study is required. A comparative study on avoidable mortality revealed that the First Nations female population was at a substantially higher risk of mortality (10-fold) due to alcohol and/or illicit drug use compared to the general Canadian population (Park et al., 2015). The substance use and mental health disparities between Indigenous and their non-Indigenous counterparts could be a result of colonial policies, which still affect the levels of access to health services, education, social, and community services, and support (Heart, 2003). This especially affects First Nations women, as it compromises their access to equitable maternal care, socio-economic welfare, and feminine autonomy and voice.

Although some recent evidence on nutrient intakes, lifestyles, and general health among First Nations women residing on reserves exists (Chan et al., 2012; 2019; Delormier & Kuhnlein, 1998; Campbell et al., 1994), there is a substantial lack of current data for pregnant First Nations women living on-reserve. Furthermore, to our knowledge, no Canadian studies have examined the influence of alcohol consumption and other risk factors on the macro- and micro-nutrient intakes of pregnant, status, First Nations women residing on-reserve. Given the evidence that nutrition plays an important role in the course of FASD, understanding all factors which

contribute to intake in a population defined as vulnerable is paramount. Therefore, this pilot study sets out to i) examine the adequacy of maternal macro- and micro-nutrient intake during gestation; and ii) identify associations between maternal nutrition and risk exposure variables with a focus on self-reported alcohol consumption for First Nations women residing in a Manitoban remote community. Learnings from this study can be used to inform the design of larger population-based research to support an equitable, evidence-based policy for maternal programming and clinical advice for First Nations women residing in communities.

Methodology and Design

Band and council approval and community profile

The study protocol was developed in consonance with the Health Research Involving Aboriginal Peoples Guidelines established by the Canadian Institutes of Health Research (CIHR) and the Tri-Council Policy (TCP) Statement, "Ethical Conduct for Research Involving Humans" (Government of Canada (a), 2018). The study protocol and consent forms were approved by the University of Manitoba Health Research Ethics Board (H2013:263), as per Tri-Council Policy on *Ethical Conduct for Research Involving Humans* requirement in June 2013. All data operation processes conformed to Indigenous research principles of Ownership, Control, Access, and Possession (OCAP) (FNIGC, 2020). The detailed process of protocol development is described previously, as this study is a sub-part of a larger project (Giesbrecht, 2015).

The study protocol was presented to the community of Chemawawin Cree Nation (CCN) members at the Band and Council meeting in July 2018. Approval of the project by the Chief and Council occurred immediately following the presentation. The data collection in this community took place in June 2018-March 2019, after the involvement with the Opaskwayak Cree Nation as stated in *Chapter 5*. Therefore, the research direction heavily relied on the formerly conducted consultations with the AMC, HIRGC, and Indigenous leaders.

Community profile

The CCN is located 440 km north of Winnipeg, next to the town of Easterville (**Figure 3-1**). CCN is a signatory to Treaty 5 territory with 1,933 registered members (Statistics Canada, 2016), which is a re-located community. The relocation occurred due to the flood that took place in 1963 during the construction of the Grand Rapids Generation Station on the Saskatchewan River between Cedar Lake and Lake Winnipeg (University College of the North, 2021). The

community developments include a nursing station, local grocery store, school, community center, and health office among others.

Unlike other First Nations communities that fall under federal jurisdiction for health services provided under *Indian Act 1876* and *Revised Statutes of Canada 1985, Chapter I-5*, health care at CCN is provided by the Province of Manitoba Health, Seniors and Active Living (MHSAL) under the 1964 Agreement. The 1964 Agreement is a Memorandum of Understanding between federal and provincial governments, wherein the primary responsibility for clinical and public health services delivery in several Manitoban First Nations communities (Fox Lake, Grand Rapids, Mosakahiken (Moose Lake) and Chemawawin (Easterville) falls under provincial jurisdiction (Cook, 2003; Lavoie & Forget 2006).

The community profile of CCN is presented in **Table 3-1**. The community has a considerably younger population compared to the Manitoba Northern region. The CCN population is also experiencing a substantially higher growth rate compared to the Northern region (6.9% vs 2.8%) (Statistics Canada, 2016). Although the information on the average number of persons per household is not available for CCN, census data indicates that the average family size is 5.1 persons compared to the 3.0 for Northern regions (Statistics Canada, 2016), suggesting the average number of individuals living in one household might also be higher.

The unemployment rate of the community is 22.6%, which is notably higher than the Northern region's estimated unemployment rate of 9.8% (Statistics Canada, 2016). The total household median income is $40,576, which is considerably lower than the region’s household median income of $72,028. Similarly to household income, the median individual income of CCN is also considerably lower than the Northern region being $9,768 and individuals $31,220 respectively (Statistics Canada, 2016).

With respect to education, the CCN community also sits unfavorably compared to overall Northern Manitoba. The rate of high school graduation or its equivalency is 22% for CCN vs 27.9% for the Northern region (Statistics Canada, 2016).

Study settings, community engagement, and recruitment

The engagement with the CCN community occurred prior to the approval of the project by CCN's Band and Council. As a result of the researcher's (OK) fellowship awarded through Manitoba Training Program for Health Services Research, the researcher became involved with CCN through the work on Jordan's Principle – a federal initiative that ensures all First Nations children have equitable access to public services. This project provided the researcher with an opportunity to regularly travel to the community and build a strong relationship with the Health Authority office, nursing station staff, school, and Jordan's Principle program directorship. Through these established partnerships the researcher had the opportunity to meet families, elders, and youths, and participate in community events and gatherings. As a result of such extensive community involvement, the researcher was invited to the Band and Council meeting to present the planned maternal study, followed by the approval. The engagement with the community continued to be strengthened over time and strong partnership ties were built with CCN nursing station staff to carry out the study.

The recruitment of the participants in this community employed two approaches. Either at the nursing station for those pregnant women who came in for their medical appointment or in-home visits by the researcher accompanied by the maternal nurse. Interested individuals were invited to the nursing station, requested to read and sign the consent form, and review an interactive study information sheet prior to the commencement of data collection. For those under 18 years old, a consent form was obtained from a caregiver and/or a parent. All information was obtained through face-to-face interviews with the researcher, which were conducted at the nursing station site. A trauma-informed approach was employed to guide the process of recruitment and in-person surveying (Province of Manitoba, 2020). Women were re-affirmed that they were free to withdraw from the study at any point without any impacts on their medical care. All recruits were notified of the confidentiality and protection of the information. Participants were ensured that if at any point they felt uncomfortable with questions they could halt their participation. Upon completion of all the steps of data collection, participants received a $25 gift card to a local grocery store.

Participants

Pregnant women of any age residing in the CCN community were eligible for study participation.

Data collection instrument

Data collection consisted of an extensive, in-depth, multi-section research instrument, which was previously developed in our laboratory (Gisbrecht, 2015). The steps and the approaches undertaken to develop and pre-test the instrument are described in detail in Master's Thesis titled *Laying the Groundwork for Prenatal Dietary Assessment Research Among First Nations Women at-risk for Alcohol Use: Implications for Fetal Alcohol Spectrum Disorder* (Giesbrecht, 2015). Briefly, the instrument was developed to conduct research on maternal dietary intake with the focus on nutrients critical for fetal growth and CNS development in First Nations women. The instrument, titled Nutrition for Two, consisted of a paper copy and an iPad version, created by software and web-developing company - Function Four Ltd (Winnipeg, MB). The development of the research instrument has been undertaken in consultation with a number of community representatives from various bands, stakeholders, and professionals engaged with maternal programs in Winnipeg and First Nations communities across Manitoba (FASD community leaders, Mount Carmel Clinic nurses and community liaison workers, Maternal and Child Health program experts). Although the instrument has not undergone extensive reliability and validity testing, the four sections within the instrument were based on well-documented research tools (Canadian Community Health Survey (CCHS), Food Choice Motives Questionnaire, Food Frequency Questionnaire (FFQ), 24-hour dietary recall), which psychometric properties have been previously well established (Statistics Canada, 2014; Steptoe et al., 1995; Cade et al., 2002; Sheehy et al., 2014, 2015; Cardoso & Stocco, 2000; Greger & Etnyre, 1978; Karvetti & Knuts, 1985).

Concisely, the research instrument was composed of four sections titled:

1) Who am I? (demographic);
2) How do I give myself and my baby energy during pregnancy? (dietary intake: FFQ and 24-hour dietary recall);
3) How is my health? (maternal health status, anthropometrics, substance use);
4) How has my pregnancy been? (pregnancy health, pregnancy outcomes).

1) *Who am I?*: This section included questions on demographics (age, First Nations status, education, employment, social assistance status, and residence). The question on income was excluded from this section during the review process. This was the recommendation of the community members and Mount Carmel Clinic personnel, due to reasons of sensitivity and comfortability of a respondent with financial information. This section also included questions related to finances available to acquire food, which is an indication of food security, as the ability to buy food is the primary factor for food security (Tarasuk et al., 2015). The options for responses to questions on financial resources were in the form of Likert scales allowing participants to indicate whether they "strongly agree", "somewhat agree", "somewhat disagree", and "strongly disagree" with the statements.

2) *How do I give myself and my baby energy during pregnancy?:* This section was composed of the FFQ and 24-hour dietary recall. The FFQ was created to collect nutrient intake information on nutrients identified as important for pregnancy and fetal CNS development. A detailed literature review was conducted to identify evidence on nutrients which play a role in embryogenesis, fetal CNS development, and fetal growth. Additionally, nutrients which were identified to have a protective effect against ethanol-induced teratogenic damage were also selected (Young et al., 2014; Giesbrecht, 2015; Kloss et al., 2022). In total, 11 micronutrients (vitamin A, vitamin C, thiamin (vitamin B1), niacin (vitamin B3), cobalamin (vitamin B12), folate, choline, calcium, iron, zinc, and DHA) were chosen to be studied based on the identified evidence. Hundred and four (104) food and beverage items, which were the top 10 sources of each of these nutrients, were included in the instrument (Dyck et a., 2016). The selection of commonly consumed food items was based on the CCHS. Traditional food sources such as game meats, lake fish, berries, and wild greens were also integrated into the FFQ. The selection of traditional food items was based using the results from the First Nations Food, Nutrition and Environment Study (FNFNES) in Manitoba (Chan, 2012).

For each food and beverage item, participants were asked to provide responses on whether they had consumed it during the studied pregnancy (Yes/No), the frequency of the consumption (rarely, 1-3 times per month; sometimes, 1-2 times a week; often 3-5 times a week; every day, once a week; all the time, 2 times or more a day), and the portion size of an item (small, medium, large). Food models were utilized to provide visual aids for participants with respect to portion

sizes, which were standard equivalents to one of Health Canada's Food Guide (CFG) servings (Health Canada, 2007).

The 24-hour dietary recall collected detailed information on each food item (including the method of preparation) and beverages consumed from the time a participant woke up until the time they went to bed, for the day prior to an interview. The researcher (OK) provided illustrations of three standard portion sizes for various food items using food models and measuring devices (cups, spoons, bowls). The researcher also further probed participants who were omitting any details with respect to specific food items, time of consumption, and the method of preparation. Through this tool information on total caloric intake and macronutrient (carbohydrate, protein, fat) intake was gathered.

3) *How is my health?:* This section of the instrument collected data on participants' health status, chronic illness before and during pregnancy, prescription and over-the-counter medication, supplement use, and anthropometric measurements, such as weight and height. While information on pre-pregnancy weight was reliant on self-report, pregnancy weight and body height were measured at the nursing station using a balanced physician scale with a mounted stadiometer. BMI was computed with the standard formula: weight in kg/height in m^2.

Assessment of risk exposures

This sub-section included questions on alcohol consumption, smoking, and drug exposures during the studied pregnancy. Alcohol use and smoking-related sub-section opened up with the confirmatory question of whether the exposure was present or absent (Yes/No) during the studied pregnancy, followed by questions on the timing, frequency, type, and amount of consumption/exposures for each risk variable. Drug exposure only provided an option for the Yes or No response with an opportunity for an open-ended follow-up question.

4) How has my pregnancy been?*:* This section was comprised of questions on certain maternal characteristics, such as parity, gravidity, number of pre-term births, miscarriages, and bed rest during pregnancy.

Study measures (derived from the research instrument)

Macronutrient intake computation

The primary tool for macronutrient intake estimation of the 24-hour recall was the Canadian Nutrient File (CNF) (Government of Canada, 2018). For 24-hour food recalls, each food item

along with its corresponding code from the CNF were recorded into an excel file. Excel sheet comments and adjustments were made when the serving sizes in the 24-hour food recall differed from those in the CNF. When the company name of a product was specified, the nutrient label of that product was preferentially used in the analysis. This process was used to record the number of kilocalories and grams of protein, total carbohydrates (which included all carbohydrate fractions: sugars, fiber, and starches), fat, and sugar (entails sum of the individual monosaccharides and disaccharides) for each food item (Government of Canada, 2018). The daily total from each category was tallied and the macronutrient intake per day calculated. The 24-hour dietary recall is most commonly used method to collect dietary information in nutrition surveys (Gibson & Ferguson, 2008), thus the performance of 24-hour dietary recall on the collection of macronutrient information is well documented (Greger & Etnyre, 1978; Karvetti & Knuts, 1985). To determine energy adjusted macronutrient intakes (Tomova et al., 2014), the macronutrient intakes in weight/day were converted to the percentage of total energy intake (to align with Acceptable Macronutrient Distribution Range (AMDR), Health Canada, 2010). The intake for fiber converted into percent AI set for pregnancy (28g/day) (Health Canada, 2010).

It was also recorded which food group each item in the 24-hour dietary recall belonged to and the portion size of a food group eaten. Food group intake was calculated by summing up the weights or reported portion sizes of each food item belonging to a respective food group and tallying that intake to identify total daily food group consumption. Health Canada's Food Guide was utilized to compare maternal intake of food groups to Health Canada's recommendations (Health Canada, 2007). To account for prenatal recommendations to increase the number of servings from any of the four food groups, an additional serving was added to Vegetable and Fruit, Milk and Alternatives, and Grain Products reference values when assessing adequacy. Although a new Canada's Food Guide was released in 2019, at the time of data collection and dietary analysis for this project the former recommendations were still in place. Therefore, the analysis and interpretation are constructed based on former recommendations. The limitation of utilizing this tool with respect to the interpretation of findings is discussed in the *Limitation* section.

Micronutrient intake computation

A reference file was created with the nutrients under investigation (vitamin A, vitamin C, thiamin, niacin, cobalamin, folate, choline, calcium, iron, zinc, and DHA) to estimate the amount of each nutrient in a one standard medium serving size for each item on the FFQ. A medium portion size for each item was established on the CFG serving size (Health Canada, 2007). CNF was then used to determine the amount of a nutrient under investigation. If a food item was not found in the CNF, the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference or the nutrition information from the company of the food item was applied (Government of Canada (b), 2018; USDA, 2015). Medium portion size was set as the 50th percentile, small as the 25th percentile, and large as the 75th percentile. Further, the frequency of consumption was translated into a weighted amount of nutrient intake per day. The values used for each frequency option were as follows: never = 0; rarely or one to three times a month = 2/30; sometimes or one to two times a week = 1.5/7; often or three to five times a week = 4/7; every day or one time a day = 1; very often or two or more times a day = 2.5. Upon determination of the serving size and frequency value, the weight of nutrient consumed per day was calculated through the multiplication of the two values. The quantity of total daily micronutrient consumption from each food item was calculated by summing up the products of frequency and food amount (weight). The methodology utilized for frequency and portion size estimations was established upon previously validated FFQ tools (Sauvageot et al., 2013; Sheehy et al., 2014, 2015; Cardoso & Stocco, 2000; Forster et al., 2014).

To determine the level of adequacy for micronutrients, the micronutrient intakes in weight/day were converted to relevant percent (%) of Dietary Reference Intake (DRI) from micronutrients (Health Canada, 2010). The micronutrient intakes for vitamin A, C, thiamin, niacin, folate, cobalamin, calcium, iron, and zinc were converted into Estimated Average Requirement (EAR), set for intake during pregnancy for the appropriate age categories (14-18, 19-30, and 31-50 years old). Due to the absence of defined EAR for choline the Adequate Intake (AI) was used (Health Canda, 2010). To determine the adequacy percent for omega-3 docosahexaenoic acid (DHA), Global Recommendations for DHA and EPA Intake were used (Institute of Medicine, 2014; International Society for the Study of Fatty Acids and Lipids (ISSFAL), 2008).

The DRIs are a comprehensive series of reference values for nutrients commonly used for the assessment of the level and prevalence of inadequacy. The EAR is the median daily intake that is deemed to be sufficient to meet a given nutrient requirement of 50% of individuals in a group (Health Canada, 2010). The EAR cut point method was used to assess the prevalence of inadequacy (Murphy & Poos, 2002: IOM, 2001; 2014). To assess dietary intake during pregnancy, the data in the present study characterize intakes of micronutrients from food consumption alone and do not take into consideration intake from dietary supplements (please see in the *Limitation* section). The median intakes accounted for supplementation are presented in **Appendix 1**.

Alcohol consumption variable

Alcohol consumption was the main exposure variable of interest. Although the research instrument collected information on the frequency, quantity, and timing of alcohol consumption, due to low response rate to these questions the quantification of use in units/day, as originally intended, was not possible. Therefore, a categorical variable (Yes-1/No-0) was employed indicating the presence or absence of use at any time during pregnancy. This is consistent with the literature on the prenatal recommendation of abstinence (Health Canada, 1996; Government of Canada, 2022). However, it is a limitation of this pilot study, which is discussed in detail in the *Limitation* section of the Chapter.

Other studied variables

Additional risk exposure variables studied include smoking tobacco and drug use. Smoking use was characterized by the number of cigarettes/day as this enabled a continuous measure of prenatal tobacco exposure. Drug use was assessed categorically (Yes-1/No-0).

To estimate effects on outcomes (dietary intake) associated with demographic and health predictors, variables such as age, employment, level of education, and social assistance; and health variables such as trimester, BMI, and chronic illness were also investigated. A new variable was created for employment, where 0 encoded the absence of employment, 1 part-time employment, and 2 full-time employment. Education was treated similarly, elementary school was encoded by - 0, middle school - 1, high school - 2, college – 3, university/professional undergraduate degree - 4, graduate degree - 5. Age, BMI, trimester, and the number of pregnancies were assessed on a continuous scale. The presence of chronic illness and social

assistance were assessed categorically (Yes-1/No-0). The rationale for the assessment of the demographic and health variables was based on the lack of literature for the First Nations maternal population, yet well-established impact of these variables is documented for other maternal populations (Oliveira et al., 2015; Sheppard et al., 2017).

Administrative data collection

Administrative hospital discharge summaries were also collected after infant delivery. The summaries included maternal and infant health indicators. Due to data fragmentation and inconsistency in clinical reporting, this information could not be properly analyzed. A general summary of post-birth hospital discharges is provided in **Appendix 2**.

Sample size determination

Due to the small-scale pilot design of the present project, convenience sampling was employed, however, sample size determination was performed to identify the feasibility of conducting the study on a larger scale. Sample size determination was estimated using SAS software. The sample size calculation was based on the multiple linear regression analysis. With alpha level of 0.05 and 3 controlled covariates in the multiple regression, the sample size required (i.e., with 80% of statistical power) to detect the small (0.1), moderate (0.3), and large (0.5) effect of partial correlation is 785, 87, and 32, respectively (Aloe, 2014). With sample size of 22, the approximate effect size that could be detected is large (partial correlation around 0.68). The implications of the sample size are further discussed in the *Methodological Considerations* section of this Chapter.

Statistical analysis

Prior to undertaking any of the statistical analysis all independent variables of interest were assessed for normality of the distributions. The normality testing was conducted using Shapiro-Wilks and Kolmogorov-Smirnov. The results revealed that DRI (%) vitamin A, cobalamin, vitamin C, iron were not normally distributed.

Descriptive statistics (means and standard deviation (SD), medians and interquartile range (IQR) were computed for the demographic, health status, and pregnancy variables for the entire sample and at-risk and non-at-risk sub-groups (as classified according to self-reported alcohol consumption during the studied pregnancy). Frequencies and proportions (%) were used to describe characteristics of the participants by group, where appropriate. To compare differences

between non-at-risk and at-risk groups, an independent t-test was used for normally distributed data. A non-parametric Wilcoxon rank-sum test was used for Likert-scale, and not normally distributed data. To test for the associations between categorical variables, the Chi-square statistic was used and the Fisher's exact test was used for those with low frequency cells. Proportions and 95% confidence intervals were constructed for the percent of individuals having inadequate intake of macronutrients (above/below) and insufficient intake of micronutrients, as assessed by the AMDR or DRIs respectively.

Proportions and 95% confidence intervals were constructed for the prevalence of inadequacy for CFG food groups (below the recommendation), macronutrients (above/below the AMDR recommendation), and micronutrients (below the %DRI).

Spearman correlation was used to test the relationships between dietary variables and individual risk, demographic, health status (BMI), and maternal variables (trimester and the number of pregnancies). Spearman correlation analysis was also employed to test the relationship between individual micronutrient DRI (%) (collected through FFQ) and macronutrient intakes (collected through 24-hr dietary recall).

A series of multiple linear regression models were constructed to examine factors associated with each outcome variable –nutrient intake (total Kcal, % energy from protein, fat, and carbohydrate, and fiber (% AI) and % DRI vitamin A, C, thiamin, niacin, folate, cobalamin, choline, calcium, iron, zinc, and DHA). The predictor variables considered included alcohol consumption, smoking, drug exposure, demographic and health variables. Multiple regression models were adjusted for the trimester and BMI, which were selected based on the literature on the impacts of various factors, including risk factors on maternal dietary intake (Ceccanti et al., 2014; Esper & Furtado, 2014; May et al., 2005) and identified to be statistically significant in Spearman correlation analysis. Assumption testing was conducted for each of these regression models. The normality of the residual distribution was assessed through Q-Q plot and results indicated that the residuals were normally or approximately normally distributed for all nutrients, except for %DRI vitamin A and cobalamin. These variables underwent natural logarithmic transformation. Interactions among the predictors were examined and none of the interactions were statistically significant.

Missing values for any variables resulted in exclusion of a subject on a case-by-case basis in each analysis. Statistical significance was set at p - <0.05. All analyses were conducted using IBM SPSS Statistic Version 26.

Results

Maternal demographic, health, and pregnancy characteristics

All 22 women from the CCN community that were pregnant during the recruitment period were screened for inclusion and participated in the study (100% participation rate). The demographic variables of the sample, and non-at-risk and at-risk groups are presented in **Table 3-2**. Eleven (11) participants reported alcohol consumption during the studied pregnancy and thus were placed in the at-risk group. The mean age of all participants was 27.2 ± 5.9 years. Over half of the participants reported unemployment and social assistance. Maternal mean pre-pregnancy BMI was 25.9 ± 7.8 kg/$m^{2,}$ which is classified as overweight (normal: 18.5 < BMI < 25 kg/m^2). About 32% of participating women reported the diagnosis of chronic illness during pregnancy. Of the total participants, 73% reported smoking cigarettes, and 36% reported exposure to drugs during the studied pregnancy. The only drug reported was marijuana. Subgroup analyses comparing not-at-risk and at-risk groups did not reveal significant between-group differences for maternal demographic, health, and pregnancy outcomes variables with the exception of the number of adult household residents, which was significantly higher in the at-risk group.

Self-reported access to finances and food

Overall, 32% of participants agreed (9% strongly, 23% somewhat) with the statement "I often run out of food". 83% of participants expressed their agreement (63% strongly, 23% somewhat) with the statement "I have enough food to eat". When asked about having a voice in the spending on food 87% of the participants agreed (51% strongly, 36% somewhat). With the last statement- "I have enough money to provide food for myself and my family" 82% of participants agreed (41% strongly, 41% somewhat). Although a high number of participants agreed with the statement, about 18% of participants strongly disagreed that they have enough money to provide for food. There were no significant differences between at-risk and non-at-risk groups for self-reported access to financial resources (**Figure 3-2).**

To gain an understanding of participants' access, adequacy, availability, and affordability of food, questions on these food security measures were asked. A high proportion (78%) of participants agreed that the food they eat is adequate to keep them healthy (37% strongly agree, 41% somewhat agree). About 54% (36% strongly agree, 18% somewhat agree) of participants agreed with the "All the food I want to eat is available from a store near my home" statement. With regard to affordability, 55% of women agreed with the statement "I can afford to buy all the foods I want to eat"; however only 14% of women strongly agreed with the statement. Similarly to affordability, about 50% of women responded favorably to questions about access to food. However, an equal proportion of participants disagreed with the aforementioned statement (50%), with 23% strongly disagreeing. There were no significant differences between at-risk and non-at-risk groups (**Figure 3-3).**

Macronutrient dietary characteristics

Detailed food group, median caloric and intake, and participants' intake inadequacy percentage for each of the macronutrients are reflected in **Table 3-3**. A high proportion of participants were not meeting Health Canada's food group recommendations. Vegetables and Fruit and Milk and Alternatives food group daily servings were not met by 95% of participants (95%CI =77-100); Grain Products by 86%, (95%CI = 65-97), and Meat and Alternatives were not met by 68%, (95%CI = 45-86) of participants. The median (IQR) percent energy intake from protein, carbohydrates, and fat was 16% (13-20), 49% (39-53), and 36% (31-43) respectively. A high proportion of the sample had inadequate (either below or above) fat intake (57%, 95%CI = 34-78).

Micronutrient dietary characteristics

Table 3-4 displays the median micronutrient intake as well as the percentage of DRI. The median (IQR) intake of all nutrients was within the recommended reference DRIs (%), except it was lower for folate 78% (57-122), iron 79% (58-126), and DHA 36% (21-77); which corresponded with a marked prevalence of inadequacy for these nutrients (73%, 95%CI = 50-89; 73%, 95%CI = 50-89; and 91%, 95% CI = 71-99, respectively).

Effect of individual predictors on percent macronutrient intake

Spearman's rank correlation was used to measure the strength and direction of the association between individual predictors (risk, demographic, health status, and maternal variables) and

percent macronutrient intake **(Table 3-5)**. This analysis showed a significant positive correlation percent energy from fat and drug use ($r = 0.462$, $p < 0.05$). With respect to maternal health variables, significant positive correlation was detected for percent energy from protein and the trimester ($r = 0.408$; $p<0.05$) and significant negative correlation was observed for the percent energy from carbohydrates and the number of pregnancies ($r = -0.477$, $p < 0.05$).

Effect of individual predictors on DRI (%) of micronutrient intake

Table 3-6 demonstrates the results from Spearman's rank correlation analysis. Significant negative relationship between alcohol consumption and the intake of thiamin ($r = -0.609$, $p < 0.01$), niacin ($r = -0.609$, $p < 0.01$), folate ($r = -0.509$, $p < 0.05$), cobalamin ($r = -0.623$, $p < 0.01$), choline ($r = -0.611$, $p < 0.01$), calcium ($r = -0.580$, $p < 0.01$), iron ($r = -0.623$, $p < 0.01$), zinc ($r = -0.652$, $p < 0.01$), and DHA ($r = -0.566$, $p < 0.01$).

In terms of demographic variables, a significant negative correlation was observed between the level of education and the intake of choline ($r = -0.446$, $p < 0.05$). Employment correlated negatively with vitamin A intake ($r = -0.429$, $p<0.05$) and positively with cobalamin intake ($r = 0.489$, $p < 0.05$).

A significant positive correlation also existed between trimester and the intakes of thiamin ($r = 0.430$, $p < 0.05$), calcium ($r = 0.577$, $p < 0.01$), and DHA ($r = 0.462$, $p < 0.05$). Similarly, the number of pregnancies correlated positively with the folate ($r = 0.426$, $p < 0.05$).

Total energy intake showed a significant positive correlation with the intake of cobalamin ($r = 0.466$, $p < 0.05$). With respect to food groups intake, calcium exhibited positive correlation with Meat and Alternative ($r = 0.479$, $p < 0.05$) and DHA with Grain Product ($r = 0.434$, $p < 0.05$).

Notably, marginal significance was identified between the following: education and thiamin, niacin, cobalamin, calcium, and zinc ($r = -0.365$, $r = -0.375$, $r = -0.388$, $r = -0.365$, $r = -0.369$, respectively $p<0.1$). Marginal significance was also noted for social assistance and thiamin, niacin, and zinc ($r = 0.372$, $r = 0.387$, $r = 0.372$, respectively, $p<0.1$). Marginal significance was detected between a number of nutrients and maternal variables. Trimester was marginally positively correlated with niacin, folate, choline, and zinc intakes ($r = 0.391$, $r = 0.414$, $r = 0.398$, $r = 0.375$, respectively, $p<0.1$); number of pregnancies with thiamin, niacin, and iron ($r = 0.417$, $r = 0.420$, $r = 0.421$, respectively, $p<0.1$). Marginal significance was detected between total energy

(kcal) and thiamin and calcium intake ($r = 0.306$, $r = 0.370$, respectively, $p<0.1$); as well as Grain Products and choline and zinc ($r = 0.364$, $r = 0.381$, respectively, $p<0.1$).

Impact of the risk and demographic variables on percent energy from macronutrient intake

Table 3-7 displays the results of multivariate linear regression models in which pregnancy trimester and BMI were used as covariates in identifying the relations between risk variables, including maternal self-reported alcohol consumption, smoking, and drug use, and demographic variables, including employment and education, with the % energy from macronutrient intake. Alcohol consumption showed significant positive associations with total energy intake ($\beta = 0.525$, $p <0.05$) and the % energy of fat intake ($\beta = 0.443$, $p <0.05$). Percent energy from fat showed significant strong positive association with the drug use ($\beta = 0.607$, $p <0.01$). Notably, the models for all of the afore-stated findings also exhibited significance for the percent of the variation explained ($R^2 = 0.315$, $R^2 = 0.172$, $R^2 = 0.340$, respectively, $p<0.05$). Demographic variables failed to show a significant association with any of the tested macronutrient parameters.

Impact of the risk and demographic variables on DRI (%) of maternal micronutrient intake

Tables 3-8a and **3-8b** present the results of multivariate linear regression models, with pregnancy trimester and BMI as covariates, which were designed to assess the relationship between risk, demographic, and dietary variables and the %DRI of micronutrient intake. Alcohol consumption showed a strong negative association with thiamin ($\beta = -0.606$, $p <0.01$), niacin ($\beta = -0.528$, $p <0.05$), folate ($\beta = -0.551$, $p <0.05$), choline ($\beta = -0.530$, $p <0.05$), calcium ($\beta = -0.506$, $p <0.05$), iron ($\beta = -0.636$, $p<0.01$), and zinc ($\beta = -0.565$, $p <0.01$). Similarly, marginal significance was detected between vitamin A intake and alcohol ($\beta = -0.469$, $p<0.1$). Neither smoking nor drug use were associated with the %DRI of any maternal micronutrient. Significant findings were also observed for the percent variance explained for the following alcohol-nutrient models (thiamin ($R^2 = 0.469$, $p<0.01$), folate ($R^2 = 0.382$, $p<0.05$), choline ($R^2 = 0.418$, $p<0.05$), calcium ($R^2 = 0.429$, $p<0.05$), iron ($R^2 = 0.355$, $p<0.05$), and zinc ($R^2 = 0.399$, $p<0.05$)). Trends toward significance ($p<0.1$) were observed for employment and vitamin A ($\beta = -0.410$); vitamin C, folate and Milk and Alternative ($\beta = -0.454$, $\beta = -0.431$, respectively); cobalamin and total energy ($\beta = 0.333$); choline and education ($\beta = -0.458$).

Discussion

This pilot study contributes to the field of maternal health and nutrition in the following aspects: It explores the dietary intake and identifies factors associated with macro- and micro-nutrient intake of status of pregnant women residing in the community of CCN, in Manitoba. The preliminary findings of this study demonstrated that expecting First Nation women living on the CCN reserve were below Health Canada's former CFG recommendations for all food groups. The findings also indicate that the micronutrients of concern for both groups were folate, iron, and DHA, where a large proportion of participants were not meeting the DRIs. The study identified statistically significant negative links between alcohol consumption and percent recommended intake (% DRI) for thiamin, niacin, folate, cobalamin, choline, calcium, and zinc.

Demographic and health characteristics

Similarly to other on-reserve maternal studies, the present study identified lower maternal age, higher prevalence of unemployment and social assistance, and higher number of household residents compared to the general Canadian population (Statistics Canada, 2016; Sheppard et al., 2017; PHAC, 2013).

A significant difference was observed in the number of people in the household between the two groups, with 3.2 ± 1.8 adult individuals in the at-risk groups compared to 1.6 ± 1.3 in the non-at-risk group. Although there is a scarcity of information on household crowding and maternal at-risk behaviors, the literature suggests strong links between these variables and their contribution to poor health outcomes, compromised mental health, and an increased risk for FASD (Abel & Hannigan 1995; May & Gossage 2011).

In this study, a pre-pregnancy maternal BMI was estimated to be 25.9 kg/m^2, which is classified as overweight. About 50% (23% overweight, 27% obese) of participants were entering this study with greater BMI compared to general Canadian women (32.8% overweight or obese) (Torrance et al., 2002: Brennand et al., 2005). Consistent with the elevated BMI findings, the prevalence of chronic conditions in this cohort was about 32% with major conditions including type 2 diabetes, GDM, and heart disease. These overall findings are consistent with other reports on Indigenous maternal populations (Dyck et al., 1995; Oliveira et al., 2013).

Any level of self-reported alcohol consumption was identified among 50% of the participants. Although the survey included questions of frequency, timing, amount, and patterns of

consumption, a large percentage of women refused to respond to these questions further. Therefore, a meaningful evaluation of consumption patterns was obstructed. Nonetheless, these findings of self-reported alcohol consumption in the present study are substantially higher compared to the general Canadian maternal population average of 9% -15% (Poole & Dell 2005, Environics Research Group 2006). Presently, there is lack of population-based information on identifying alcohol consumption during pregnancy for First Nations on-reserve women. There is also a lack of information on demographic, lifestyle, health, and cultural factors, which have been shown to differentiate and influence alcohol consumption patterns in various populations (Salmon, 2011; Adlafet al., 2005).

About 73% of participants reported smoking cigarettes during the studied pregnancy. This is substantially higher than other prenatal smoking rates in First Nations women during pregnancy (35%-68%, depending on the study) (Oliveira et al., 2013; Heaman & Chalmers 2005). These statistics are even further apart from the 10.5% of the self-reported general Canadian maternal population (Al-Sahab et al., 2010). The prevalence of marijuana use, the only drug reported in the present study, was 36%. This is markedly higher than the general Canadian estimate of 1% (PHAC, 2009), however, an updated national statistic is required.

Concomitant and individual substance exposures are significant risk factors for inadequate dietary intake and FASD, independently. Physiologically, the recruitment of metabolic pathways resulting from substance use, such as appetite incitement or suppression (Schrieks et al., 2015; Hillemacher et al., 2007) alongside endocrine and metabolic factors up- and down-regulation (Farokhnia et al., 2020) contribute to the disruptions in metabolic homeostasis impacting the dietary under- or over-consumption – a modifiable risk factor for FASD (May & Gossage, 2011; Young et al., 2014; Kloss et al., 2022). The aggravated substance exposure increases fetal susceptibility to ROS damage through hypoxia and prolonged decreased blood flow and nutrition supply to the fetus (Kuhnert et al., 1987; Koop, 2006; Castles et al., 1999). Epidemiologically, it is broadly acknowledged that substance use is often concomitant (Poole & Dell, 2006; May & Gossage, 2011). Several studies reported higher frequency of tobacco use among pregnant women who had FASD diagnosed children or confirmed alcohol consumption during pregnancy (May et al., 2000; 2005; 2008; Miller et al., 1995; Coyne et al., 2008). Similar findings were observed by Viljoen and colleagues, (2002) and Cannon and colleagues (2012), for the

concomitant drug use. While the impacts of smoking during pregnancy are well documented, limited evidence exists with respect to drug use, including concomitant use, specifically marijuana. Singular reports link marijuana usage to low birth weight (Janisse et al., 2014; Hayatbakhsh et al., 2012), premature birth (Mark et al., 2016), stillbirth (National Institutes of Health (NIH), 2013); and the newly published report finds an association between prenatal cannabis use and incidence of autism spectrum disorder (Corsi et al., 2020). The evidence with respect to the physiological effect of concomitant use in the context of FASD is required. The impact of substance exposures and dietary intake are discussed further in the *Macro- and Micronutrient* subsections.

Access to resources

Food insecurity is a term ascertained to describe the situation of not having enough food to consume or consuming food items of lower nutritional quality, typically as a result of financial hardship. It is closely connected to the right to food, which is a human right. Food insecurity is widely prevalent in remote and northern Canadian locations. According to the FNFNES, a ten-year-long study that investigated the lifestyle, dietary habits, environmental pollution, and quality of the ecosystems in First Nations communities across Canadian provinces, found that 48% of First Nations households are food insecure (Chan et al., 2019). Although the present study did not distinctly aim to identify food security status, its survey asked questions concerning access to resources and food. Close to 20% reported that they "strongly disagree" with having enough money for food items, and enough food to eat on the daily basis; over 30% reported running out of food before having the ability to purchase more. The more disparaging statistic was observed for food access variables. Close to 50% of participating women disagreed with having access to food on a regular basis and over 60% disagreed with food being available at a nearby location. The findings from this Manitoban community align with the commonly reported pervasive barriers to food security in First Nations reserves (Chan et al., 2019; Thompson et al., 2012).

Taken together, this study preliminary findings carry special implications for risk exposure and FASD outcomes. Epidemiological evidence on the risk factors associated with the diagnosis of FASD identifies food insecurity as one of the major contributing factors (May & Gossage, 2011). A South African study of pregnant women reported greater likelihood of food insecurity among

women exposed to prenatal alcohol consumption (AOR: 1.03, 95%CI: 1.02–1.04) compared to women who were not exposed (Eaton et al., 2014). These findings are consistent with the findings of other research groups identifying FASD risk factors epidemiologically (May et al., 2011; 2014; Abel & Hannigan, 1995; Popova et al., 2020; Esper & Furtado, 2014).

Food group and macronutrient intake

In the present study, Health Canada's former CFG daily serving recommendations (Health Canada, 2007) were not met by high proportion of participants. These proportions were translated into low median intakes, which were consistent with a couple of prenatal studies in the rural First Nations communities (Back et al., 2012; Johnson-Down & Egeland, 2013). Macronutrient energy intakes of the participants were congruent with food group intakes and similar to that of women reported by Back and colleagues (2013) and Delormier and Kuhnlein (1999). Both reports focused on identifying the dietary characteristics of First Nations women living in rural locations. The findings were also similar to other rural reports, which did not specifically study gestation period or childbearing age. A study on dietary practices in a Woodland Cree woman, with a very similar sample size (n=19), revealed almost identical mean percent energy intake from macronutrients to the ones reported in this study (48.7 ± 11.8% from carbohydrates, 18.3 ± 8.7% from protein, 33.0 ± 9.1% from fat) (Bruner & Chad, 2013). Such consistencies between the present study and other reports point to the consensus of collective findings to other maternal on-reserve populations in similar areas.

Although information on traditional food items was collected, women had not reported the intakes of these foods. Therefore, the women in the study would be even further with respect to the types of food items recommended by First Nations, Inuit, and Métis (CFG (Health Canada, 2007)). While this scenario could be the result of the state of pregnancy, small sample size, data collected through fall and winter seasons, these findings might also have been provoked by a nutrition transition – a phenomenon characterized by increased intakes of western food items and decreased intakes of traditionally harvested foods (Earl, 2011; Johnson-Down & Egeland, 2012). This phenomenon is not studied amongst marginalized First Nations women who might be at-risk of carrying a child with FASD. Therefore, the exploration of the impacts of nutrition transition and the factors which promote the intake of traditional food items among pregnant women is needed.

The Spearman correlation revealed significant positive correlations between the self-reported alcohol consumption and total caloric intake and percent energy intake from fat and drugs (marijuana) exposures and percent energy intake from fat. The associations maintained their significance even when BMI and trimester were included as covariates in the linear regression model ($p<0.05$). These results were not unexpected, as both alcohol and marijuana are known metabolic regulators, with diverse regulatory actions, including the regulation of total caloric intake and body weight (Sansone & Sansone, 2014; Schrieks et al., 2015). There are inconsistencies between results from different studies investigating macronutrient intake in relation to risk exposures and FASD. While some international reports identify associations between risk exposures and lower caloric intake or significant differences between cases and control groups (May et al., 2014), others report no meaningful or clinically significant associations between exposures and total caloric and macronutrient intakes (Carter et al., 2017; Coathup et al., 2017). High-powered studies, studies on specific food items consumed in the presence of alcohol and drug exposures are needed in the general Canadian and First Nations populations to elucidate relevant conclusions on the subject matter.

Micronutrient intake

Results for median micronutrient intakes indicate that pregnant women living in the CCN reserve have adequate intakes for most of the studied nutrients (vitamin A, vitamin C, thiamin, niacin, cobalamin, choline, calcium, and zinc). The median intake of DHA fell substantially below the recommendation, demonstrating a need for an improved intake. Although human data are scarce on the impacts of DHA deficiency, experimental studies reveal that deprivation of DHA during gestation is linked to visual and behavioral impairments and disturbances in brain development affecting cognitive function later in life (Innis et al., 2008; Huffman et al 2011). Lower median intakes were also observed for folate and iron, which is problematic during gestation, as they are required for cell division, proliferation, growth, and proper neural tube formation (Ballard et al., 2012). These findings are consistent with the findings of two studies where First Nations women of childbearing age (19-50 years old) were included. A study of Cree communities in Northern Quebec, reported a lower intake of folate and iron, 497 ± 556 μg/day and 22.5 ± 56.0 mg/day, respectively (Johnson-Down & Egeland, 2012). A large Manitoban study conducted in a number of First Nations communities, similarly, reported moderately lower intakes of folate and iron (~304 μg/day and 13.9 mg/day, respectively) for women of childbearing age (19-50 years) (Chan

et al., 2012). Additionally, the present study found that a high proportion of participants was not meeting the recommendations for folate, iron, and DHA. Only one Canadian comparative study reported on similar subject matter (Berti et al., 2008). The authors observed high prevalence of dietary inadequacy for folate, ranging from 89.8-96.2%, and iron 84.1%. The authors did not study DHA intake. These findings suggest that a special emphasis needs to be placed on health promotion, ensuring food security, and nutrition education for women of childbearing years.

The investigation of the associations between dietary intake and risk variables revealed significant negative relationship between self-reported alcohol consumption and the DRI (%) met for thiamin niacin, folate, cobalamin, choline, calcium, iron, and zinc. Additionally, significant negative relationship was identified between smoking and the DRI (%) met for thiamin and choline. The associations maintained its significance between alcohol and nutrients after adjustments for trimester and BMI for thiamin folate, cobalamin, choline, calcium, and zinc. Although no known Canadian studies have reported and compared the micronutrient intakes for pregnant women at-risk, a South African study by May and colleagues (2014) investigated intakes of women who consumed alcohol prenatally. The authors reported significantly lower micronutrient intakes in alcohol-exposed women for choline, calcium, riboflavin, and docosapentaenoic acid (DPA) (n-3) ($p<0.05$) compared to the control group. Carter and colleagues (2017) also found positive associations ($p<0.05$) between the intake of methyl donor-related micronutrients and self-reported alcohol consumption (choline ($\beta = 0.09$) and cobalamin ($\beta = 0.12$) in Cape Coloured pregnant women.

There is a common misconception that FASD outcomes may be associated with the ethnic and cultural backgrounds (Chudley, 2005). As previously stated, a multitude of proximal (PAE, maternal health) and distal factors (food security, socioeconomic status, rural place of residence, etc), which are impacting First Nations communities at a greater rate, are associated with the outcome (May & Gossage 2011). Notably, nutrition is directly involved in both of these groups of factors. Therefore, establishing baseline nutrition intake and status for various Canadian maternal populations identified as at- and non- at-risk is crucial for public health maternal programming and health policy. The resulting recommendation of this study is to conduct a community-based, large scale research study with the focus on maternal health indicators, nutrition, and risk variables.

Adequate consumption of high-fiber foods, fruits and vegetables, healthy fats, and micronutrients, is recognized as a health-promoting behavior in all life phases and ages, however, it is especially accentuated during pregnancy, lactation, and child-bearing years (Gruszfeld & Socha, 2013). Given a higher prevalence of chronic illness and risk exposures of the sample, the findings of not meeting dietary recommendations are concerning in the context of other FASD predisposing factors studied in this work (and unfavourable socio-demographic position, maternal health, food security). These concerns are intensified when taken together with the finding of an inverse relationship between micronutrients studied and the presence of alcohol consumption. Although the timing and frequency of alcohol consumption was not possible to confirm in our study, alcohol consumption during pregnancy is most commonly reported during the first trimester when pregnancy may not yet be recognized (O'Keeffe et al., 2015; Rossen et al., 2018). Two large sample (n = 6,822; n = 2,006) international reports found that high proportion (23% and 53%) of women reporting consumption in the first trimester, the reporting of consumption was substantially lower in the subsequent trimester (13% and 12%) (Rossen et al., 2018; O'Keeffe et al., 2015). Considering that risk-associated behaviours occur at a higher rate prior to pregnancy recognition (1st trimester) (Rossen et al., 2018) the compounding effects of all the risk exposures, poor health, and poor dietary intakes may aggravate pregnancy outcomes and translate into higher incidences of FASD. Therefore, one of the major recommendations of this pilot work is to build on the presented design incorporating follow-up assessments throughout the trimesters.

The maternal health disparities in the studied First Nations community are shaped by structural inequities that formed as a result of historical, economic, and social policies targeted at assimilation of Indigenous population (Kendal, 2009; MacDonald 2015). The history of colonization and residential school system lay at the foundation of inequities in the health and well-being of the Indigenous population (Kendal, 2009). Historic and social analysis provide evidence on how cumulative effects of colonial infringements engendered loss of identity, resources, agency, and language, culture, and land (Heart, 2003). Specifically, in gendered dimension, the colonial infringements greatly impacted Indigenous women's autonomy, motherhood, and feminine capacity (Bourassa et al., 2004). An example of a policy in gendered dimension is birth evacuation policy. This mandate requires First Nations women living on reserves and in remote locations to evacuate to urban medical centres for the purposes of

childbirth and immediate antenatal care (Lawford et al., 2018). The medical confinement starts at 36-38 weeks of gestation and ends shortly after birth. This policy has greatly affected the women of this remote community, as all mothers were expected to deliver in the birthing centers away from their home community (Winnipeg, Brandon, or the Town of The Pas). Literature on the outcomes of locally available prenatal care and prenatal evacuation identifies mostly negative, long-lasting impacts on First Nations women, infant, and overall community health (Riddell et al., 2016; Kornelsen et al., 2010; Lawford et al., 2018).

Strengths

The strengths of this research include relationship building with the community, the presence of the same researcher (OK) during questionnaire completion, which assisted with the interpretation and clarification of the tool, and the adaptation of the research tool to study nutrients important for CNS development prenatally. An additional strength of the study is the assessment of the face and content validity of the research instrument. Although the instrument did not undergo extensive validity testing for the population studied, it founded upon validated instruments such as CCHS, Food Choice Motives Questionnaire, FFQ, and 24-hour dietary recall (Giesbrecht, 2015). Furthermore, key stakeholders including dietitians, Indigenous community leaders, experts in the field, had an opportunity to review the instrument and include extensive feedback to improve the content and readability for the population studied. These methods of face and content validation align with the with the methodology of similar studies in the field of nutrition (Czuber-Dochan et al., 2014; Gleason et al., 2010).

Limitations

Several limitations have affected the results of this study. Most notable limitations were small sample size, the use of self-reported data and absence of recovery biomarkers for the micronutrients studied, the lack of clinically confirmed PAE, and the lack of follow-up throughout trimesters. Although this was a pilot design, small sample size precluded various forms of sub-analysis and more extensive covariate adjustments in regression models.

The use of self-report affected nearly all of the studied variables (dietary, health, and risk-exposures). This is a common limitation of many studies that capture information on lifestyle parameters retrospectively (Stevens et al., 2020), especially for the reports of risk exposures including alcohol consumption. This limitation applies not only to this study, but greatly affects

the field of FASD diagnosis (Cook, 2003; Joya et al., 2012; Stevens et al., 2020). Confirmation of prenatal exposure is necessary for the diagnosis of the forms of FASD where the is no presentation of the three sentinel facial features (Chudley et al., 2005; Cook et al., 2016). Thus, self-report remains to be a critical tool for information gathering on all aspects of alcohol consumption (pattern, quantity, timing, and frequency) (Stevens et al., 2020). Although the elucidation of an objective consumption recovery biomarker is required, reducing the stigma associated with consumption, may improve the reporting during pregnancy, especially for First Nations women where stigma and fear appear to be common and often attributable factors (Tait, 2000). This was the situation for the present study, where women had identified the feeling of discomfort with some questions regarding risk quantity, timing, and frequency of exposures. When probed by the researcher (OK), some women had identified that the hesitations were due to involvements with Child and Family Services (CFS), family conflicts, and other social issues which may impact their motherhood.

Likewise, the use of self-reported information for dietary data collection also impacted the accuracy of results. As most research that uses FFQ and 24-hour dietary recall indicates, the reliance on memory, responders' biases, and social desirability aspects contribute to over- and under-estimations of intakes (Gleason et al., 2010; Prentice, 1996). Over- and under-reporting introduce measurement error, which impacts statistical power (Freedman et al., 2011). This limitation is especially considered in this study, as an already existing small sample size also impacts power and precludes meaningful conclusions. This limitation is recommended to be addressed through greater sample size, repeated measures of intakes throughout the trimesters, and the use of biomarkers in future studies.

The present study has not taken into account supplement intake when assessing the DRI (%). It was the primary objective of this pilot study to identify specifically the influence of alcohol consumption on dietary behaviours and the level of nutrient adequacy coming from food to establish the baseline. Further to that, it was stated by medical professionals of the nursing station that although women may report taking supplements, the regularity of it not certain. Since the research instrument did not assess the frequency of supplement intake it was excluded from the analysis. It is recommended to launch a study specifically aimed at examining various supplement intakes of First Nations maternal population.

The use of the former CFG is a limitation posing challenges to the knowledge translation and wholistic interpretation of the findings with the updated recommendations. The CFG has been substantially revised. Whereas former CFG (2007) contained four distinct food groups with prescriptive recommendations for the number of daily servings for each food group (Health Canada, 2007), the recommendations in novel Guide were replaced with visual plate representation (Government of Canada, 2019). Furthermore, the Milk & Alternatives and Meat & Alternatives food groups were imploded into one Protein Foods food group (Government of Canada, 2019). Therefore, the interpretation of our finding for the protein-based foods may present challenges for practicing clinicians and community programming personnel, when providing an advice to women in the community. However, the overall recommendations for increased fruit and vegetable intake and fiber-rich whole grains remain conventional and well interpretable.

Conclusion

The objective of this project was to evaluate the nutrition status, a major risk modifier for FASD, of First Nations women residing in remote communities. This pilot study contributes to the field of maternal health and nutrition by identifying the factors associated with macro- and micro-nutrient intake of status of pregnant women residing in the community of CCN, in Manitoba. The findings demonstrated that expecting First Nation from the CCN reserve had poor intakes of all four food groups, as postulated by the former recommendations; lower intakes of folate, iron, and DHA, where a large proportion of participants were not meeting the DRIs. The study also detected association between alcohol consumption and percent recommended intake (% DRI) for thiamin, niacin, folate, cobalamin, choline, calcium, and zinc. These findings are consistent with the limited number of other studies. Large prospective data is needed to identify dietary intake and nutrition status of at-risk women.

Table 3-1. Chemawawin Cree Nation community profile

Parameter	CCN[1]	Division No. 21, (Nor-man region)[2]
Total population	1,639[1]	21,983
Population change since 2011	6.9%[2]	2.8%
Total no. of households	265	9,162
Ave. family size	5.1	3.0
Ave. persons per household	N/A	2.7
Median population age (years)	19.7	34.8
Educational attainment (individuals ≥25 years of age):	22.0%	27.9%
Secondary	11.0%	49.9%
Post-secondary		
Total median household income	$40,576	$72,028
Total median individual income	$9,768	$31,220
Unemployment rate (individuals ≥25 years of age):	22.6%	9.8%

[1]Census Profile, 2016 Census. Chemawawin Cree Nation (CCN), Tribal Council area
[2]Census Profile, 2016 Census. Chemawawin Cree Nation 21E, Indian reserve.

Table 3-2. Maternal demographic, health, and pregnancy characteristics for all participants and by self-reported alcohol consumption

Characteristic	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value
Age (years)[a]	27.2 ± 5.9	27.3 ± 4.5	27.1 ± 7.3	0.944
≤18	1 (5)	1 (9)	0	
19-30	15 (68)	6 (55)	9 (82)	
31-39	6 (27)	4 (36)	2 (18)	
Education[b]				0.151
Elementary	0	0	0	
Junior high	7 (32)	2 (18)	5 (46)	
High school	13 (59)	7 (64)	6 (54)	
Certificate	2 (9)	2 (18)	0	
University	0	0	0	
Employment[b]				0.519
Unemployed	13 (59)	8 (73)	5 (46)	
Employed part-time	0	0	0	
Employed full-time	4 (18)	0	4 (36)	
Student	1 (5)	1 (9)	1 (9)	
Maternity Leave	2 (9)	2 (18)	1 (9)	
Social Assistance[c]	14 (64)	9 (82)	5 (45)	0.091
# of household residents[b]	4 (3-6)	4 (3-5)	6 (4-10)	0.192
# of Adults (18+ years)	2 (1-4)	1 (1-2)	3 (2-4)	**0.030**
# of Children (<18 years)	2 (2-3)	2 (2-3)	3 (2-5)	0.730
Pre-pregnancy BMI[a]	25.9 ± 7.8	23.9 ± 6.8	27.7 ± 8.6	0.280
Below	4 (18)	2 (18)	2 (18)	
Normal	7 (32)	3 (27)	4 (36)	
Overweight	5 (23)	3 (27)	2 (18)	
Obese	6 (27)	3 (27)	3 (27)	
Chronic illness:				
Before pregnancy[c]	5 (23)	2 (18)	3 (27)	0.611
During pregnancy[c]	7 (32)	2 (18)	3 (27)	0.611
Medications:				
Prescribed[c]	2 (18)	1 (9)	1 (9)	1.000
Over-the-counter[c]	6 (27)	4 (36)	2 (18)	0.338
Vit & min supplements[c]	14 (64)	5 (45)	9 (82)	0.183
Smoking[c]	16 (73)	7 (64)	9 (82)	0.330
Drugs (marijuana)[c]	8 (36)	4 (36)	4 (36)	1.000
Trimester				
1st	6 (27)	2 (18)	4 (36)	
2nd	13 (59)	6 (55)	7 (64)	
3rd	3 (14)	3 (27)	0	
Pregnancy outcomes[b]:				
# of pregnancies	4 (2-6)	4 (3-7)	4 (2-6)	0.575
# of miscarriages	0 (0-2)	0 (0-2)	1 (0-2)	0.690
# of stillbirths	0 (0-0)	0 (0-0)	0 (0-0)	1.000
# of abortions	0 (0-0)	0 (0-0)	0 (0-0)	1.000
# of full-term births	2 (0-3)	2 (1-3)	1 (0-3)	0.800
# of pre-term births	0 (0-1)	0 (0-1)	0 (0-1)	0.690
Self-reported health[b]				1.000
Excellent	2 (9)	2 (18)	0	
Very Good	4 (18)	1 (9)	3 (27)	
Good	11 (50)	5 (45)	6 (55)	
Fair	4 (18)	2 (18)	2 (18)	
Poor	1 (5)	1 (9)	0	
Bed rest during pregnancy[c]	2 (9)	1 (9)	1 (9)	1.000

Values are means ± SD, n (percentages), and medians (Q1-Q3). The differences between the two groups were tested by an independent t-test[a], Wilcoxon rank-sum test[b] or a Chi-square or Fisher's exact tests of independence[c].

Table 3-3. Maternal macronutrient intake for all participants (n=22)

Dietary intake	Median (Q1-Q3)	% Inadequate (95% CI)
Food group (#/day)[†]		
Vegetable and Fruit	3 (3-5)	95 (77-100)
Grain Products	4 (2-6)	86 (65-97)
Milk and Alt.	1 (0-2)	95 (77-100)
Meat and Alt.	3 (2-4)	68 (45-86)
Macronutrient (g/day)		
Protein	77 (63-104)	
Carbohydrate	216 (166-258)	
Fat	80 (55-108)	
Fiber	16 (8-20)	
Sugar	56 (39-76)	
Energy (kcal/day)	1953 (1528-2466)	
Protein	308 (253-417)	
Carbohydrate	866 (665-1033)	
Fat	729 (497-971)	
Sugar	225 (156-302)	
Energy from macronutrients (%)[††]		
Protein	16 (13-20)	5 (0-24)
Carbohydrate[†††]	49 (39-53)	48 (26-70)
Fat	36 (31-43)	57 (34-78)

Data derived from 24-hour dietary recall. [†]Prevalence of inadequacy was assessed using former Health Canada's Eating Well with Canada's Food Guide (2007). [††]Prevalence of inadequacy was assessed using Health Canada's Acceptable Macronutrient Distribution Range (2022). [†††]Carbohydrate includes sugar.

Table 3-4. Maternal micronutrient intake for all participants (n=22)

Dietary intake	Median intake (Q1-Q3)	Median (IQR) %DRI[1]	% Inadequate (95% CI)
Vitamin A (RE) (mcg)	1555 (769-2434)	255 (145-346)	27 (11-50)
Vitamin C (mg)	141 (69-174)	200 (135-252)	22 (7-45)
Thiamin (Vit B1) (mg)	3 (1-4)	201 (156-302)	14 (3-35)
Niacin (Vit B2) (mg)	31 (17-46)	226 (168-304)	9 (0-29)
Folate (Vit B9) (mcg)	438 (243-638)	78 (57-122)	73 (50-89)
Cobalamin (Vit B12) (mcg)	8 (4-17)	476 (249-592)	0
Choline (mg)	484 (341-894)	128 (81-205)	36 (17-59)
Calcium (mg)	961 (535-1592)	134 (74-190)	46 (24-68)
Iron (mg)	17 (12-32)	79 (58-126)	73 (50-89)
Zinc (mcg)	15 (9-27)	165 (105-248)	36 (17-59)
DHA (mcg)	69 (39-143)	36 (21-77)	91 (71-99)

Data derived from FFQ. Nutrient inadequacy was assessed using Health Canada's Dietary Reference Intake, Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50 for vitamins A, B12, C, folate, thiamin, niacin, zinc, calcium, iron; Adequate Intake (AI) for choline. Recommendations for DHA (docosahexaenoic acid, C22:6n-3) were obtained from Global Recommendations for DHA and EPA Intake (2008).

Table 3-5. The effect of individual predictors (Spearman's r) on maternal macronutrient intake (% energy) (n=22).

	Total Energy (kcal)	Protein (%)	Fat (%)	CHO (%)	Fiber (%)†
Risk variables					
Alcohol (Yes/No)	0.308	0.099	0.380+	-0.048	0.165
Smoking (# of cigarettes/d)	0.055	-0.398	-0.242	0.376	-0.256
Drugs (marijuana) (Yes/No)	-0.134	-0.164	**0.462***	0.313	0.030
Demographic variables					
Age	0.072	-0.009	-0.307	-0.303	-0.131
Education (level)††	0.010	0.259	0.158	0.029	0.249
Employment†††	-0.059	-0.196	-0.006	0.050	0.010
Soc. Assistance (Yes/No)	0.074	-0.102	0.073	0.119	0.089
Health status variables					
BMI††	0.197	0.048	0.143	-0.252	-0.143
Maternal variables					
Trimester	0.190	**0.408***	0.044	0.021	-0.085
# of pregnancies	0.222	0.252	0.321	**-0.477***	-0.204

†Fiber - (%) Adequate Intake (AI) (Health Canada, 2010). ††Levels of education included the following variables in ordinal progression: elementary school, middle school, high school, college, university/ professional undergraduate degree, graduate degree. †††Employment included the following variables in ordinal progression unemployed, employed part-time, employed full-time. Pre-pregnancy BMI. Note: *p <0.05; +trending toward significance, p<0.1.

Table 3-6. The effect of individual predictors (Spearman's r) on the maternal micronutrient intake (%, DRI) (n=22).

	Vitamin A	Vitamin C	Thiamin (Vit B1)	Niacin (Vit B2)	Folate (Vit B9)	Cobalamin (Vit B12)	Choline	Calcium	Iron	Zinc	DHA
Risk variables											
Alcohol (Yes/No)	-0.365	-0.236	**-0.609****	**-0.609****	**-0.509***	**-0.623****	**-0.611****	**-0.580****	**-0.623****	**-0.652****	**-0.566****
Smoking (# of cigarettes/d)	0.106	0.133	0.059	0.112	-0.001	-0.019	-0.055	-0.275	0.037	0.001	0.256
Drugs (Yes/No)	0.053	-0.238	0.074	0.089	0.015	0.060	0.119	0.045	0.104	0.104	0.015
Demographic variables											
Age	0.024	-0.141	-0.091	-0.052	-0.071	0.023	-0.045	-0.007	-0.016	-0.024	-0.066
Education (level)†	-0.097	-0.103	-0.365^{+}	-0.375^{+}	-0.323	-0.388^{+}	**-0.446***	-0.365^{+}	-0.314	-0.369^{+}	-0.294
Employment††	**-0.429***	0.082	-0.110	-0.168	-0.236	**0.489***	-0.180	-0.178	-0.220	-0.196	-0.237
Soc. Assistance (Yes/No)	0.000	0.089	0.372^{+}	0.387^{+}	0.357	0.149	0.253	0.328	0.313	0.372^{+}	0.194
Health status variables											
BMI†††	-0.053	0.013	-0.190	-0.253	-0.130	-0.003	-0.283	-0.121	-0.162	-0.262	-0.426^{+}
Maternal variables											
Trimester	0.161	0.347	**0.430***	0.391^{+}	0.414^{+}	0.196	0.398^{+}	**0.577****	0.269	0.375^{+}	**0.462***
# of pregnancies	0.207	0.132	0.417^{+}	0.420^{+}	**0.426***	0.254	0.212	0.326	0.421^{+}	0.347	0.011
Dietary variables											
Total energy	-0.097	0.101	0.306^{+}	0.072	0.172	**0.466***	0.143	0.370^{+}	-0.027	0.084	0.111
V & F†††† (# of servings/d)	-0.197	0.077	-0.102	-0.094	-0.098	-0.323	0.121	-0.034	-0.172	0.007	0.137
Grain (# of servings/d)	0.045	0.245	0.314	0.329	0.313	0.037	0.364^{+}	0.339	0.281	0.381^{+}	**0.434***
Milk & Alt (# of servings/d)	-0.193	-0.192	-0.050	-0.056	-0.119	-0.305	0.003	0.090	-0.154	-0.075	0.073
Meat & Alt (# of servings/d)	0.101	0.200	0.186	0.089	0.274	-0.043	0.289	**0.479***	0.115	0.208	0.106

Levels of education included the following variables in ordinal progression: elementary school, middle school, high school, college, university/ professional undergraduate degree, graduate degree. ††Employment included the following variables in ordinal progression unemployed, employed part-time, employed full-time. †††Pre-pregnancy BMI. ††††Vegetable & Fruit. Nutrient inadequacy was assessed using Health Canada's Dietary Reference Intake, EAR for pregnant women aged 14-18, 19-30, and 31-50 for vitamins A, B12, C, folate, thiamin, niacin, zinc, calcium, iron; AI for choline. Recommendations for DHA (docosahexaenoic acid, C22:6n-3) were obtained from Global Recommendations for DHA and EPA Intake (2008). Note: *p <0.05, ** p <0.01; $^{+}$trending toward significance, p<0.1.

Table 3-7. The impact of the risk and demographic variables on the maternal macronutrient intake (%, energy) (n=22).

	Total Energy (kcal)	Protein (%)	Fat (%)	CHO (%)	Fiber (%)
Alcohol (Yes/No)	**R^2 = 0.315*** **β = 0.525***	R^2 =0.202 β = 0.292	**R^2 = 0.172*** **β = 0.443***	R^2 = 0.232$^+$ β = 0.171	R^2 = 0.207 β = -0.248
Smoking (# of cigarettes/day)	R^2 = 0.137 β = 0.006	R^2 = 0.274 β = -0.372	R^2 = 0.114 β = -0.284	R^2 = 0.458 β = 0.060	R^2 = 0.233$^+$ β = -0.537$^+$
Drugs (Yes/No)	R^2 = 0.181 β = -0.324	R^2 = 0.134 β = -0.054	**R^2 = 0.340*** **β = 0.607****	R^2 = 0.174 β = 0.096	R^2 = 0.141 β = 0.134
Employment†	R^2 =0.089 β = 0.037	R^2 = 0.198 β = 0.262	R^2 = 0.017 β = 0.111	R^2 = 0.186 β = 0.080	R^2 = 0.126 β = 0.008
Education (level)††	R^2 = 0.089 β = -0.022	R^2 = 0.133 β = -0.050	R^2 =0.035 β = -0.185	R^2 =0.171 β = -0.004	R^2 =0.166 β = -0.234

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. †Employment included the following variables ordinal progression: unemployed, employed part-time, employed full-time. ††Levels of education included the following variables in ordinal progression: elementary school, middle school, high school, college, university/professional undergraduate degree, graduate degree. Results are presented as coefficient of determination (R2) and standardized regression coefficient (β). Note: *p <0.05, ** p <0.01; +trending toward significance, p<0.1.

Table 3-8a. The impact of the risk and demographic variables on the maternal micronutrient intake (%, DRI) (n=22).

	Vitamin A	Vitamin C	Thiamin (Vit B1)	Niacin (Vit B2)	Folate (Vit B9)
Alcohol (Yes/No)	$R^2 = 0.192$ $\beta = -0.469^{+}$	$R^2 = 0.265$ $\beta = -0.376$	**$R^2 = 0.469$**** **$\beta = -0.606$****	$R^2 = 0.327^{+}$ **$\beta = -0.528$***	**$R^2 = 0.382$*** **$\beta = -0.551$***
Smoking (# of cigarettes/d)	$R^2 = 0.208$ $\beta = 0.272$	$R^2 = 0.091$ $\beta = 0.165$	$R^2 = 0.086$ $\beta = -0.027$	$R^2 = 0.130$ $\beta = 0.098$	$R^2 = 0.076$ $\beta = 0.055$
Drugs (Yes/No)	$R^2 = 0.011$ $\beta = 0.031$	$R^2 = 0.181$ $\beta = -0.191$	$R^2 = 0.174$ $\beta = 0.095$	$R^2 = 0.100$ $\beta = -0.056$	$R^2 = 0.141$ $\beta = 0.108$
Employment†	$R^2 = 0.173$ $\beta = -0.410^{+}$	$R^2 = 0.155$ $\beta = -0.084$	$R^2 = 0.192$ $\beta = -0.165$	$R^2 = 0.136$ $\beta = -0.200$	$R^2 = 0.190$ $\beta = -0.248$
Education (level) ††	$R^2 = 0.042$ $\beta = -0.209$	$R^2 = 0.151$ $\beta = -0.063$	$R^2 = 0.218$ $\beta = -0.266$	$R^2 = 0.231$ $\beta = -0.426$	$R^2 = 0.173$ $\beta = -0.240$
Total Energy (kcal)	$R^2 = 0.112$ $\beta = -0.335$	$R^2 = 0.168$ $\beta = -0.146$	$R^2 = 0.183$ $\beta = -0.137$	$R^2 = 0.111$ $\beta = -0.125$	$R^2 = 0.138$ $\beta = -0.086$
Food groups (# servings/day)					
V & F†††	$R^2 = 0.124$ $\beta = -0.358$	$R^2 = 0.148$ $\beta = -0.003$	$R^2 = 0.227$ $\beta = -0.263$	$R^2 = 0.138$ $\beta = -0.214$	$R^2 = 0.180$ $\beta = -0.236$
Grain Products	$R^2 = 0.017$ $\beta = -0.093$	$R^2 = 0.161$ $\beta = 0.129$	$R^2 = 0.218$ $\beta = 0.265$	$R^2 = 0.156$ $\beta = 0.279$	$R^2 = 0.205$ $\beta = 0.313$
Milk & Alternative	$R^2 = 0.054$ $\beta = -0.243$	$R^2 = 0.300$ $\beta = -0.454^{+}$	$R^2 = 0.282$ $\beta = 0.397$	$R^2 = 0.194$ $\beta = -0.362$	$R^2 = 0.268$ $\beta = -0.431^{+}$
Meat & Alternative	$R^2 = 0.055$ $\beta = -0.234$	$R^2 = 0.181$ $\beta = -0.202$	$R^2 = 0.209$ $\beta = -0.229$	$R^2 = 0.136$ $\beta = -0.219$	$R^2 = 0.145$ $\beta = -0.132$

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. †Employment included the following variables ordinal progression: unemployed, employed part-time, employed full-time. ††Levels of education included the following variables in ordinal progression: elementary school, middle school, high school, college, university/professional undergraduate degree, graduate degree. †††V & F – Vegetable & Fruit. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: *p <0.05, ** p <0.01; $^{+}$trending toward significance, p<0.1

Table 3-8b. continued

	Cobalamin	Choline	Calcium	Iron	Zinc	DHA
Alcohol (Yes/No)	R^2 = 0.293 β = -0.380	**R^2 = 0.418*** **β = -0.530***	**R^2 = 0.429*** **β = -0.506***	**R^2 = 0.355*** **β = -0.636****	**R^2 = 0.399*** **β = -0.565***	R^2 = 0.153 β = -0.357
Smoking (# of cigarettes/d)	R^2 = 0.196 β = 0.102	R^2 = 0.164 β = -0.119	R^2 = 0.188 β = -0.119	R^2 = 0.035 β = 0.102	R^2 = 0.125 β = -0.134	R^2 = 0.384$^+$ β = -0.040
Drugs (Yes/No)	R^2 = 0.016 β = -0.036	R^2 = 0.194 β = 0.099	R^2 = 0.244 β = 0.175	R^2 = 0.025 β = -0.069	R^2 = 0.140 β = 0.066	R^2 = 0.073 β = -0.167
Employment (Yes/No)	R^2 = 0.132 β = -0.346	R^2 = 0.240 β = -0.237	R^2 = 0.242 β = -0.161	R^2 = 0.100 β = -0.285	R^2 = 0.180 β = -0.213	R^2 = 0.081 β = -0.185
Education (level) †	R^2 = 0.094 β = -0.328	R^2 = 0.339$^+$ β = -0.458$^+$	R^2 = 0.239 β = -0.172	R^2 = 0.108 β = -0.345	R^2 = 0.264 β = -0.417	R^2 = 0.165 β = -0.400
Total Energy (kcal)	R^2 = 0.187 β = 0.333$^+$	R^2 = 0.190 β = 0.072	R^2 = 0.297 β = 0.214	R^2 = 0.086 β = -0.267	R^2 = 0.146 β = -0.109	R^2 = 0.048 β = -0.022
Food Groups (# of servings/d)						
V & F††	R^2 = 0.090 β = -0.289	R^2 = 0.201 β = -0.135	R^2 = 0.253 β = -0.202	R^2 = 0.097 β = -0.293	R^2 = 0.170 β = -0.197	R^2 = 0.054 β = -0.082
Grain Products	R^2 = 0.016 β = -0.027	R^2 = 0.246 β = 0.285	R^2 = 0.269 β = 0.262	R^2 = 0.037 β = 0.148	R^2 = 0.208 β = 0.309	R^2 = 0.100 β = 0.264
Milk & Alternative	R^2 = 0.065 β = -0.259	R^2 = 0.306 β = -0.405	R^2 = 0.271 β = -0.270	R^2 = 0.093 β = -0.313	R^2 = 0.228 β = -0.354	R^2 = 0.102 β = -0.271
Meat & Alternative	R^2 = 0.073 β = -0.267	R^2 = 0.198 β = -0.126	R^2 = 0.219 β = 0.053	R^2 = 0.079 β = -0.267	R^2 = 0.149 β = -0.130	R^2 = 0.064 β = -0.140

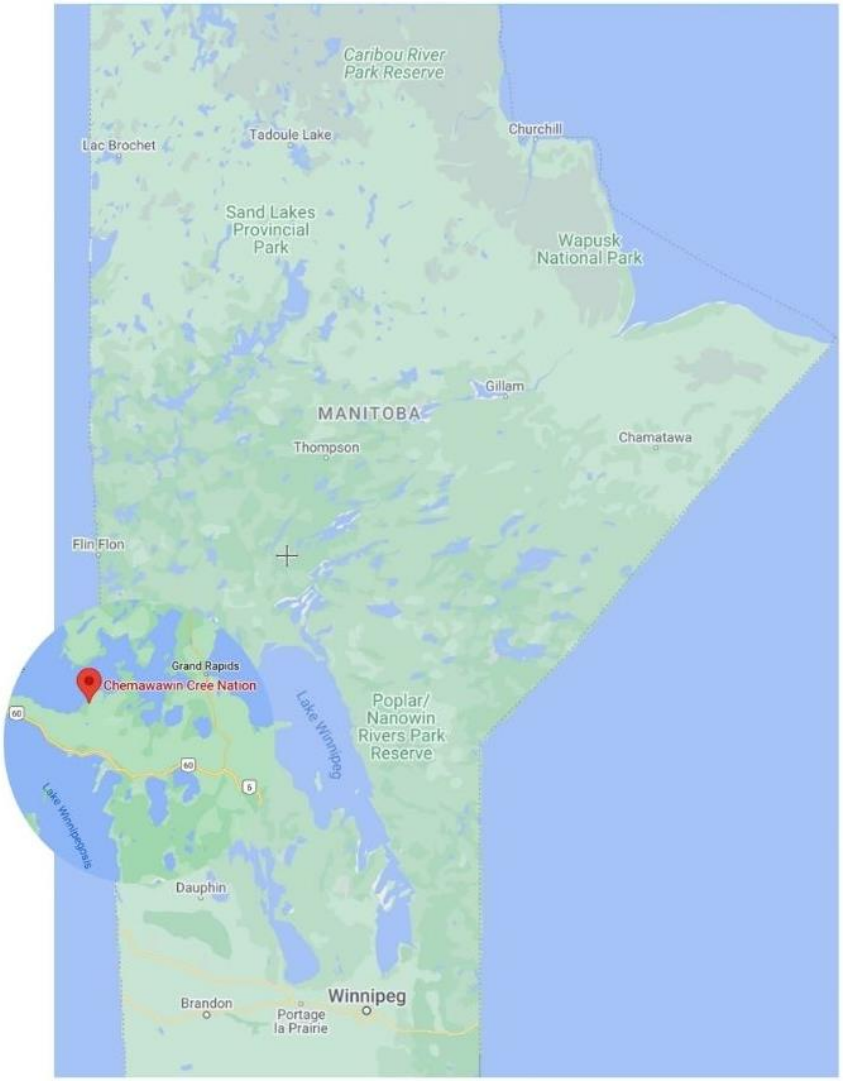
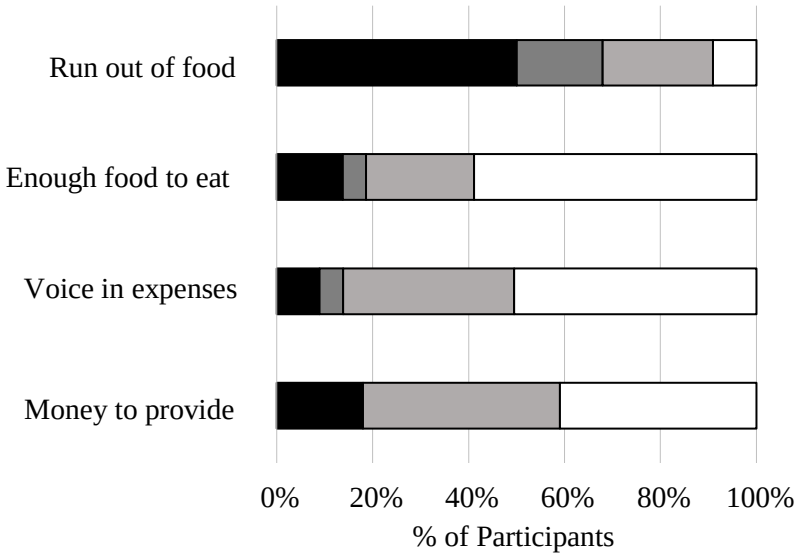


Figure 3-1. Chemawawin Cree Nation Location. Adapted from Google Maps.

a)



b)

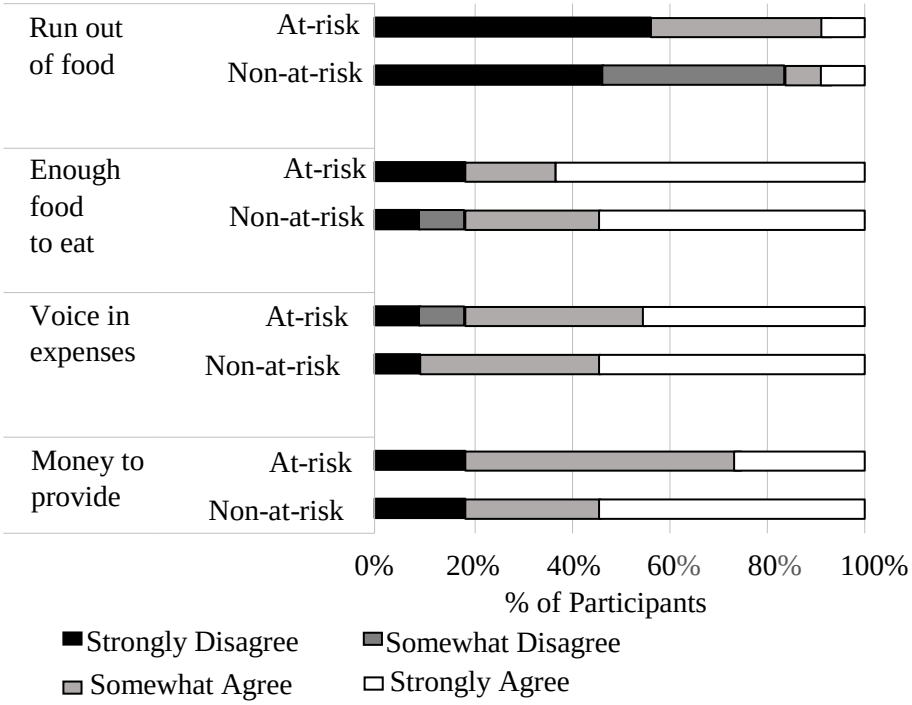
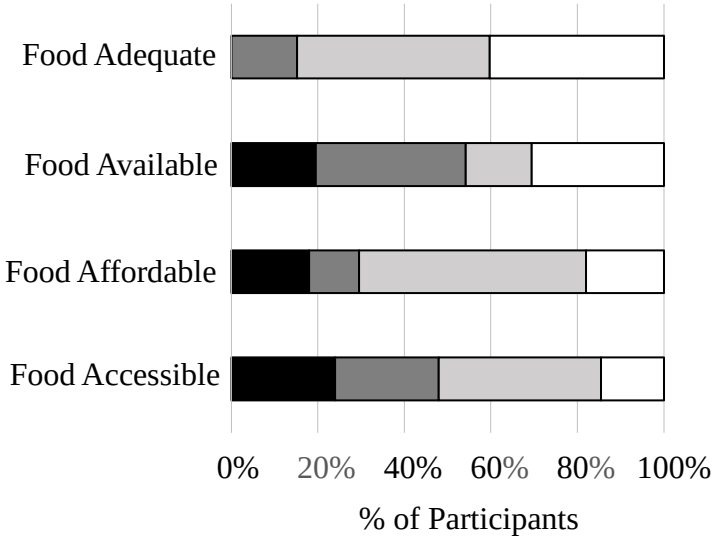


Figure 3-2. Self-reported access to financial resources a) all women (n=22); b) non-at-risk (n=11) and at-risk women (n=11). Data presented in proportion of participants. No significant difference was identified between non-at-risk and at-risk groups.

a)



b)

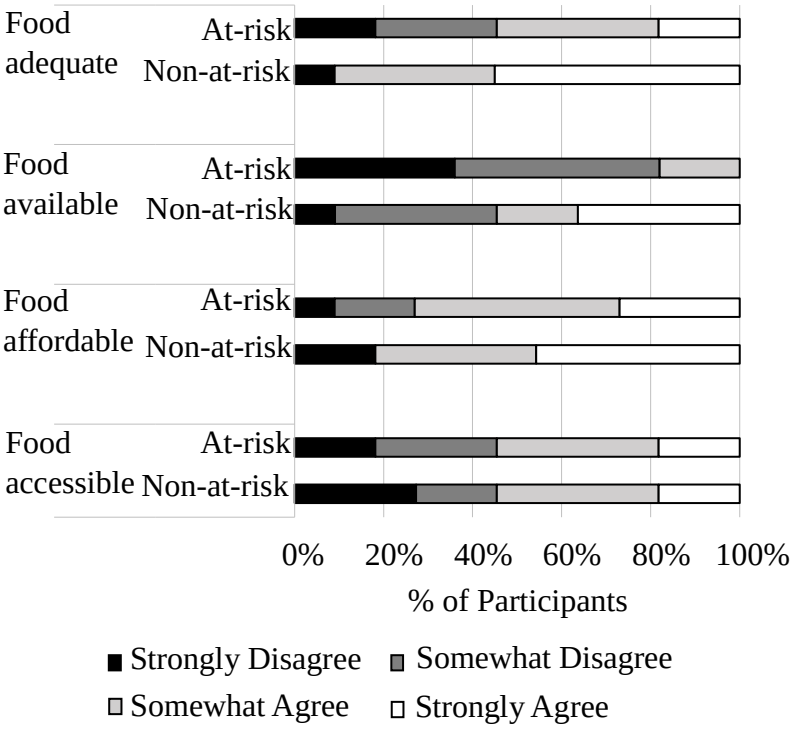


Figure 3-3. Self-reported access to food a) all women (n=22); b) non-at-risk (n=11) and at-risk women (n=11). Data presented in proportion of participants. No significant difference was identified between non-at-risk and at-risk groups.

CHAPTER IV: A PILOT STUDY ON THE ASSOCIATIONS BETWEEN MATERNAL NUTRITIONAL BIOMARKERS AND SELF-REPORTED ALCOHOL CONSUMPTION IN PREGNANT FIRST NATION WOMEN LIVING ON RESERVE

Abstract

Background: Fetal Alcohol Spectrum Disorder (FASD) is a spectrum of anomalies caused by prenatal alcohol consumption. Maternal nutrition has been postulated to be one of the most impactful FASD factors, however, the degree of interaction between various plasma nutritional indicators and alcohol consumption is unknown, especially in First Nations women living in remote locations. ***Objective:*** This study examined the relationship between maternal self-reported alcohol consumption and nutrition-related, inflammatory, and general health biomarkers in pregnant First Nations women. ***Methods:*** Twenty-two pregnant women, ages 14-39 participated in the study. The participants part-took in an in-person nutrition questionnaire and provided a sample of 15 mL of urine and 20 mL of blood. Plasma concentrations of metabolic parameters, fatty acids, cytokines, and mineral profiles were determined utilizing Cobas c 111 analyzer, gas chromatography instrument (Vista 6010 GLC), custom made Meso Scale Discovery (MSD) biomarker assays, and Thermo Finnigan Element 2 High Resolution-Inductively Coupled Plasma-Mass Spectrometry respectively. Linear regression models were applied to test the relationship between the risk, maternal health, nutrient intake and the studied biomarkers. ***Results:*** Self-reported alcohol consumption during pregnancy presented a significant positive relationship with plasma glucose (β= 0.446, $p<0.05$) with BMI and age as covariates in the model. After adjustments for trimester and BMI, alcohol consumption decreased erythrocyte C18:2n6 and C20:4n6 (β= -0.444, β= -0.419, respectively $p <0.05$). Self-reported drug use decreased plasma C20:4n6, C22:6n3, and total n-3 fatty acids (β= -0.428, β= -0.433, β= -0.474; respectively, $p<0.05$). Alcohol consumption also decreased plasma IL-10 (β= -0.444, $p<0.05$). Among the maternal health indicators, gravidity showed negative associations with erythrocyte C20:4n6 (β= -0.419, $p <0.01$) and positive associations with plasma and urine MCP-1 (β= 0.484, β= 0.487, respectively, $p <0.05$). ***Conclusion:*** Collectively, the results of this pilot study provide insight into the relationship between self-reported alcohol consumption and nutrition and inflammatory markers for First Nations pregnant women. Further research with a larger sample size is needed to solidify the current findings which will provide the basis of developing maternal nutrition strategies for preventing FASD.

Key words: Nutritional biomarkers, Risk exposures, Dietary intake, Alcohol consumption, FASD

Introduction

Maternal risk exposures, specifically alcohol consumption during pregnancy, impacts the regulation and homeostasis of fetal growth and neurodevelopment, resulting in a spectrum of anomalies termed fetal alcohol spectrum disorder (FASD) (CanFASD, 2013). Alcohol induces the restriction of placental blood flow, consequently leading to fetal hypoxia, reduced flow of nutrients (Schenker et al., 1990; Kennedy, 1984), and production of reactive oxygen species (ROS) (Nordmann et al., 1992; Koop, 2006). FASD is an umbrella term which encompasses all alcohol-related birth defects (Chudley et a., 2005). FASD is the most common cause of neurodevelopmental disability affecting children in the Western world, with a prevalence of 1-2% of the total population in Canada and 2-3% among school aged children (Popova et al., 2019).

Although alcohol consumption is the primary risk factor for FASD, it has been postulated that maternal nutrition is one of the most important modulators of the course and manifestation of FASD (Jones, 1973; Abel & Hannigan, 1995; Young et al., 2014; May & Gossage, 2011). Cohort and clinical studies reveal interactions between macro- and micro-nutrients and alcohol in maternal plasma and placental tissues (Miller et al., 1983; Ojeda et al., 2009; Moos et al., 2018; Summer et al., 2009; Carrey et al., 2003; Stark et al., 2005; Sowell et al., 2020). For example, ethanol has been found to impede the mobilization of folate at the placental level (Hutson et al., 2012), to reduce maternal fetal cord plasma zinc concentrations in dyads (Flynn and colleagues, 1981), and to alter docosahexaenoic acid (DHA) status in women at risk of carrying a child with FASD (Sowell and colleagues, 2020).

Pre-clinical studies using animal models demonstrate ethanol's action on various nutrients. Ethanol amplifies teratogenic effects in the absence of proper nutrition (Young et al., 2014), but conversely, the effect is reduced when nutrient supplementation is introduced (Ballard et al., 2012; Keen et al., 2010). These findings could be explained, in part, by the important role nutrients play in mitigating ROS-mediated cellular damage (Thoen et al., 2023), as well as their effects on growth factors synthesis, cellular mitosis and apoptosis (McMahan et al., 2020). These critical cellular functions are impaired in the presence of nutrient deficiencies, compounded by the presence of ethanol (Keen et al., 2010; Miller et al., 1995; Gloria et al., 1997).

The exact mechanisms of alcohol-nutrition interaction in the developing fetus has not yet been comprehensively elucidated. It is known that alcohol consumption increases lipid peroxidation of polyunsaturated fatty acids (PUFA), impacts glycemic control, reduces duodenal uptake of micro-elements such as minerals, electrolytes, and co-enzymes, induces liver damage, obstructs the production of hormones that regulate nutrient absorption and metabolism, increases pro-inflammatory signaling and generally dysregulates nutrient metabolism (Young et al., 2014; Saha & Mayhan, 2022). Specifically, a number of investigations have observed alterations in lipids, lipoproteins, and mineral status during prenatal ethanol consumption (Keen et al., 2010; Sowell et al., 2020; Stark et al., 2005; Valimaki et al., 1990). A study done by Stark and colleagues (2005) detected low concentrations of DHA in plasma and erythrocytes of pregnant black women who consumed alcohol during the second trimester. Similarly, a more recent study observed differences in the status of stearic acid (C18:0), eicosenoic acid (C20:1n9), arachidonic acid (AA) (C20:4n6), and docosapentaenoic acid (DPA) (C22:5n-6) between ethanol exposed and non-ethanol exposed women during pregnancy (Sowell et a., 2020). An earlier study identified disturbances in normal gestational increases of low-density lipoprotein (LDL) cholesterol, LDL-phospholipids, and proteins when alcohol was consumed during the third trimester (Valimaki et al., 1990). Reductions in maternal-fetal zinc, iron, and selenium levels during prenatal alcohol exposure (PAE) have been reported by many earlier studies (Miller et al.,1983; Ruth & Goldsmith,1981; Flynn et al., 1981; Halmesmäki et al., 1986; and Keen et al., 2010). In contrast, prenatal mineral supplementation restored concentrations of minerals in maternal-fetal plasma, attenuating the damaging effects of alcohol (Ojeda et al., 2009; Moos et al., 2018; Summers et al., 2009).

PAE has also been implicated in the alteration of adaptive immunity and inflammatory cellular responses. Preclinical and clinical studies have demonstrated that ethanol is a direct modulator of inflammatory mediators, specifically cytokines, small signaling peptides that act as biological messengers in the immune system (Ahluwalia et al., 2000; Adams et al., 2020). Homeostatic immune responses are critical for a healthy pregnancy. Throughout pregnancy cytokines assist in mediation of crucial processes such as maternal-fetal immune communication (Interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, IL-15, IL-17A, IL-1β) (Yockey & Iwasaki, 2018; Vilotic et al., 2022), implantation (IL-2, IL-6, IL-10, IL-15, tumor necrosis factor-TNF-α (TNF- α)) Mor et al., 2011; Lin et al., 1993), placental remodeling (IL-15, IL-10, (Ashkar et al., 2003; Liu et al., 2017; Roth

et al., 1996), and parturition (IL-8, IL-1β) (El Maradny et al., 1996; Tribe et al., 2003), and others. Therefore, disturbances in cytokine balance can be damaging to both mother and fetus, contributing to pregnancy complications and congenital disorders (Yockey & Iwasaki, 2018). Since both ethanol and diet can influence cytokine levels, a greater understanding of the interplay between alcohol consumption during pregnancy, nutritional biomarkers, and cytokine profiles is expected to shed light on how these factors jointly contribute to disease manifestation.

Furthermore, there is a paucity of information on concomitant and singular additional risk exposures such as cigarette smoking and/or drug use during pregnancy, which have confirmed negative nutritional consequences (Al-Sahab et al., 2010; Sowel et al., 2020). These data are even more limited for First Nations women residing on reserves, as there is lack of clinical infrastructure, services, and nutrition research while having high incidence of FASD. For this reason, we aim to conduct an exploratory pilot study to examine whether there is a relationship between self-reported alcohol consumption during pregnancy and nutritional biomarkers (plasma metabolic parameters, plasma and erythrocytes FAs, and inflammatory markers and plasma minerals). Additionally, the study explores the relationship between other risk exposures (smoking, drug use) and the afore-stated biomarkers. The results of this study may have important implications for greater-scale clinical research, public health programming, and nutrition intervention at the individual and population levels.

Methodology and Design

The process of community engagement, study settings, participants, consent, dietary research instrument development, anthropometric data collection, risk assessment, macro- and micronutrient intake evaluation are described in detail in *Chapter 3*. Band and Council approval from the First Nation Community and The University of Manitoba human ethics approval are also stated in the previous *Chapter 3*.

Biological sample collection

Every woman participating in the verbal data collection was invited to partake in providing biological samples. Over night fasted blood and urine were collected in the morning (8:00–10:00 a.m.) during participants' preceding visits with the maternal nurse, after they fasted for 8-12 hours. Plasma and erythrocytes were collected by centrifugation at 1500 g for 10 min at 4°C,

then aliquoted before storing in a -20° C freezer. The samples were then transported in the portable dry freezer packed with dry ice to Dr. Suh's laboratory located at Albrechtsen Research Centre, St. Boniface Hospital, Winnipeg. Upon arrival, the blood samples were kept in a -80° C freezer and urine was kept in a -20° C freezer until analysis.

Biomarker selection

The biomarker selection relied on two extensive literature reviews previously published (Young et al., 2014; Kloss et al., 2022); as well as on the on-going research on ethanol-lipid interactions conducted in our laboratory (Feltham et al., 2019; 2020). Cytokine selection was based on literature which indicated a relationship between alcohol and pregnancy (Bodnar et al., 2019, Sowell et al., 2018; Ahluwalia et al., 2000).

The biomarkers were selected as follows:

- General health and nutritional biochemistry markers:
  - Glucose, total cholesterol (TC), high density lipoprotein (HDL), LDL, triglycerides,
  - liver enzymes: ALT (alanine transaminase), AST (aspartate aminotransferase), and gamma-glutamyl transferase (GGT), and
  - C-reactive protein-high sensitivity (CRP-hs).
- Plasma and erythrocyte FA profiles:
  - Saturated (SFA): C14:0, C16:0, C18:0, C20:0 C22:0, C24:0;
  - Monounsaturated (MUFA): C16:1, C18:1n-7, C18:1n-9, C20:1, C22:1, C24:1;
  - n-6 polyunsaturated (n-6 PUFA): C18:2, 18:3, 20:2, 20:3, 20:4, 22:4;
  - n-3 polyunsaturated (n-3 PUFA): C18:3, C20:3, C20:5, C22:5, C22:6.
- Cytokines:
  - Interleukins: IL-2, IL-6, IL-8, IL-10, IL-15, IL-17A, IL-1β
  - TNF- α
  - Monocyte chemoattractant protein-1 (MCP-1)
- Trace minerals: chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se).

Plasma biochemistry and metabolic parameters analysis

Maternal plasma biochemistry and metabolic parameters, glucose, TC, HDL-C, LDL-C, TAG, the liver function enzymes, ALT, AST, GGT; and hs-CRP, were measured using Cobas c 111 analyzer (Roche Diagnostics, Indianapolis, IN, USA) available at the Alberchtsen Research Centre.

Fatty acid analysis

Erythrocyte membrane FAs are known to be long-term makers of FA status in vivo, due to is life span of an average of 120 days in blood circulation (Klei et al., 2019). Thus, both maternal plasma, a short-term marker, and erythrocyte FAs were analyzed, employing a direct saponification and methylation method, as was established in our lab, which was based on the modified method described in Kang and Wang (2005). 50ul of each plasma and erythrocyte, containing 5 μg of C23:0 standard, were saponified with 0.5 M methanolic KOH at 110° C for 1 hour, followed by methylation with the 14% w/w boron trifluoride in methanol (Sigma-Aldrich, St. Louis, MO) at 110° C for 1 hour. The FA methyl esters in the upper hexane phase were separated on gas chromatography (a Vista 6010 GLC and Vista 402 data system, Varian 58 Instruments, Mississauga, ON, Canada), equipped with a BPX70 micro-column (10m x 0.1 mm diameter, SGE Analytical Science, Carrboro, NC, USA). The detailed running condition was 130° C for 0 min, raised to 175° C at 20° C/min held for 1 min, 200° C at 6 min held for 0 min, and raised to 280° C at 30° C /min. Carrier gas and hydrogen at a flow rate of 0.5 ml/min. FA identification was based on standard FAME 461 *Nucheck Prep Inc., Waterville, Mann, USA).

Inflammatory marker analysis

Maternal inflammatory status was measured in maternal plasma and urine. Cytokine selection was based on its role in the modulation of the maternal immune system and the maintenance of a healthy pregnancy. The quantification of cytokines was performed through the electrochemiluminescence detection using the Mesoscale Discovery (MSD) V-PLEX Human Cytokine kit (MSD, Montreal, QC, Canada), according to manufacturer protocol. Briefly, plasma samples were thawed at room temperature and diluted twice, as per indication. After dilution, samples were incubated on the precoated multi-spot V-Plex plates for 2 hours at room temperature. Following the incubation, plates were washed and incubated again. After a second incubation period, a buffer was added to each well and the plates were analyzed in a Sector

Imager. Cytokine concentration was determined through standard curves for each analyte with a coefficient of variation <25% and an accuracy of 80%–120%. Samples were processed in duplicates.

Trace mineral analysis

The quantification of plasma trace minerals was performed using Thermo Finnigan Element 2 High Resolution-Inductively Coupled Plasma-Mass Spectrometry (HR-ICP-MS), which was completed in collaboration with Dr. Feiyue Wang (the Department of Geological Sciences, University of Manitoba). 0.5 mL of thawed plasma was dissolved in 5 mL of 2% nitric acid (HNO_3). The sample digestion was based on Harrington et al.'s method (2014), which used the CEM microwave digestion of bovine blood. Elemental analysis was performed HR-ICP-MS according to the ICP protocol. Limits of blank, limits of detection, and limits of quantification were calculated through standard calibration curves. Samples were processed in duplicates.

Elements vanadium (V), cobalt (Co), nickel (Ni), arsenic (As), barium (Ba), which are not defined as dietary, are available in **Appendix 3**.

Statistical analysis

The statistical methodology employed for this chapter is largely similar to the one described in *Chapter 3*. Briefly, the differences between the risk groups for continuous clinical and biochemical variables were assessed using independent student t-test for normally distributed and Wilcoxon rank-sum test for not non-normally distributed data. All data was assessed for normality using the Kolmogorov-Smirnov and Wilks-Shapiro tests. GGT, plasma IL-2, IL-17, and urine IL-2, IL-6, IL-8, IL-10, TNF -α were not normally distributed.

To test the associations between risk exposures and all biomarkers studied, including health and dietary variables, a series of multiple linear regression models was applied. All models were adjusted for trimester and BMI. The selection of the covariates for the models was based on substantive literature and the pre-testing of the associations with the bivariate (Spearman and Pearson's, where appropriate) correlations. The models for general nutritional metabolic markers included age and trimester as covariates. All remaining models included BMI and trimester as covariates. To test the assumption of residual normality, Q-Q plots were assessed. The residual testing indicated normal or nearly normal distribution for residuals from these regression models. The independent variable and covariates in all models with 3 covariates were tested for

multicollinearity through the variance inflation factor (VIF). VIF under 2 was determined as the cut off. No multicollinearity problem was identified.

This data set had no missing values. Statistical significance was set at p-value <0.05. All analyses were conducted using IBM SPSS Statistic Version 26.

The computation of the sample size with various effect sizes and three predictor variables in the regression model is described in detail in *Chapter 3*. The measurement assessment and the selection of the dependent variables is also described in detail in *Chapter 3, Methodology and Design* section.

Results

Plasma metabolic markers

Maternal plasma markers (glucose and lipid profiles) and liver function enzymes were assessed in all participants and compared between non-at-risk and at-risk groups (**Table 4-1)**. While the majority of the maternal biochemical variables were within the Medical Council of Canada reference ranges, triglycerides and CRP-hs concentration were above the references in this cohort. No differences in any markers were noted between non-at-risk and at-risk groups.

Correlations were tested to determine whether these metabolic markers were affected by the risk variables (alcohol consumption, smoking and drug usage), maternal health and dietary intake (**Table 4-2)** after adjusting age and trimester as covariates. A significant positive relationship was identified between the glucose and alcohol ($\beta = 0.446$, $p<0.05$). Marginal significance was noted between smoking and LDL cholesterol ($\beta = 0.532$, $p<0.1$). No other risk variables were associated with any of the other metabolic biomarkers. In relation to nutrient intake, HDL-cholesterol showed positive association with vitamin C intake ($\beta = 0.493$, $p <0.05$). Significant negative association was detected between AST and vitamin C intake ($\beta = -0.449$, $p <0.05$). Marginal significance ($p<0.1$) was detected for between LDL cholesterol and BMI, LDL cholesterol and vitamin C intake, and total cholesterol and protein intake ($\beta = 0.441$, $\beta = 0.277$, $\beta = 0.448$, respectively, $p<0.1$). Marginal significance was also detected for triglycerides and vitamin C, and CRP-hs and vitamin C intake ($\beta = -0.417$, $\beta = 0.467$, respectively $p<0.1$).

Fatty acid profiles in plasma and erythrocytes

Circulating plasma and erythrocyte FA profiles are presented for the whole participants and the risk groups in **Table 4-3**. The major plasma FAs were C16:0, C18:1n9, and C18:2n6, contributing ~75% (w/w, total FAs) to all FAs. No significant differences were observed for most of the plasma FAs, with the exception of C14:0, C18:3n3, and total n-3 PUFA, being significantly higher ($p < 0.05$) in the at-risk group compared to the non-at-risk group. Similarly, the major erythrocyte FAs were C16:0, C18:0, and C18:1n9, followed by n-6 PUFA, C20:4n6, and C18:2n6. No significant differences were identified between the at-risk and non-at-risk groups in erythrocyte FAs.

Correlations were tested to inquire whether plasma FAs are affected by the risk variables (alcohol consumption, smoking and drug usage), maternal health and dietary intake (**Table 4-4**) after adjusting for BMI and trimester. Alcohol did not affect the FA profiles, but self-reported drug use exhibited significant negative association with the level of PUFA for C20:4n6, C22:6n3, and total n-3 FAs ($\beta = -0.428$, $\beta = -0.433$, $\beta = -0.474$; respectively, $p<0.05$). Out of the dietary variables, Milk and Alternatives intake was shown to have a negative association with total n-3 PUFA ($\beta = -0.527$, $p <0.05$). A trend toward significance was observed between drug use and total monounsaturated FA level ($\beta = -0.410$, $p<0.1$). Marginal significance was also noted for the C20:4n6, C22:6n3, and total MUFAs and number of servings of Milk and Alternatives ($\beta = -0.480$; $\beta = -0.480$, $\beta=-0.465$, respectively, $p<0.1$).

In addition to plasma, erythrocyte FA and various maternal variables were examined with the same adjustors of BMI and trimester (**Table 4-5**). Self-reported alcohol consumption reduced erythrocyte C18:2n6 and C20:4n6 level ($\beta = -0.444$, $\beta = -0.419$, respectively $p <0.05$). Marginally significant negative association was also observed for alcohol consumption on C22:6n3 ($p<0.1$). Number of pregnancies showed a negative association with C20:4n6 ($\beta = -0.419$, $p <0.01$). Total caloric intake was negatively associated with erythrocyte C20:4n6 and C22:6n3 FAs ($\beta = -0.391$, $\beta = -0.401$, respectively, $p <0.05$). Fat intake affected reduction in erythrocyte C18:3n3 and C22:6n3 (g/d) ($\beta = -0.466$, $\beta = -0.405$, respectively, $p<0.05$).

Inflammatory markers

Maternal plasma and urine cytokine concentrations are presented for all participants and the risk groups in **Table 4-6**. Examining if the observed values are within a normal range was not

possible due to a lack of reference values available for plasma or urine in the literature and any clinical guidelines to date. When compared to the urine, plasma concentrations of IL-1β, IL-2, IL-6, IL-8, IL-10, IL-15, IL-17A, and TNF-α were lower, and the concentration of MCP-1 was higher. Significant differences between the two risk groups were not detected for any of the cytokines studied in plasma and urine.

Correlation was tested to ascertain whether cytokine concentrations are affected by the risk variables (alcohol consumption, smoking and drug usage), maternal health and dietary intake (**Table 4-7; 4-8**) after adjusting for BMI and trimester as covariates. Self-reported alcohol consumption affected negatively on plasma IL-10 (β= -0.444, $p<0.05$). The total number of pregnancies and chronic illness during pregnancy increased plasma MCP-1 concentration in the plasma (β= 0.484, β= 0.568, respectively, $p<0.05$). Trends toward significance were noted for the plasma IL-15, MCP-1 and the number of cigarettes/day (β= 0.394, β= 0.457, respectively) ($p<0.1$). With respect to the dietary intake variables, marginal significance was detected between IL-6 and dietary fat (g/day) (β= 0.296, $p<0.1$) and IL-2, IL-6 and protein (β= 0.561, β= 0.482, respectively) ($p<0.1$).

Similarly to plasma observations, positive associations were identified between MCP-1 and the number of pregnancies, and MCP-1 and the diagnosis of chronic illness in urine (β= 0.487, β= 0.569, respectively) ($p <0.05$) **(Table 4-8)**. Marginal significance ($p<0.1$) were observed between MCP-1 and smoking (β = 0.457), IL-8, IL-10 and chronic illness during pregnancy (β= 0.386 and β = 0.414, respectively). Finally, a negative relationship trending toward significance was noted between IL-15 and the number of pregnancies (β= -0.346, $p<0.1$).

Minerals

Maternal plasma trace mineral concentrations were measured for the whole participants and the risk groups **(Table 4-9)**. No differences were detected in mineral concentrations between non-at-risk and at-risk groups. While the results of multiple linear regression modelling revealed no significant relationship between mineral status and the variables stated above, trends toward significance ($p<0.1$) were noted for copper and cigarette smoking (β= -0.443), zinc and drug exposure (β= 0.529), and manganese and the number of pregnancies and (β= 0.396).

Discussion

The present pilot study explored the relationship between maternal nutritional and inflammatory biomarkers and self-reported risk exposures, especially alcohol consumption, of pregnant First Nations women, living in Manitoban remote location. In this study self-reported alcohol consumption was associated with plasma glucose, erythrocyte PUFA, C18:2n6 and C20:4n6, and plasma IL-10. Self-reported risk exposures, such drug use, was associated with alterations in plasma PUFA, C20:4n6, C22:6n3, and total n-3 FAs. Additionally, the maternal health indicators also showed associations with erythrocyte C20:4n6 with plasma and urine MCP-1. Although the study had small sample size, these preliminary findings contribute to the overall body of clinical research and provide information for community health programming.

Plasma metabolic markers

This study found that our participants had higher triglycerides and CRP-hs than the upper range of the reference values. Fasting glucose and total cholesterol were also marginally elevated. This data aligns with the maternal health status results displayed in *Chapter 3,* which demonstrated high prevalence of chronic conditions in this cohort (32%). Elevated levels of triglycerides and cholesterols is considered to be a normal physiological response to elevated levels of estrogen and reduced insulin sensitivity during gestation to assist with proper energy and nutrient supply to the developing fetus (Chiang et al., 1995). Although elevated maternal lipids are normal during gestation, it is possible that observed elevation in triglycerides may be associated with the diagnosis of type 2 diabetes and GDM in this study cohort (*Chapter 3*). This observation is especially plausible in-light of substantially elevated CRP-hs, a marker which is sensitive to disruptions in the homeostasis of glucose control (Kumari & Singh, 2017; Babu & Joshi, 2017). Therefore, the biochemical values observed among the participants are consistent with the information available for chronic illness during gestation.

Maternal risk variables such as alcohol, smoking, and drugs were largely unassociated with alterations in maternal basic metabolites, with the exception of glucose. This is consistent with the literature on glucose homeostasis and alcohol consumption (Leggio et al., 2009; Athyros et al., 2007). Interestingly, not only glucose levels, but various modulators involved in glucose homeostasis (ghrelin, leptin, thyroid hormones) are also linked to pattern of alcohol consumption (Addolorato et al., 2006; Hillemacher et al., 2007, Leggio et al., 2009). These observations carry

special implications for expecting women and women planning to conceive as fetal growth relies on the maternal energy status. Further investigation of the maternal adjustment of nutrient metabolism and risk exposures is required.

Both plasma HDL-C levels were closely associated with vitamin C intake. Although there is paucity of these data for pregnant women, these results are consistent with published studies for general population (McRae, 2008). More published clinical work in pregnant population and population exposed to various risk factors is required. Consideration should also be given to incorporating vitamin C metabolites in clinical investigations on the topic, to confirm the relationship between the status of the nutrient and cholesterol fraction. Provided that vitamin C is an antioxidant and alcohol upregulates a series of reactions responsible for the ROS production and lipid peroxidation, investigating the relationship between the vitamin C intake, active forms of plasma vitamin C (L-ascorbic acid) and lipoprotein interactions may have merit for FASD implications.

Fatty acids

While there are a number of scientific reports identifying FA status during gestation, there is lack of understanding and scientific consensus for the normal reference values of plasma and erythrocyte FAs concentration. To our knowledge, this is the first report that contributed information on the status and associations between risk exposures and maternal FAs profiles in both plasma and erythrocytes for the First Nations maternal population. The plasma FA levels are similar to those reported in Sowel and colleagues (2020), a study conducted in Ukrainian women exposed to various levels of alcohol during gestation. In our cohort of First Nations women, myristic acid (C14:0), α-linolenic acid **(**LNA**,** C18:3n3) and total n-3 PUFA were significantly higher in at-risk group compared to the non-at-risk group. Similar findings were identified in a small African American study (Beblo et al., 2005). The authors reported a significant elevation in n-3 PUFAs and DHA concentration in umbilical cords of women exposed to moderate and high alcohol levels during gestation (10% and 14% respectively), compared to women not exposed to alcohol. Mechanisms are unknown of how alcohol increases n-3 FA. DHA is known to be semi-essential FAs for fetal brain development and decreased in fetal brain membrane with alcohol (Feltham et al., 2020), implying there is gap in transportation of n-3 FA from maternal to fetus.

While no significant associations were noted for alcohol and the plasma FAs, significant findings were observed between erythrocyte, a long-term indicator of dietary FA status. Alcohol was negatively linked with the erythrocyte linoleic acid (LA, C18:2n6) and AA, the model maintained its significance after trimester and BMI adjustments. Comparable FA alterations were also noted by Sowell and colleagues (2020). The authors detected a significant negative Spearman rank-order correlation coefficient between ethanol intake and percent plasma concentration for LA ($r = -0.373$, $p<0.05$), AA ($r = -0.410$, $p<0.05$). However, the authors have not studied erythrocyte FAs profile, which poses a challenge in meaningful comparison and interpretation due to mechanistic differences. The LA is an essential FA supplied through the diet. Sequentially, the AA is derived from LA through a series of desaturation and elongation steps. Therefore, the congruency in findings for both n-6 FAs may be pointing toward a reduced long-term dietary intake of n-6 rich foods. While the present study conducted an analysis of fat intake, we did not analyze the dietary contribution of individual fatty acids such as LA and AA, which could have been of help for finding direct relations with plasma and erythrocyte FA. Furthermore, the larger body of literature focuses on the ethanolic influences on the n-3 FAs (Beblo et al., 2005; Feltham et al., 2018; Innis et al., 2008; Huffman et al 2011), due their well-defined role in CNS development. Provided that all PUFAs are powerful endogenous signaling molecules, delineation of mechanisms responsible for alterations in all lipids, including n-6 and n-3 FA, in the presence of ethanol is critical. This may provide insights into the pathogenesis, novel biomarkers, and therapeutic targets for the mitigation of damage during risk exposure and FASD.

Changes in plasma lipid concentrations were also detected for AA, DHA, and total n-3 FAs and self-reported drug use, which was marijuana. Interestingly, we did not observe similar trends for these FAs in the erythrocyte, which points toward a short-term presence of the alteration. This finding is not surprising, as cannabinoids have been shown to be potent metabolic modulators which have been implicated in FA regulation (Rossi et al., 2016). While cannabinoid receptors (CB1) and (CB2) are most abundant in the CNS, both types of receptors have been detected in the liver and cannabis have been shown to have a direct influence on hepatic lipid alterations in synthesis and metabolism (Trebicka et al., 2011). Another possible explanation for the alterations in FA profiles with cannabis use is the involvement of cannabinoids in the homeostatic hunger mechanisms. CB1 and CB2 are expressed in high levels in hunger control centers such as limbic

system (Berrendero et al., 1999) hypothalamus (Edwards et al., 2006) and hindbrain (Miller et al., 2004). The data on the precise influences of cannabis on FA profile in human populations are scarce, especially in maternal population.

Number of pregnancies or gravida is a maternal health indicator which has not been widely studied in relation to dietary intake and status. This is the first attempt to study the relationship between gravida and maternal dietary intake and nutrient biomarkers. We observed a significant negative correlation between AA levels and gravida. While this information is limited, a recent longitudinal study of 479 pregnant women found a significant decrease in AA during gestation (Aparicio, 2021). Therefore, it is reasonable to deduce that lower levels of plasma AA might occur with each subsequent pregnancy. More research is needed to validate this hypothesis.

Inflammatory markers

A number of studies demonstrated that alcohol affects adaptive and innate immune system, elevating the concentrations of numerous plasma immune factors and pro-inflammatory signaling. Specifically, animal studies revealed that acute and chronic high blood alcohol concentration leads to an upregulation of IL-1β, TNF-α, IL-6 mRNA in plasma and various tissues (Bjørkhaug et al., 2020; Achur et al., 2010). These findings have been confirmed in pregnant women. Ahluwalia and colleagues, (2000) found that chronic alcohol use (more than 60 drinks per month) resulted in elevated plasma levels of IL-1β, TNF-α, IL-6. Elevated levels of the same cytokines were also observed in the cord blood upon delivery (Ahluwalia et al., 2000). While the present study did not observe any statistically significant relationships between self-reported alcohol consumption and IL-1β, TNF-α, IL-6, a negative relationship has been identified between alcohol exposure and IL-10. This is an interesting finding, as elevation of IL-10, an anti-inflammatory interleukin, has been observed in binging patterns of alcohol consumption in human subjects (Ashfar et al., 2015).

The investigation of systemic and central cytokine signaling in the context of FASD has received little attention. A limited number of reports identified that alcohol-affected children have increased risk for various infections, such as bacterial pneumonia, meningitis, otitis media (Johnson et a., 1981; Popova et al., 2016), and associated complications – sepsis (Gauthier et al., 2004, 2005). This suggests disturbances in humoral immunity and cell-mediated immunity, which may be a result of an in-utero, ethanol-induced programming (Bodnar et al., 2020). These

critical clinical research indications point toward the need of a large-scale clinical trial longitudinal, and cohort studies investigating the relationship between PAE and immune-related alterations in exposed dyads. Although the current study did not find significant alterations in immune factors in women who consumed alcohol, these findings may not be due to a direct lack of associations. As noted in *Chapter 3*, this cohort has high proportion of women with diagnosis of chronic illness, miscarriage, and other adverse health outcomes (high BMI, stillbirth, etc). All these conditions produce inflammatory states, which might be impacting the data hygiene for the association analysis. Greater sample size which would allow for the incorporation of more predictors in the multiple linear regression model may resolve this limitation.

The findings of our study identified significant positive association between the diagnosis of chronic illness and plasma and urine MCP-1. MCP-1 is a major promoter of inflammation, renal damage, and nephropathy associated with diabetes (Dragomir & Simionescu, 2006). Trends toward significance ($p<0.1$) were also observed between IL-8, a chemoattractant cytokine which activates neutrophils in inflammatory regions (Bickel, 1993). Collectively, these findings are especially concerning in light of self-reported risk exposures as these metabolic disturbances might be further aggravated.

Minerals

Maternal microelement status has been identified to play a role in FASD-associated biological mechanisms (Carey et al., 2003; Miller et al., 1983; Moghimi et al., 2017). A number of preclinical and clinical studies have demonstrated negative compounding effects of iron, zinc, selenium, and copper deficiencies and ethanol exposure on the development of a fetus (Flynn et al., 1981; Keen et al., 2010; Miller et al., 1995; Rufer et al., 2012; Halmesmäki et al., 1986; Ojeda et a., 2009). Although marginal significance ($p<0.1$) were observed for copper and smoking and number of pregnancies and manganese, this study found no significant associations between maternal plasma mineral status and risk or health variables. Warranted small sample pilot, lack of follow-up observations, the use of novel and not well-tested methodology in pregnant human subjects (Ivanenko et al., 2013), and inconsistencies in self-reported data on alcohol exposure contribute to power reduction and thus lack of weighty conclusion. Mechanistically, microelement deficiency exacerbated by alcohol consumption leads to an impaired oxidative defense system, reduced cell differentiation and proliferation consequently

leading to impediments in protein synthesis and impaired hormone production (Keen et al., 2010; Chrisman et al., 2004; Huebner et al., 2016). These unfavorable events result in various system malfunctions, especially CNS, therefore large-scale cohort studies are needed to establish clear relationship between risk exposures and mineral status.

An important point to consider is the use of relatively novel methodology HR-ICP-MS for the assessment of women's trace mineral status. Although the performance of this methodology has been well documented for in the geological and environmental field, more validation studies are required for the biological use, especially in pregnant population (Bolann et al., 2007; Choi et al., 2016). Limited number of studies utilized this methodology to examine plasma, blood, or urine trace mineral status of pregnant women (Choi et al., 2016). Further investigations are required to test this methodology for the biological laboratory use in various tissues.

Dietary Sub-analysis

In addition to the relationship between maternal risks and health variables, the present study attempted to conduct a sub-analysis on the relationship between relevant dietary intake variables and the plasma and urine levels of nutritional biomarkers. The identification of the relationship between self-reported dietary intake and biochemical measure (ideally recovery biomarker or a biomarker of long-term nutrient status) (Mossavar-Rahmani et al., 2015) is suggested to be a golden standard for methodological validation of various dietary research instruments (National Cancer Institute, 2022; Mossavar-Rahmani et al., 2015). Significant or marginally significant dietary correlate for plasma AA, DHA, total MUFA and total n-3 FAs was Milk and Alternatives food group intake (servings/day), which was conducted through 24-hour dietary recall. These results were unanticipated for the FAs and Milk and Alternatives food group, as the main n-6 FA in dairy products is LA, which greatly contributes to the synthesis of C20:4n-6.

Total energy intake (computed through 24-hour dietary recall) was identified as a negative predictor for the erythrocyte AA, DHA, and marginal negative predictor for ALA. This is an interesting and unanticipated finding. There is a lack of comparative studies investigating the relationship total energy intake and erythrocyte FAs, therefore comparisons are difficult to make. While correlates of plasma and erythrocyte FAs were identified, the results are inconclusive due to a multitude of limitations. This warrants further investigation with larger sample size and more biochemical parameters included.

Strengths

The present study has a number of strengths. To our knowledge this is the first report focusing on the identification of the relationship of maternal risks with biological nutrient and immuneand inflammatory markers and alcohol consumption, as well as other risk variables for First Nations maternal population. Although the sample size is low, the researcher has collected data on all of the pregnant women living in the same locality during the duration of the project. This nuance allowed for better estimations of the relationships between the afore-stated indicators. Another strength is the collection of detailed information on maternal dietary intakes which were correlated with biochemical nutritional and inflammatory biomarkers.

An additional study strength is the collaboration with the nursing station and access to participants medical files. This was a considerable strength, as the self-reports of chronic illness, pre-pregnancy BMI, maternal health indicators (number of pregnancies, number of previous births, miscarriages, etc), and risk-exposure history for some participants were clinically confirmed.

Methodological strengths include the use of direct saponification and methylation, which has been well established in our laboratory throughout the year, rendering better and more precise results. The use of custom-designed V-PLEX Human Cytokine kits allowed for increase throughput, higher volume efficiency, and a high number of data points. This methodology has been widely employed in the identification of inflammatory status, therefore method testing data is available (Bastarache et al., 2014).

Limitations

This cross-sectional pilot study has a number of limitations, which are recommended to be addressed through the future research. First, the small sample size has impacted the results, analysis, and various forms of sub-analysis in this study. As indicated by the power analysis, this study is underpowered, which leads to difficulty with the generalization and interpretation of results. A larger sample size would allow for a better powered study, proper control of confounding variables such as age and chronic illness and others, as all of these factors influence the circulating levels of the studied plasma and urine markers.

Another limitation is a lack of confirmation of ethanol exposure during the studied pregnancy. All maternal substance use variables are based on self-reports, therefore the control for frequency, timing, duration could not be properly quantified, assessed, and compared. Provided that the study is aiming to set the foundation for FASD prevention research with nutrition strategies, this limitation is consequential for the verification of the study findings. As identified in the *Limitations* section of *Chapter 3*, the confirmation of consumption and the pattern of consumption is the limitation affecting the FASD research and medical field (Cook et al., 2016; Stevens et al., 2020). Therefore, the next steps of the research team include identifying ways to safely and respectfully collect information on dietary intake and nutritional biomarkers with the clinical confirmation of prenatal alcohol exposure. However, it may not be feasible for the studied populations.

Blood collection throughout the three trimesters was also a limitation for this study. Changes and adaptations in maternal biochemistry in response to physiological changes and fetal demands are normal throughout trimesters (Mor et al., 2007). Therefore, blood samples from various trimesters may show fluctuating concentrations of various markers. This limitation is specific to FAs, cytokines and mineral measurements, as methodological validations and reference values exist for other biochemical parameters studied. The limitations specific to verbal data collection on dietary variables included in this chapter are described in detail in *Chapter 3*.

Conclusion

Identification of alcohol exposure during pregnancy related with biological markers, dietary and maternal variables was the primary objective of this study. While small sample precluded some forms of analysis, significant associations between a number of plasma and erythrocyte FAs, and cytokine levels were detected with the self-reported risk exposures and maternal health factors. As a future perspective, all findings from this study should be integrated into further maternal research in Indigenous communities. Additionally, the information from this study, specifically, increased levels of maternal metabolic markers (glucose and lipids) should be an immediate focus of maternal programming in the community. The findings should also be a priority for provincial maternal programming to attenuate the rates of adverse maternal and infant outcomes associated with the findings.

Table 4-1. Fasting glucose, lipid panel, and liver enzymes for the sample and by self-reported alcohol consumption

Biomarker	Reference values[†]	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value
Glucose (mmol/L)	4 - 7	6.2 ± 2.4	5.9 ± 2.6	6.6 ± 2.3	0.504
HDL-C (mmol/L)	above 1.3	1.5 ± 0.4	1.4 ± 0.4	1.5 ± 0.5	0.744
LDL-C (mmol/L)	below 3.5	2.5 ± 0.7	2.6 ± 0.8	2.4 ± 0.8	0.713
TC (mmol/L)	below 5.2	5.0 ± 1.1	5.1 ± 1.2	5.0 ± 1.0	0.790
Triglycerides (mmol/L)	below 1.7	2.2 ± 1.0	2.2 ± 1.1	2.1 ± 1.0	0.952
ALT (units/L)	2-33	10.7 ± 3.2	9.5 ± 2.3	11.9 ± 3.6	0.873
AST (units/L)	3-33	16.1 ± 3.7	16.0 ± 4.0	16.1 ± 3.5	0.943
GGT (units/L)	2-26	13.7 ± 13.0	14.7 ± 16.1	12.7 ± 9.7	0.738
CRP-hs (mg/L)	below 5	6.1 ± 4.8	7.4 ± 5.4	4.9 ± 3.9	0.529

Values are expressed as means ± SD. The significant differences between the risk groups were tested by an Independent t-test for normally distributed data and Wilcoxon rank-sum test for not non-normally distributed data. [†]Reference values are from Medical Council of Canada. HDL-High density lipoprotein; LDL-low density lipoprotein; TC-total cholesterol; ALT-alanine transaminase; AST-aspartate aminotransferase; GGT-gamma-glutamyl transferase; -CRP-hs-C-reactive protein-high sensitivity.

Table 4-2. The impact of risk, maternal health, and dietary variables on basic biochemistry (n=22).

	Glucose (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Total chol (mmol/L)	Triglycerides (mmol/L)	ALT (units/L)	AST (units/L)	GGT (units/L)	CRP-hs (mg/L)
Alcohol (Yes/No)	$R^2 = 0.289^+$ $\beta =$ **0.446***	$R^2 = 0.162$ $\beta = 0.045$	$R^2 = 0.122$ $\beta = -0.147$	$R^2 = 0.079$ $\beta = 0.130$	$R^2 = 0.047$ $\beta = -0.009$	$R^2 = 0.164$ $\beta = 0.126$	$R^2 = 0.244$ $\beta = 0.236$	$R^2 = 0.098$ $\beta = 0.112$	$R^2 = 0.124$ $\beta = -0.020$
Smoking (# of cigarettes/d)	$R^2 = 0.063$ $\beta = 0.036$	$R^2 = 0.252$ $\beta = 0.264$	$R^2 = 0.335$ $\beta = 0.532^+$	$R^2 = 0.188$ $\beta = -0.488$	$R^2 = 0.127$ $\beta = -0.083$	$R^2 = 0.202$ $\beta = 0.250$	$R^2 = 0.270$ $\beta = -0.114$	$R^2 = 0.049$ $\beta = -0.174$	$R^2 = 0.179$ $\beta = 0.214$
Drug use (Yes/No)	$R^2 = 0.146$ $\beta = 0.267$	$R^2 = 0.166$ $\beta = 0.080$	$R^2 = 0.104$ $\beta = 0.028$	$R^2 = 0.018$ $\beta = -0.113$	$R^2 = 0.095$ $\beta = 0.228$	$R^2 = 0.198$ $\beta = 0.226$	$R^2 = 0.199$ $\beta = -0.013$	$R^2 = 0.189$ $\beta = -0.355$	$R^2 = 0.183$ $\beta = 0.312$
BMI	$R^2 = 0.098$ $\beta = 0.163$	$R^2 = 0.250$ $\beta = -0.204$	$R^2 = 0.298$ $\beta = 0.441^+$	$R^2 = 0.065$ $\beta = 0.244$	$R^2 = 0.081$ $\beta = 0.121$	$R^2 = 0.164$ $\beta = -0.130$	$R^2 = 0.212$ $\beta = -0.216$	$R^2 = 0.099$ $\beta = -0.035$	$R^2 = 0.159$ $\beta = -0.085$
Pregnancies (#)	$R^2 = 0.187$ $\beta = -0.337$	$R^2 = 0.197$ $\beta = -0.221$	$R^2 = 0.107$ $\beta = -0.067$	$R^2 = 0.007$ $\beta = 0.039$	$R^2 = 0.048$ $\beta = 0.033$	$R^2 = 0.161$ $\beta = 0.038$	$R^2 = 0.198$ $\beta = 0.062$	$R^2 = 0.114$ $\beta = 0.145$	$R^2 = 0.204$ $\beta = -0.329$
Chronic illness (Yes/No)	$R^2 = 0.063$ $\beta = -0.097$	$R^2 = 0.161$ $\beta = -0.035$	$R^2 = 0.112$ $\beta = 0.093$	$R^2 = 0.077$ $\beta = -0.277$	$R^2 = 0.047$ $\beta = -0.004$	$R^2 = 0.175$ $\beta = 0.159$	$R^2 = 0.254$ $\beta = -0.251$	$R^2 = 0.124$ $\beta = -0.166$	$R^2 = 0.134$ $\beta = 0.111$
Exercise (min/d)	$R^2 = 0.060$ $\beta = -0.048$	$R^2 = 0.042$ $\beta = -0.043$	$R^2 = 0.091$ $\beta = -0.029$	$R^2 = 0.054$ $\beta = 0.117$	$R^2 = 0.039$ $\beta = -0.113$	$R^2 = 0.103$ $\beta = 0.122$	$R^2 = 0.167$ $\beta = -0.039$	$R^2 = 0.128$ $\beta = 0.030$	$R^2 = 0.121$ $\beta = -0.001$
Total Kcal (kcal/d)	$R^2 = 0.064$ $\beta = -0.088$	$R^2 = 0.189$ $\beta = 0.176$	$R^2 = 0.158$ $\beta = 0.238$	$R^2 = 0.027$ $\beta = 0.147$	$R^2 = 0.071$ $\beta = -0.159$	$R^2 = 0.104$ $\beta = -0.114$	$R^2 = 0.207$ $\beta = -0.109$	$R^2 = 0.178$ $\beta = 0.283$	$R^2 = 0.123$ $\beta = -0.023$
Protein (g/day)	$R^2 = 0.105$ $\beta = 0.167$	$R^2 = 0.160$ $\beta = 0.004$	$R^2 = 0.128$ $\beta = -0.166$	$R^2 = 0.186$ $\beta = 0.448^+$	$R^2 = 0.112$ $\beta = 0.272$	$R^2 = 0.156$ $\beta = -0.076$	$R^2 = 0.252$ $\beta = -0.254$	$R^2 = 0.243$ $\beta = -0.225$	$R^2 = 0.125$ $\beta = -0.053$
Fat (g/day)	$R^2 = 0.087$ $\beta = -0.096$	$R^2 = 0.170$ $\beta = 0.100$	$R^2 = 0.177$ $\beta = 0.279$	$R^2 = 0.007$ $\beta = 0.109$	$R^2 = 0.082$ $\beta = -0.203$	$R^2 = 0.154$ $\beta = -0.047$	$R^2 = 0.209$ $\beta = -0.549$	$R^2 = 0.222$ $\beta = 0.361$	$R^2 = 0.157$ $\beta = -0.191$
CHO (g/day)	$R^2 = 0.094$ $\beta = -0.106$	$R^2 = 0.277$ $\beta = 0.347$	$R^2 = 0.225$ $\beta = 0.355$	$R^2 = 0.006$ $\beta = 0.025$	$R^2 = 0.076$ $\beta = -0.174$	$R^2 = 0.156$ $\beta = -0.064$	$R^2 = 0.196$ $\beta = 0.006$	$R^2 = 0.133$ $\beta = 0.190$	$R^2 = 0.173$ $\beta = 0.230$
Fiber (g/day)	$R^2 = 0.084$ $\beta = 0.049$	$R^2 = 0.138$ $\beta = -0.027$	$R^2 = 0.094$ $\beta = 0.028$	$R^2 = 0.041$ $\beta = -0.210$	$R^2 = 0.131$ $\beta = -0.199$	$R^2 = 0.141$ $\beta = 0.008$	$R^2 = 0.208$ $\beta = -0.152$	$R^2 = 0.082$ $\beta = 0.047$	$R^2 = 0.113$ $\beta = -0.036$
Vitamin A (mcg/d)	$R^2 = 0.092$ $\beta = -0.110$	$R^2 = 0.160$ $\beta = -0.012$	$R^2 = 0.103$ $\beta = 0.002$	$R^2 = 0.007$ $\beta = -0.041$	$R^2 = 0.048$ $\beta = 0.030$	$R^2 = 0.175$ $\beta = -0.161$	$R^2 = 0.254$ $\beta = -0.295$	$R^2 = 0.098$ $\beta = -0.012$	$R^2 = 0.125$ $\beta = 0.057$
Vitamin C (mg/d)	$R^2 = 0.129$ $\beta = -0.222$	**$R^2 = 0.310$*** **$\beta = 0.493$***	$R^2 = 0.273^+$ $\beta = 0.277^+$	$R^2 = 0.008$ $\beta = -0.040$	$R^2 = 0.189$ $\beta = -0.417^+$	$R^2 = 0.198$ $\beta = -0.236$	**$R^2 = 0.370$*** **$\beta = -0.449$***	$R^2 = 0.188$ $\beta = 0.345$	$R^2 = 0.213$ $\beta = 0.467^+$
Folate (mcg/d)	$R^2 = 0.145$ $\beta = -0.272$	$R^2 = 0.178$ $\beta = 0.141$	$R^2 = 0.105$ $\beta = 0.020$	$R^2 = -0.039$ $\beta = -0.143$	$R^2 = 0.067$ $\beta = -0.148$	$R^2 = 0.180$ $\beta = -0.165$	$R^2 = 0.280$ $\beta = -0.308$	$R^2 = 0.112$ $\beta = 0.122$	$R^2 = 0.159$ $\beta = 0.204$

Results are from multivariable linear regression models in which age and trimester were included as covariates. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: *p <0.05; $^+$trending toward significance, p<0.1. HDL-High density lipoprotein; LDL-low density lipoprotein; TC-total cholesterol; ALT-alanine transaminase; AST-aspartate aminotransferase; GGT-gamma-glutamyl transferase; -CRP-hs-C-reactive protein-high sensitivity; BMI-body mass index.

Table 4-3. Maternal plasma and RBC fatty acid profile for the sample and by self-reported alcohol use (% of total FAs)

	Plasma				RBC			
FA	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value
C14:0	0.92 ± 0.54	0.70 ± 0.46	1.16 ± 0.55	**0.050**	0.30 ± 0.11	0.28 ± 0.11	0.32 ± 0.12	0.358
C16:0	25.56 ± 2.48	25.12 ±1.67	26.04 ± 3.17	0.409	26.71 ± 4.18	25.58 ± 2.85	27.73 ± 5.02	0.249
C16:1	1.88 ± 0.58	1.66 ± 0.44	2.13 ± 0.64	0.058	ND	ND	ND	
C18:0	6.39 ± 0.84	6.31 ± 1.12	6.48 ± 0.42	0.672	18.23 ± 2.57	17.43 ± 1.88	18.95 ± 2.98	0.184
C18:1n-9	24.69 ± 3.44	25.62 ± 3.06	23.66 ± 3.69	0.198	15.37 ± 1.32	15.23 ± 1.07	15.50 ± 1.56	0.661
C18:1n7	3.00 ± 1.14	2.82 ± 0.81	3.21 ± 1.43	0.450	1.48 ± 0.19	1.46 ± 0.16	1.49 ± 0.22	0.708
C18:2n6	25.65 ± 3.08	25.79 ± 3.23	25.50 ± 3.07	0.836	7.56 ± 1.96	8.02 ± 1.82	7.14 ± 2.07	0.315
C18:3n6	0.08 ± 0.09	0.08 ± 0.09	0.08 ± 0.08	0.943	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.961
C18:3n3	1.04 ± 0.36	0.84 ± 0.28	1.27 ± 0.29	**0.003**	0.24 ± 0.11	0.24 ± 0.12	0.24 ± 0.10	0.960
C20:3n6	1.74 ± 0.38	1.67 ± 0.33	1.82 ± 0.43	0.408	1.44 ± 0.57	1.48 ± 0.42	1.41 ± 0.69	0.800
C20:4n6	4.85 ± 1.45	5.18 ± 1.40	4.48 ± 1.49	0.283	8.59 ± 4.10	9.72 ± 3.24	7.56 ± 4.65	0.237
C20:3n3	ND	ND	ND		0.18 ± 0.08	0.21 ± 0.06	0.16 ± 0.09	0.137
C20:5n3	ND	ND	ND		1.40 ± 0.32	1.34 ± 0.17	1.45 ± 0.42	0.258
C22:0	0.12 ± 0.04	0.13 ± 0.05	0.11 ± 0.03	0.318	0.31 ± 0.10	0.29 ± 0.08	0.34 ± 0.11	0.451
C22:4n6	0.16 ± 0.05	0.15 ± 0.06	0.17 ± 0.04	0.281	1.86 ± 1.04	2.01 ± 0.81	1.73 ± 1.23	0.543
C24:0	0.23 ± 0.08	0.23 ± 0.10	0.23 ± 0.05	0.941	4.40 ± 0.94	4.24 ± 0.50	4.54 ± 1.23	0.479
C24:1	0.16 ± 0.07	0.14 ± 0.08	0.18 ± 0.05	0.131	5.58 ± 0.93	5.64 ± 0.79	5.53 ± 1.08	0.793
C22:5n3	0.24 ± 0.08	0.26 ± 0.09	0.22 ± 0.07	0.292	1.07 ± 0.60	1.18 ± 0.49	0.98 ± 0.70	0.470
C22:6n3	1.57 ± 1.51	1.64 ± 0.43	1.49 ± 0.38	0.422	1.96 ± 1.01	2.04 ± 0.78	1.89 ± 1.22	0.752
ΣSAT	33.82 ± 2.73	33.07 ± 1.80	34.65 ± 3.39	0.462	52.94 ± 7.63	50.83 ± 5.15	54.86 ± 9.16	0.236
ΣMUFA	30.56 ± 3.14	31.06 ± 3.06	30.02 ± 3.30	0.598	23.45 ± 1.81	23.35 ± 1.47	23.54 ± 2.14	0.818
Σn-6 PUFA	32.76 ± 3.29	33.13 ± 3.00	32.35 ± 3.71	0.286	19.82 ± 7.43	21.61 ± 5.93	18.20 ± 8.53	0.307
Σn-3 PUFA	2.86 ± 0.53	2.74 ± 0.62	2.99 ± 0.40	**0.004**	3.79 ± 1.76	4.22 ± 1.15	3.40 ± 2.16	0.298

Values are expressed as means ± SD. P-values indicated are for the differences between the groups assessed using the t-test of independence for normally distributed data and Wilcoxon's rank-sum test for not normally distributed data. Bolded p-values denote statistical significance, with p-values less than 0.05. RBC-red blood cell; FA-fatty acid; ΣSAT, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; Σn-6 PUFA, total n-6 polyunsaturated fatty acids; Σn-3 PUFA, total n-3 polyunsaturated fatty acids; ND, not detected

Table 4-4. The impact of risk, maternal health, and dietary variables on major plasma polyunsaturated fatty acids (n=22)

	C18:2n6	C18:3n3	C20:4n6	C22:6n3	Tot Mono	Total n-6	Total n-3
Alcohol (Yes/No)	$R^2 = 0.038$ $\beta = -0.136$	$R^2 = 0.074$ $\beta = 0.288$	$R^2 = 0.078$ $\beta = -0.257$	$R^2 = 0.076$ $\beta = -0.228$	$R^2 = 0.023$ $\beta = -0.071$	$R^2 = 0.033$ $\beta = -0.152$	$R^2 = 0.015$ $\beta = 0.018$
Smoking (# cigarettes/day)	$R^2 = 0.051$ $\beta = -0.093$	$R^2 = 0.011$ $\beta = -0.049$	$R^2 = 0.118$ $\beta = -0.276$	$R^2 = 0.114$ $\beta = -0.217$	$R^2 = 0.046$ $\beta = -0.210$	$R^2 = 0.052$ $\beta = -0.116$	$R^2 = 0.052$ $\beta = -0.116$
Drug use (Yes/No)	$R^2 = 0.118$ $\beta = -0.327$	$R^2 = 0.204$ $\beta = -0.262$	$R^2 = 0.188$ **$\beta = -0.428$***	$R^2 = 0.201$ **$\beta = -0.433$***	$R^2 = 0.169$ $\beta = -0.410^{+}$	$R^2 = 0.127$ $\beta = -0.354$	$R^2 = 0.216$ **$\beta = -0.474$***
Pregnancies (#)	$R^2 = 0.025$ $\beta = 0.051$	$R^2 = 0.009$ $\beta = 0.060$	$R^2 = 0.073$ $\beta = 0.225$	$R^2 = 0.046$ $\beta = 0.113$	$R^2 = 0.074$ $\beta = 0.239$	$R^2 = 0.020$ $\beta = 0.078$	$R^2 = 0.031$ $\beta = 0.131$
Chronic illness (Yes/No)	$R^2 = 0.102$ $\beta = -0.302$	$R^2 = 0.027$ $\beta = -0.155$	$R^2 = 0.043$ $\beta = -0.147$	$R^2 = 0.062$ $\beta = -0.180$	$R^2 = 0.065$ $\beta = -0.231$	$R^2 = 0.087$ $\beta = -0.288$	$R^2 = 0.035$ $\beta = -0.154$
Exercise (min/d)	$R^2 = 0.104$ $\beta = -0.091$	$R^2 = 0.066$ $\beta = -0.003$	$R^2 = 0.065$ $\beta = 0.009$	$R^2 = 0.085$ $\beta = -0.002$	$R^2 = 0.008$ $\beta = -0.010$	$R^2 = 0.073$ $\beta = -0.028$	$R^2 = 0.133$ $\beta = 0.071$
Total energy (kcal/d)	$R^2 = 0.039$ $\beta = 0.137$	$R^2 = 0.067$ $\beta = 0.260$	$R^2 = 0.024$ $\beta = 0.000$	$R^2 = 0.034$ $\beta = 0.011$	$R^2 = 0.045$ $\beta = 0.170$	$R^2 = 0.028$ $\beta = 0.122$	$R^2 = 0.040$ $\beta = 0.167$
Meat (servings/d)	$R^2 = 0.046$ $\beta = 0.170$	$R^2 = 0.169$ $\beta = 0.418$	$R^2 = 0.052$ $\beta = 0.185$	$R^2 = 0.059$ $\beta = 0.177$	$R^2 = 0.102$ $\beta = 0.320$	$R^2 = 0.038$ $\beta = 0.169$	$R^2 = 0.103$ $\beta = 0.330$
Milk (servings/d)	$R^2 = 0.111$ $\beta = -0.346$	$R^2 = 0.138$ $\beta = -0.423$	$R^2 = 0.194$ $\beta = -0.480^{+}$	$R^2 = 0.203$ $\beta = -0.480^{+}$	$R^2 = 0.178$ $\beta = -0.465^{+}$	$R^2 = 0.123$ $\beta = -0.383$	**$R^2 = 0.220$*** **$\beta = -0.527$***
Fat (g/d)	$R^2 = 0.041$ $\beta = 0.137$	$R^2 = 0.062$ $\beta = 0.238$	$R^2 = 0.030$ $\beta = 0.079$	$R^2 = 0.036$ $\beta = 0\ .048$	$R^2 = 0.070$ $\beta = 0.227$	$R^2 = 0.033$ $\beta = 0.136$	$R^2 = 0.051$ $\beta = 0.191$
Vitamin A (mcg/d)	$R^2 = 0.024$ $\beta = -0.037$	$R^2 = 0.006$ $\beta = -0.026$	$R^2 = 0.034$ $\beta = -0.099$	$R^2 = 0.038$ $\beta = -0.066$	$R^2 = 0.019$ $\beta = -0.006$	$R^2 = 0.017$ $\beta = -0.046$	$R^2 = 0.018$ $\beta = -0.063$
Choline (mg/d)	$R^2 = 0.044$ $\beta = 0.152$	$R^2 = 0.017$ $\beta = -0.115$	$R^2 = 0.024$ $\beta = 0.015$	$R^2 = 0.041$ $\beta = 0.083$	$R^2 = 0.022$ $\beta = 0.061$	$R^2 = 0.029$ $\beta = 0.134$	$R^2 = 0.015$ $\beta = -0.008$
DHA (mg/d)	$R^2 = 0.023$ $\beta = -0.038$	$R^2 = 0.006$ $\beta = -0.027$	$R^2 = 0.028$ $\beta = -0.076$	$R^2 = 0.035$ $\beta = -0.044$	$R^2 = 0.023$ $\beta = -0.067$	$R^2 = 0.016$ $\beta = -0.044$	$R^2 = 0.017$ $\beta = 0.051$

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: *$p < 0.05$; $^{+}$trending toward significance, $p<0.1$. Meat-Meat and Alternative; Milk-Milk and Alternative; DHA-docosahexaenoic acid.

Table 4-5. The impact of risk, maternal health, and dietary variables on RBC polyunsaturated fatty acids (n=22).

	C18:2n6	C18:3n3	C20:4n6	C22:6n3	Tot Mono	Total n-6	Total n-3
Alcohol (Yes/No)	**R^2 = 0.493**** **β = -0.444***	R^2 = 0.177 β = -0.218	**R^2 = 0.610**** **β =-0.419***	**R^2 = 0.469*** β = -0.365$^+$	R^2 = 0.330$^+$ β = -0.067	**R^2 = 0.424*** β = -0.075	**R^2 = 0.451*** β = -0.052
Smoking (# cigarettes/day)	R^2 = 0.235 β = 0.013	R^2= 0.145 β = 0.057	R^2 = 0.388$^+$ β = -0.068	R^2 = 0.309 β = -0.075	**R^2 = 0.478*** β = 0.112	**R^2 = 0.673**** β = 0.086	**R^2 = 0.673**** β = 0.123
Drug use (Yes/No)	R^2 = 0.336 β = -0.079	R^2 = 0.167 β = -0.178	R^2 = **0.466*** β = 0.101	**R^2 = 0.368*** β = -0.100	R^2 = 0.352$^+$ β = -0.169	**R^2 = 0.419*** β = 0.011	**R^2 = 0.454*** β = 0.071
Pregnancies (#)	R^2 = 0.336 β = 0.078	R^2 = 0.161 β = -0.154	**R^2 = 0.610**** **β = -0.419***	R^2 = 0.360 β = -0.034	R^2 = 0.329 β = 0.054	**R^2 = 0.426*** β = -0.084	**R^2 = 0.449*** β = 0.013
Chronic illness (Yes/No)	R^2 = 0.338 β = -0.095	R^2 = 0.163 β = -0.168	**R^2 = 0.467*** β = 0.048	**R^2 = 0.364*** β = -0.070	R^2 = 0.330$^+$ β = -0.060	**R^2 = 0.449*** β = 0.014	**R^2 = 0.422*** β = -0.061
Exercise (min/d)	R^2= 0.194 β = -0.010	R^2= 0.131 β =-0.029	R^2= 0.365$^+$ β = -0.001	R^2= 0.224 β = -0.093	R^2=0.303$^+$ β=-0.009	**R^2=0.396*** β =-0.001	**R^2 = 0.413*** β = 0.005
Total energy (kcal/d)	**R^2 = 0.415*** β = -0.304	R^2= 0.275$^+$ β = -0.387$^+$	**R^2= 0.605**** **β = -0.391***	**R^2= 0.506**** **β = -0.401***	R^2= 0.327$^+$ β =-0.030	**R^2 = 0.421*** β = 0.050	**R^2= 0.456*** β = 0.085
Meat (servings/d)	R^2=0.340$^+$ β =-0.109	R^2= 0.145 β = -0.092	**R^2= 0.474*** β = -0.104	**R^2= 0.364*** β = -0.074	R^2= 0.335$^+$ β =-0.02	**R^2= 0.420*** β=-0.040	**R^2 = 0.454*** β = -0.079
Milk (servings/d)	R^2 = 0.333$^+$ β = 0.063	R^2 = 0.141 β = -0.056	**R^2 = 0.465*** β = 0.004	R^2 = 0.370 β = 0.121	R^2=0.347$^+$ β = -0.137	**R^2 = 0.427*** β = 0.101	**R^2 = 0.461*** β = 0.128
Fat (g/d)	R^2 = **0.406*** β = -0.276	R^2 = 0.354$^+$ **β = -0.466***	**R^2 = 0.570**** β = -0.326$^+$	**R^2= 0.522**** **β = -0.405***	R^2 = 0.330$^+$ β = -0.058	**R^2 = 0.424*** β = -0.069	**R^2= 0.449*** β = 0.003
Vitamin A (mcg/d)	R^2= 0.343$^+$ β = 0.114	R^2= 0.162 β = 0.155	**R^2= 0.471*** β= 0.078	R^2= 0.361$^+$ β= 0.039	R^2= 0.327$^+$ β =-0.022	**R^2 = 0.449*** β =-0.173	**R^2 = 0.481*** β =-0.179
Choline (mg/d)	R^2 = 0.331$^+$ β = 0.019	R^2 = 0.140 β = 0.020	**R^2 = 0.466*** β = -0.052	R^2 = 0.371 β = -0.120	R^2= 0.328$^+$ β = -0.048	**R^2= 0.423*** β=-0.072	**R^2 = 0.459*** β = -0.109
DHA (mg/d)	R^2= 0.338$^+$ β = -0.088	R^2 = 0.139 β = 0.008	**R^2 = 0.490**** β = 0.163	**R^2 = 0.386*** β = -0.004	R^2 = 0.333$^+$ β = 0.039	**R^2 = 0.464*** β = -0.219	**R^2 = 0.510**** β = -0.232

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: *p <0.05, ** p <0.01; $^+$trending toward significance, p<0.1. Meat-Meat and Alternative; Milk-Milk and Alternative; DHA-docosahexaenoic acid.

Table 4-6. Maternal plasma and urine cytokine profiles by ethanol exposure

	Plasma Cytokine				Urine Cytokine			
Cytokine (pg/mL)	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value	All women (n=22)	Not-at-risk (n=11)	At-risk (n=11)	P-value
IL-1β	0.19 ± 0.07	0.17 ± 0.07	0.20 ± 0.07	0.469	7.23 ± 5.86	5.48 ± 3.42	9.17 ± 7.43	0.154
IL-2	0.98 ± 1.11	0.75 ± 0.16	1.15 ± 1.00	0.463	10.02 ± 11.56	12.05±14.93	7.80 ± 6.24	0.415
IL-6	1.05 ± 0.80	0.99 ± 0.59	1.11 ± 1.00	0.743	11.87 ± 13.03	12.23 ± 14.58	11.47 ± 11.87	0.898
IL-8	2.33 ± 0.71	2.34 ± 0.74	2.33 ± 0.72	0.960	4.54 ± 4.85	5.11 ± 5.77	3.92 ± 3.79	0.587
IL-10	0.42 ± 0.29	0.49 ± 0.38	0.36 ± 0.15	0.311	21.39 ± 25.26	26.09 ± 33.12	16.22 ± 11.95	0.716
IL-15	4.17 ± 1.74	3.66 ± 1.31	4.68 ± 0.45	0.173	9.20 ± 5.70	21.27 ± 24.00	14.75 ± 8.16	0.425
IL-17	3.55 ± 6.87	2.27 ± 1.13	2.53 ± 3.33	0.853	18.17 ± 18.14	21.25 ± 24.00	14.75 ± 8.16	0.425
MCP-1	68.95 ± 13.93	69.7 ± 16.03	68.22 ± 13.93	0.811	5.87 ± 3.95	6.33 ± 4.74	5.37 ± 3.04	0.874
TNF-α	1.83 ±1.17	1.67 ±0.37	2.00 ± 1.64	0.529	17.51 ± 17.18	20.27 ± 21.19	14.48 ± 11.73	0.455

Values are expressed as means ± SD. The significant differences between the risk groups were tested by an Independent t-test for normally distributed data and Wilcoxon rank-sum test for not non-normally distributed data. IL-interleukin; MCP-1-monocyte chemoattractant protein-1; TNF- α-tumor necrosis factor α.

Table 4-7. The impact of risk, maternal health, and dietary variables on maternal cytokine plasma profile (n=22).

	IL-1β	IL-2	IL-6	IL-8	IL-10	IL-15	IL-17	MCP-1	TNF-α
Alcohol (Yes/No)	R^2 = 0.011 β = 0.084	R^2= 0.121 β = 0.363	R^2= 0.004 β = 0.048	R^2= 0.097 β =-0.035	R^2= 0.208 **β =-0.444***	R^2=0.116 β= 0.280	R^2= 0.043 β =-0.180	R^2= 0.127 β =-0.180	R^2= 0.208 β = -0.074
Smoking (#cigarettes/day)	R^2= 0.031 β = 0.169	R^2= 0.011 β =-0.015	R^2= 0.003 β =-0.052	R^2=0.371$^+$ β =-0.216	R^2= 0.192 β =-0.225	R^2= 0.324$^+$ β = 0.394$^+$	R^2= 0.214 β = -0.067	R^2= 0.394$^+$ β = 0.457$^+$	R^2= 0.228 β =-0.279
Marijuana (Yes/No)	R^2= 0.146 β = 0.398$^+$	R^2= 0.013 β = -0.025	R^2= 0.074 β =-0.283	R^2= 0.099 β =-0.064	R^2= 0.058 β =-0.047	R^2= 0.054 β = 0.059	R^2= 0.015 β =-0.015	R^2= 0.138 β = 0.205	R^2 = 0.212 β = -0.085
Pregnancies (#)	R^2= 0.027 β =-0.150	R^2= 0.044 β = 0.180	R^2= 0.048 β = 0.116	R^2= 0.126 β =-0.177	R^2= 0.048 β =-0.061	R^2= 0.086 β =-0.188	R^2= 0.090 β =-0.277	**R^2= 0.329*** **β = 0.484***	R^2= 0.278 β =-0.271
Chronic illness (Yes/No)	R^2= 0.005 β = -0.017	R^2= 0.151 β = 0.390	R^2= 0.019 β = 0.113	R^2= 0.139 β =-0.246	R^2= 0.062 β = 0.141	R^2= 0.133 β = 0.305	R^2= 0.073 β = 0.094	**R^2= 0.383*** **β = 0.568***	R^2= 0.258 β = 0.244
Exercise (min/d)	R^2=0.046 β= 0.021	R^2=0.042 β=0.084	R^2=0.099 β = 0.033	R^2=0.154 β = 0.091	R^2= 0.091 β = -0.053	R^2=0.098 β = 0.101	R^2=0.102 β = 0.128	R^2=0.132 β = -0.031	R^2= 0.201 β = 0.053
Fat intake (g/d)	R^2= 0.018 β = 0.114	R^2=-0.097 β = 0.287	R^2= 0.089 β = 0.296$^+$	R^2= 0.161 β = 0.030	R^2= 0.031 β =-0.080	R^2= 0.053 β = -0.067	R^2= 0.036 β =-0.149	R^2= 0.111 β =-0.105	R^2= 0.215 β =-0.097
CHO intake (g/d)	R^2= 0.059 β = 0.043	R^2= 0.022 β = 0.104	R^2= 0.002 β = 0.017	R^2= 0.194 β =-0.339	R^2= 0.044 β =-0.064	R^2= 0.063 β=0.119	R^2= 0.015 β= 0.034	R^2= 0.116 β=-0.136	R^2= 0.207 β = 0.032
Protein intake (g/d)	R^2= 0.009 β = 0.064	R^2=0.285 β = 0.561$^+$	R^2=0.204 β = 0.482$^+$	R^2= 0.129 β =-0.197	R^2= 0.019 β =-0.008	R^2= 0.070 β =-0.039	R^2= 0.045 β =-0.186	R^2= 0.106 β = 0.099	R^2= 0.206 β = 0.013

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: *p <0.05; $^+$trending toward significance, p<0.1. IL-interleukin; MCP-1-monocyte chemoattractant protein-1; TNF- α-tumor necrosis factor α; CHO-carbohydrate.

Table 4-8. The impact of risk, maternal health, and dietary variables on maternal urine cytokine profile (n=22)

	IL-1β	IL-2	IL-6	IL-8	IL-10	IL-15	IL-17	MCP-1	TNF-α
Alcohol (Yes/No)	R^2 = 0.125 β = 0.230	R^2 = 0.179 β=-0.008	R^2= 0.006 β = 0.126	R^2= 0.031 β =-0.107	R^2= 0.072 β =-0.144	R^2= 0.214 β = 0.192	R^2= 0.002 β =-0.012	R^2= 0.122 β =-0.157	R^2= 0.208 β =-0.059
Smoking (#cigarettes/day)	R^2= 0.070 β =-0.231	R^2= 0.377$^+$ β =-0.073	R^2= 0.047 β = -0.087	R^2= 0.072 β = 0.105	R^2= 0.092 β = 0.152	R^2= 0.281 β = 0.196	R^2= 0.046 β = -0.121	R^2= 0.394$^+$ β = 0.457$^+$	R^2= 0.228 β =-0.279
Marijuana (Yes/No)	R^2= 0.206 β =-0.360	R^2= 0.182 β = 0.054	R^2=-0.049 β = 0.219	R^2= 0.023 β = 0.039	R^2= 0.034 β =-0.044	R^2= 0.278 β = 0.318	R^2= 0.003 β = 0.042	R^2= 0.138 β =-0.205	R^2= 0.212 β =-0.085
Pregnancies (#)	R^2= 0.089 β = 0.078	R^2= 0.296 β = 0.346	R^2= 0.085 β =-0.292	R^2= 0.054 β = 0.182	R^2= 0.070 β =-0.016	R^2= 0.302$^+$ β =-0.346$^+$	R^2= 0.037 β =0.188	R^2= 0.370$^+$ β = **0.487***	R^2= 0.278 β =-0.271
Chronic illness (Yes/No)	R^2= 0.215 β = 0.349	R^2= 0.210 β = 0.186	R^2= 0.002 β = 0.005	R^2= 0.153 β = 0.386$^+$	R^2= 0.221 β = 0.414$^+$	R^2= 0.192 β =-0.092	R^2= 0.007 β =-0.080	**R^2=0.390*** **β = 0.569***	R^2= 0.258 β= 0.244
Exercise (min/d)	R^2= 0.088 β = 0.075	R^2= 0.169 β = -0.004	R^2= 0.005 β = 0.015	R^2= 0.011 β = 0.061	R^2= 0.038 β = -0.018	R^2= 0.182 β = 0.018	R^2= 0.006 β = 0.049	R^2= 0.143 β = 0.092	R^2= 0.130 β = 0.007
Fat intake (g/d)	R^2= 0.099 β = 0.127	R^2= 0.180 β =-0.032	R^2= 0.058 β=-0.241	R^2= 0.029 β = 0.076	R^2= 0.084 β=-0.121	R^2= 0.188 β = 0.087	R^2= 0.003 β =-0.045	R^2= 0.111 β = -0.107	R^2= 0.197 β = 0.015
CHO intake (g/d)	R^2= 0.074 β =-0.101	R^2= 0.180 β =-0.019	R^2= 0.002 β= 0.014	R^2= 0.022 β = -0.005	R^2= 0.080 β = 0.115	R^2= 0.143 β = 0.038	R^2= 0.013 β =-0.056	R^2= 0.135 β = 0.167	R^2= 0.207 β =-0.053
Protein intake (g/d)	R^2= 0.111 β = 0.182	R^2= 0.253 β =0.298	R^2= 0.076 β = 0.251	R^2= 0.022 β =-0.069	R^2= 0.044 β = 0.078	R^2= 0.190 β = 0.079	R^2= 0.026 β =-0.069	R^2= 0.119 β = 0.146	R^2= 0.206 β =-0.031

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: *p <0.05; $^+$trending toward significance, p<0.1. IL-interleukin; MCP-1-monocyte chemoattractant protein-1; TNF- α-tumor necrosis factor α; CHO-carbohydrate.

Table 4-9. Maternal plasma trace mineral profile (ug/L) for the sample and by self-reported alcohol use (n=22).

Biomarker	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value
Chromium	0.50 ± 0.20	0.52 ± 0.16	0.49 ± 0.24	0.787
Manganese	0.20 ± 0.08	0.20 ± 0.08	0.21 ± 0.09	0.913
Iron	73.40 ± 36.98	70.80 ± 43.21	76.00 ± 31.47	0.265
Copper	193.91 ± 59.68	190.65 ± 80.37	197.17 ± 31.60	0.175
Zinc	75.36 ± 21.00	75.32 ± 27.58	75.40 ± 12.88	0.632
Selenium	10.01 ± 2.59	9.99 ± 3.55	10.03 ± 1.21	0.442

Values are expressed as means ± SD. The significant differences between the risk groups were tested by an Independent t-test for normally distributed data and Wilcoxon rank-sum test for not non-normally distributed data.

Table 4-10. The impact of risk, maternal health, and dietary variables on maternal plasma trace mineral profile (ug/L) (n=22).

	Chromium	Manganese	Iron	Copper	Zinc	Selenium
Alcohol (Yes/No)	R^2 = 0.020 β =-0.078	R^2=0.043 β = 0.186	R^2=-0.154 β = 0.279	R^2=-0.015 β = 0.078	R^2= 0.024 β = 0.016	R^2= 0.063 β = 0.152
Smoking (# cigarettes/day)	R^2= 0.124 β = -0.020	R^2=0.130 β= 0.346	R^2=-0.209 β = -0.056	R^2= 0.223 β= -0.443$^+$	R^2= 0.049 β = -0.162	R^2= 0.075 β = -0.288
Marijuana (Yes/No)	R^2= 0.045 β =-0.178	R^2=0.023 β =-0.065	R^2= 0.178 β = 0.314	R^2= 0.011 β = 0.035	R^2= 0.271 β = 0.529$^+$	R^2= 0.160 β = 0.369
Pregnancies (#)	R^2= 0.076 β = -0.248	R^2= 0.168 β = 0.396$^+$	R^2= 0.117 β=0.191	R^2= 0.015 β= 0.077	R^2=0.029 β=0.074	R^2=0.050 β=0.081
Chronic illness (Yes/No)	R^2= 0.017 β = 0.016	R^2= 0.027 β = 0.114	R^2= 0.036 β = -0.091	R^2= 0.017 β =-0.032	R^2= 0.034 β =-0.075	R^2= 0.009 β = -0.001
Fat intake (g)	R^2= 0.060 β = 0.151	R^2= 0.017 β= -0.017	R^2= 0.090 β= 0.030	R^2= 0.087 β= 0.280	R^2= 0.065 β = 0.024	R^2= 0.052 β = 0.091
CHO intake (g)	R^2= 0.143 β = 0.318	R^2= 0.078 β= -0.247	R^2= 0.088 β = -0.070	R^2= 0.013 β = 0.061	R^2= 0.095 β = -0.250	R^2= 0.009 β = 0.004
Protein intake (g)	R^2 = 0.016 β = 0.035	R^2 = 0.014 β = -0.083	R^2= 0.143 β = 0.259	R^2= 0.011 β = 0.016	R^2= 0.049 β= -0.203	R^2= 0.046 β = -0.043
Zinc (mg)	R^2 = 0.046 β = -0.122	R^2 = 0.010 β = -0.035	R^2 = 0.043 β = 0.071	R^2 = 0.121 β = 0.057	R^2= 0.102 β = 0.298	R^2= 0.122 β = 0.302
Iron (mg)	R^2 = 0.019 β = -0.052	R^2 = 0.050 β = 0.190	R^2 = 0.128 β = 0.199	R^2= 0.048 β = 0.089	R^2= 0.087 β = 0.224	R^2= 0.075 β = 0.176

Results are from multivariable linear regression models in which BMI and trimester were included as covariates. Results are presented as coefficient of determination (R^2) and standardized regression coefficient (β). Note: $^+$trending toward significance, $p<0.1$. CHO-carbohydrate.

CHAPTER V: PILOT STUDY ON PRENATAL DIETARY INTAKE AND ITS ASSOCIATIONS WITH SELF-REPORTED ALCOHOL CONSUMPTION IN FIRST NATIONS WOMEN LIVING IN A NORTHERN MANITOBAN COMMUNITY

Part of the data were published:

Kloss O., Jebb M., Chartrand L., Chudley A., Eskin NAM., & Suh M. (2022). Dietary intake patters and lifestyle behaviors of pregnant women living in a Manitoba First Nations community: implications for fetal alcohol spectrum disorder. Nutrients, 14 (15), 3233:1-16.

Abstract

Background: Compromised maternal nutrition is identified as one of the major factors contributing to FASD due to alcohol's properties interfering with nutrient metabolism. Although nutrition is identified as a major contributing factor to FASD, information on nutrition is scarce, particularly in First Nations populations living on reserve. ***Objective:*** This pilot study aims to explore the relationship between the intake of macro- and micronutrients, important for fetal central nervous system development, and its association with maternal risk exposures and lifestyle predictors in First Nations women living in a northern Manitoban reserve. ***Methods:*** Through partnerships with Opaskwayak Cree Nation community in Manitoba, 37 pregnant women (aged 14-42 years) were recruited to participate in the study. An interactive, in-person questionnaire collected information on participants' demographics, anthropometrics, dietary intake. risk exposures, pregnancy outcomes, and maternal health. Hierarchical linear regression modelling was performed to evaluate the contributions of risk, demographic, and health predictors in blocks***. Results:*** A high proportion of participants were not meeting Health Canada's food group recommendations. Vegetables and Fruit recommended daily servings were not met by 94% (95%CI = 81-99), Grain Products by 89% (95%CI = 74-97), Milk and Alternatives by 94%, (95%CI = 81-99); and Meat and Alternatives by 86% (95%CI = 71-95) of participants. High prevalence of inadequacy was identified for folate, calcium, iron, and DHA (68%, 95%CI = 50-82; 57%, 95%CI = 40-73; 68%, 95%CI = 50-82; and 97%, 95%CI = 86-100, respectively). Self-reported alcohol consumption was associated with increased fat intake (%), in all models ($\beta = 0.256$; SE, 0.086; $p < 0.01$; $\beta = 0.275$; SE, 0.100; $p < 0.05$; $\beta = 0.257$; SE, 0.111; $p < 0.05$). A decrease in %DRI vitamin C and niacin was observed with alcohol consumption ($\beta = -120$; SE, 56; $p < 0.05$; $\beta = -90$; SE, 43; $p < 0.05$, respectively) in the block adjusted for dug use; and calcium in the block adjusted for drug use and demographic factors ($\beta = -56$; SE, 26; $p < 0.05$). ***Conclusion:*** The findings of this pilot study contribute important information for the future research designs in the context of FASD risk factors in similar populations and to the development of nutrition-based programming for First Nations pregnant women living in Northern Manitoba.

Key words: Maternal nutrition; First Nations; Macronutrients; Micronutrients, FASD.

Introduction

Fetal alcohol spectrum disorder (FASD), defined as spectrum of physical and cognitive anomalies associated with alcohol exposure in utero, is recognized to be the most prevalent and preventable cause of mental disability in Canada and the western world (Popova et al., 2019). The most cited estimate for FASD prevalence in Canada is 9 in 1,000 births (Government of Canada, 2017). Various reports indicate that the proportion of pregnancies affected by alcohol among Indigenous populations may be higher (50.8% Manitoba (Williams & Gloster, 1999); 25.3% Ontario (Kelly et al., 2001); 60.5% Nunavut (Fraser et al., 2012)) than the general Canadian population, with the overall national prevalence of affected pregnancies being 10-15% (Public Health Agency of Canada (PHAC), 2010; Popova et a., 2019).

Although alcohol consumption is the number one risk factor for FASD, factors such as socioeconomic status, psychiatric and neuropsychological health, concomitant substance use and smoking, maternal health and chronic conditions, and nutrition and food security are predictive of the development of the disorder (Esper & Furtado, 2014; May & Gossage, 2011). Experimental, cohort, and clinical research studies reveal that the afore-stated factors increase the vulnerability of a fetus to the teratogenic effects of ethanol, thus elevating the risk for FASD outcome (Bignol et al., 1987; May et al., 2005; Singal et al., 2017; Jacobson et al., 1996).

To date, the major FASD preventative approaches have centered around promoting alcohol abstinence during pregnancy (CanFASD, 2013; Poole et al., 2016). However, emerging research and the findings of the Truth and Reconciliation Commission (TRC) make it clear that a focus on abstinence alone does not reduce the rates of FASD in Indigenous communities and in some instances, may promote colonial practices (TRC, 2015; Wilkinson & Room, 2009). Therefore, a novel effective prevention strategy is required (TRC, 2015).

Maternal nutrition has been identified as one of the major modifiable factors contributing to the severity of FASD, as it is the primary agent controlling the intrauterine environment and genome expression of the placenta, embryo, and fetus (Chudley 2005, May & Gossage, 2011; Ballard et al., 2011; Abel & Hannigan, 1995). Chronic, heavy, and binge patterns of alcohol consumption can lead to primary and secondary malnutrition by displacing energy from macronutrients and interfering with nutrient uptake, transport, metabolism, and utilization (Abel & Hannigan, 1995). Thus, ensuring adequate maternal nutrition is especially critical for alcohol-affected pregnancy.

Although the concomitant influences of alcohol and macro-and micro-nutrient deficiencies on the developing fetus and central nervous system (CNS) are not well understood, a body of experimental research has shown that alcohol-induced teratogenic insults are exacerbated by inadequate nutrient status (Thomas et al., 2000; Hewitt et al., 2011; Martin et al., 1993). Moreover, a line of research demonstrates that prenatal nutrient supplementation (vitamins A and C, choline, zinc, iron) alleviates alcohol-induced damage in animal models and humans, reducing the severity of alcohol-related birth anomalies (Young et al., 2014). Despite the existence of data on the feasibility of nutrient supplementation as a potential FASD intervention, there is a lack of information on maternal nutrition intake and the interplay between nutrition and various risk exposures. Lack of information is particularly critical for First Nations pregnant women residing on reserves, due to the exclusion of this population from large national surveillance programs and the overall dearth of studies investigating this topic in this population.

Indigenous community leaders, elders, maternal and child health clinical professionals, and the Canadian government have drawn increasing attention to the necessity of identifying and implementing targeted preventative initiatives for FASD in Indigenous communities. In pursuit of this goal, this pilot study aims to lay the groundwork for exploring the relation between nutrition – a protective FASD factor – and social and lifestyle predictors associated with dietary intake. Specifically, the study's objective is to examine maternal dietary intake and evaluate the link between the intake of nutrients known to be important for fetal central nervous system development and maternal risk and health factors. Contributing with baseline information to establish the foundation through future research and closing this knowledge gap is the first step to fulfilling national health priorities and guiding public health policy planning to improve health in First Nations communities.

Methodology and Design

Band and council approval

The study protocol development is described in detail in *Chapter 3*. All research was performed in accordance with the Health Research Involving Aboriginal Peoples Guidelines established by the Canadian Institutes of Health Research (CIHR) and the Tri-Council Policy (TCP) Statement, "Ethical Conduct for Research Involving Humans" (Government of Canada, 2020). The study protocol and consent forms were approved by the University of Manitoba Health Research

Ethics Board (H2013:263), as per Tri-Council Policy on *Ethical Conduct for Research Involving Humans* requirement in June 2013. All data operation processes conformed to the Indigenous research principles of ownership, control, access, and possession (OCAP) (First Nations Information Governance Centre (FNIGC), 2020). The detailed protocol development process is described elsewhere, as this study is a sub-part of a larger project (Giesbrecht, 2015).

The study protocol was presented to community of Opaskwayak Cree Nation (OCN) members and elders in June 2015, and obtained Band and Council approval on July 08, 2015. Prior to the presentation of the study to the community, the study team consulted with the advisors from the Assembly of Manitoba Chiefs (AMC) and presented the research protocol at the AMC Health Information Research Governance Committee (HIRGC) semi-annual board meeting (Giesbrecht, 2015).

Community profile

The OCN, formerly known as The Pas Band, is located 630 km northwest of Winnipeg (**Figure 5-1**) (Swampy Cree Tribal Council (SCTC), 2020). It is Treaty 5 territory with 6,285 registered members and an on-reserve population of 3,355 individuals (First Nations Land Management Resource Centre, 2020; Statistics Canada, 2016). The reserve borders the Town of the Pas and Rural Municipality (RM) of Kelsey (SCTC, 2020). The nation's prominent developments include Joe A. Ross School, Oscar Lathlin Collegiate, University College of the North (UCN), Beatrice Wilson (BW) Health Centre, Community Centre, hotel-Kikiwak Inn, and Otineka Shopping Centre.

As demonstrated through a comparison to Division No. 21 or informally called as Manitoba Northern region, the OCN community has a different population growth rate and age distribution. The OCN community differs from the Northern region with respect to average persons per household (3.0-4.1 vs 2.7). The unemployment rate of the community's most populated division (21E) is 18.2%, which is higher than the Northern region's estimated unemployment rate of 9.8% (Statistics Canada, 2016). The total household median income of the OCN's division 21E is $48,256 and individual median income is $18,288. This is substantially lower than the region's household ($72,028) and individual ($31,220) incomes (Statistics Canada, 2016).

Study settings, community engagement and recruitment

A targeted community engagement process was initiated after receiving Band and Council Resolution (BCR). The process focused on building relationships with the community members, elders, band, and council. The engagement was strengthened through partnerships and significant involvement with the Beatrice Wilson (BW) Health Centre. The BW Health Centre offers a variety of programs and services to support the health of the OCN community, with the mission of incorporating mental, physical, spiritual, and emotional well-being while maintaining and respecting traditional and cultural values and beliefs (Beatrice Wilson Health Centre, 2023). The health center is heavily involved with the OCN community and collaborating with them allowed researchers to build on existing relationships with community members, recognize community health needs and priorities, and conduct research in culturally grounded ways relevant to the local population. Involvement with the health center included development and the facilitation of maternal health workshops at the request of the health center team, as they identified a need in the community for this type of programming. The researcher (OK) delivered workshops as part of the community's existing Prenatal Program. In collaboration with the BW Health Centre team, the research team employed traditional teachings and perspectives to build-in cultural inclusivity and traditional diversity into the workshops. The workshops were held monthly at the BW Health Centre (7-hour commute one way from Winnipeg), for the duration of data collection (3.5 years, 2015-2019). Workshop topics included gestational diabetes, diet during pregnancy, a sushi-making session, healthy baking, drug use during pregnancy, dietary myths and facts, and others. To measure the success of the workshop sessions, evaluation forms were presented at the end of each workshop and participant feedback was collected and considered when preparing the next workshop. In addition, upon the completion of each community visit to conduct a workshop and gather study data, the report was provided to the OCN Health Leadership team to communicate the level of workshop attendance, study recruitment progress, and overall workshop acceptance and success. The on-going communication and reporting strengthened the relationships between the OCN's Health Center and the research team and ensured accountability for improved results. To further foster an environment of mutual respect, the researcher (OK) attended community events, provided support for the BW Prenatal Program, and met with leaders, elders, and community members.

In addition to the community engagement, to improve the recruitment and participation rate, a community member was hired as a Project Assistant. The Project Assistant was trained on data collection (collected data on 4 participants) and helped with the development of study posters, community newspaper announcements, and door-to-door visits.

Upon completion of this pilot study, reporting meetings were held to provide the community leadership, members, and BW Health Center staff with the study results. A final report and an infographic were distributed to the BW Health Center staff and community members during the OCN Indian Days, Aug 16-19, 2022. **Figure 5-2** depicts a logic model of the community engagement process and the development of cross-sectoral partnerships between the research team and the community, as described in this section.

Participants

The inclusion criteria for the study were pregnant women aged 14-50 years, who were members of the OCN community, and able to communicate in English. Women who did not belong to the OCN community were not able to participate in the study as the BCR received was for the community members only.

Data collection, research tool

The data collection process, research tool, quantification of dietary intake and adequacy is described in detail in *Chapter 3* (maternal micronutrient intake from supplementation and diet is available in **Appendix 4**). Unlike with the CCN, hospital discharge summaries were not obtained for the OCN participants due to jurisdictional barriers and a newly built health office that lacked data infrastructure and staffing.

Sample size determination

Convenience sampling was utilized to recruit participants who received prenatal care at the BW Health Center. The sample size calculation for this community employed the same determination method as in *Chapter 3*. With the present sample, large effects (partial correlation 0.5) could be detected.

Statistical analysis

The statistical methodology employed for this study is similar to that described in *Chapter 3*. Briefly, the normality testing for variables distributions was performed by the Kolmogorov-

Smirnov and Shapiro-Wilks tests, presenting nonparametric distribution for total energy (kcal), (%) energy from fat, (DRI%) vitamin A, thiamin, folate, cobalamin, iron, and DHA.

Sample characterization was described by means of descriptive statistics (mean, SD, median, IQR) for continuous variables and proportions with 95% CIs for categorical variables. Student's t-test, Wilcoxon rank-sum test, and Chi-square test of independence ($\chi 2$) were used to assess the differences between the two groups (non-at-risk and at-risk) for normally distributed data, non-normally distributed and ordered categorical data, and nominal data, respectively.

To test the strength and direction of associations between the nutrients (% energy for macronutrients, %DRI for micronutrients) and risk, maternal health, and demographic factors a Spearman correlation test was employed.

Hierarchical regression analysis with forward selection was performed to evaluate the level of contribution of independent variables to the macro- and micro-nutrient % energy and % DRI intake, respectively. In this analysis, dependent variables included were total energy (kcal), energy (%) from protein, carbohydrate, fat, and the DRI (%) intake of vitamin A, vitamin C, thiamin, niacin, folate, cobalamin, choline, calcium, iron, zinc, and % recommendations for docosahexaenoic acid (DHA) (Global Recommendations for DHA and EPA Intake, 2008). Each of the dependent variables underwent hierarchical regression with forward selection separately. The independent variables were added in the following order of blocks.

- Block 1/Model 1: included risk exposures (alcohol consumption, drug use);
- Block 2/Model 2: to the afore-stated risk exposures, demographic factors (age and employment) were added;
- Block 3/Model 3: maternal health variables (trimester, pre-pregnancy BMI, chronic illness) were added to the risk exposures and demographic factors. Block 3/Model 3 represents an omnibus model for each of the macronutrient variables.
- Block 4/Model 4: total energy (kcal) (applicable to micronutrients). This independent variable was added to identify the relationships between total energy collected from 24-hour dietary recall and FFQ. Block 4/Model 4 represents an omnibus model for each of the micronutrient variables.

The assumptions for the multiple linear regression were analyzed using the following tests: Variance Inflation Factor (VIF) for multicollinearity; and Q-Q plots for the assessment of the

normality of the residual distribution. Due to small sample size and the observations of outliers through Q-Q plot, additional normality of the residuals testing was performed with a Wilks-Shapiro test, which confirmed a non-normal distribution of residuals for total energy (kcal), (%) energy from fat, (DRI%) vitamin A, thiamin, folate, cobalamin, iron, and DHA. These variables underwent natural logarithmic transformation. The variables were also tested for interactions, no interactions were detected.

Results

Maternal demographic, health, and pregnancy characteristics

Table 5-2 presents the sociodemographic characteristics of all women and women who were defined as non-at-risk and at-risk. Fifty-two (52) women were approached during recruitment, and thirty-eight (38) women participated in the study (73% participation rate). One participant provided a limited number of responses and thus, was not accounted for in the analysis. All participants were First Nations status women residing on the OCN reserve land with an average age of 24.4 ± 7.0 years old. Education and employment status varied greatly among all women, with the majority of women having junior high and high school education (44% and 41%, respectively); and unemployed and student status (39% and 28%, respectively). About half of the participants reported being on social assistance (51%). When non-at-risk and at-risk groups were compared there were no significant differences with respect to education, employment, social assistance, and the number of household residents. However, significant differences were noted for age, with the at-risk women having higher than average age ($p < 0.01$).

The average maternal pre-pregnancy BMI was estimated to be 26.5 ± 8.8 kg/m^2, which is classified as overweight. Chronic illness before and during pregnancy was reported by 15% and 21% of participants, respectively. Vitamin and other supplements were taken by 94% of participants, with the major supplement being maternal multivitamins received at the clinic. About 37% of participants reported smoking cigarettes during the given pregnancy, as well as 19% reported using illicit drugs such as marijuana (at the time of the data collection marijuana was illicit) and cocaine. The majority of the participating women were in the first and third trimesters (36% and 51%, respectively) and only 12% of women were in their second trimester. There were no significant differences between non-at-risk and at-risk women for maternal health and pregnancy characteristics.

Self-reported access to finances and food

Overall, 11% and 19% of participants stated that they "strongly agree" and "somewhat agree", respectively, with the statement "I often run out of food". When asked whether they had enough food to eat, 89% of participants agreed (64% strongly agreed, 25% somewhat agreed). A high number of participants also agreed to having a say in the way money is spent (47% strongly agreed, 31% somewhat agreed). Lastly, participants were asked if they had enough money to provide for food, with the majority of participants agreeing that they did (56% strongly agreed, 19% somewhat agreed). There were no significant differences between at-risk and non-at-risk groups (**Figure 5-3**).

The majority of participants responded favorably when asked whether the food they eat is adequate to keep them healthy 79% agreed (38% strongly agreed, 41% somewhat agreed). As for the availability question, 80% of participants agreed that the food was available from the store near to their home (33% strongly agreed, 47% somewhat agreed). Similar to the availability question, 80% of participants responded favorably when asked about the affordability of food (33% strongly agreed, 47% somewhat agreed). When asked if they feel the food they have access to all the foods they would like to eat, 84% of participants agreed (42% strongly agreed, 42% somewhat agreed). There were no significant differences between at-risk and non-at-risk groups. Results are displayed in **Figure 5-4**.

Macronutrient dietary characteristics

Detailed food group, macronutrient and energy intake, and participants' intake inadequacy, percentage contribution from each of the macronutrients are reflected in **Table 5-3**. A high proportion of participants were not meeting Health Canada's food group recommendations. Vegetables and Fruit recommended daily servings were not met by 94%, (95% CI = 81-99) of participants; Grain Products by 89%, (95% CI = 74-97), and Milk and Alternatives by 94%, (95% CI = 81-99); and Meat and Alternatives were not met by 86% (95% CI = 71-95) of participants.

The median (IQR) percent energy intake from protein was 16% (11-19) and 54% (42-59) for carbohydrates. The median (IQR) percent energy intake from fat was 14% (10-17). Lastly, half of the participants had inadequate fat intake (50%, 95% CI = 34-65).

Micronutrient dietary characteristics

Table 5-4 displays the median micronutrient intake as well as the DRI (%). The median (IQR) intake of all nutrients was within the recommended reference %DRIs, except it was lower for folate 84% (59-108), calcium 87% (81-149), iron 84% (62-118), and DHA 38% (20-51); which corresponded with high proportions of participants falling below the recommendations for these nutrients (68%, 95%CI = 50-82; 57%, 95%CI = 40-73; 68%, 95%CI = 50-82, and 97%, 95%CI = 86-100, respectively).

Effect of individual predictors on the (%) macronutrient intake

Spearman's rank correlation was computed to measure the strength and direction of association between individual predictors (risk exposure, demographic, health status, and maternal variables) and each macronutrient intake (% energy) **(Table 5-5)**. This analysis showed a significant positive correlation between total caloric intake and alcohol consumption ($r = 0.428$, $p<0.05$), energy from fat intake (%) and alcohol consumption ($r = 0.551$, $p <0.01$), as well as energy from fat (%) and illicit drug use ($r = 0.395$, $p <0.05$), maternal age ($r = 0.380$, $p <0.05$), number of pregnancies ($r = 0.368$, $p <0.05$), and chronic illness ($r = 0.345$, $p <0.05$). On the other hand, energy (%) from fat intake correlated negatively with participants' employment status ($r = -0.357$, $p <0.05$).

A significant positive correlation was also seen between maternal age and energy (%) from protein intake ($r = 0.376$, $p <0.05$); and the employment and fiber intake ($r = 0.395$, $p <0.05$). An inverse relationship was identified for chronic illness and fiber ($r = -0.381$, $p <0.05$).

Effect of individual predictors on the DRI (%) of micronutrient intake

Spearman's rank correlation analysis identified a significant negative relationship between alcohol consumption and the intake of niacin ($r = -0.210$, $p <0.05$). No other significant associations were identified between risk variables and DRI (%) of the micronutrient intake (**Table 5-6**).

Thiamin intake correlated negatively with maternal age ($r = -0.360$, $p <0.05$) and level of education ($r = -0.380$, $p <0.05$). A significant positive correlation was detected between maternal pre-pregnancy BMI and the intake of cobalamin ($r = 0.361$, $p <0.05$). In addition, the pregnancy trimester correlated negatively with vitamin C ($r = -0.440$, $p <0.05$).

Total energy intake showed a significant negative correlation with calcium intake ($r = -0.409$, $p < 0.05$). Lastly, the intake of vegetables and fruits (# of servings/day) correlated negatively with iron intake ($r = -0.367$, $p < 0.05$).

Effect of combined predictors on macro- and micronutrient (%) intake

Hierarchical linear regression modelling was performed to evaluate the contributions of risk, demographic, and health predictors in blocks (**Tables 5-7**). The first block of regression analyses examined the impact of two risk variables on maternal energy (%) from each macronutrient intake. Increased total energy intake was associated with alcohol consumption ($\beta = 0.256$; SE, 0.086; $p < 0.01$).

The second block of regression analyses examined the impact of risk exposure and demographic variables (alcohol, drug, age, employment) on maternal energy (%) from micronutrient intake. The result (Model II) shows that after controlling for the effect of age and employment status, the effect of alcohol consumption on the fat intake (%) maintained its significance ($\beta = 0.275$; SE, 100; $p < 0.05$). When we further controlled for the effect of the trimester, BMI and chronic illness, the effect of drug use on the fat intake (%) intake (Model III) was still significant ($p < 0.05$).

Tables 5-8a-f presents the results of hierarchal linear regressions for % DRI for micronutrients. A decrease in vitamin C and niacin was observed with alcohol consumption ($\beta = -120$; SE, 56; $p < 0.05$; $\beta = -90$; SE, 43; $p < 0.05$, respectively) in Model I; and calcium in Model 2 ($\beta = -56$; SE, 26; $p < 0.05$). Percent DRI intake for thiamin and folate showed a positive association with total energy intake ($\beta = 0.148$; SE, 0.062; $p < 0.05$; $\beta = 0.033$; SE, 0.012; $p < 0.05$, respectively).

Discussion

The present cross-sectional pilot study in First Nations maternal population living on OCN reserve contributes to the maternal health and nutrition field in the following ways: It presents the information on maternal baseline dietary intake of macro- and micro-nutrients, prevalence of inadequacy for eleven micronutrients and DHA, and demonstrates the influence of risk-exposure predictors, especially self-reported alcohol consumption on the %DRI intake for micronutrients and energy adjusted macronutrients. This information may serve as the baseline for further

research on maternal dietary intake, prenatal nutrition programming, and FASD prevention planning.

Demographic and health characteristics

Demographic information demonstrates that the participating cohort is relatively homogenous with all women reporting First Nations Status and residence on the OCN reserve land. The average age of the sample was 24.4 ± 7.0 years old, suggesting that the age of first-time motherhood is below the average maternal age of the general Canadian population (29.6 years of age) (PHAC, 2010). This is consistent with the findings of other studies which point toward high fertility rates at an earlier age among First Nations teenage girls (Oliveira et al., 2013; Wenman et al., 2004). Education and employment status were wide-ranging among all participants. A high proportion of women reported being unemployed and/or on social assistance (51%). These statistics are consistent with other on-reserve studies (Oliveira et al., 2013; Hui et al., 2014;) and government reports (Government of Canada, 2020; PHAC, 2010).

The present study found our cohort had high pre-pregnancy BMI (21% overweight, 33% obese) and high proportion of chronic illness during the studied pregnancy. Likewise, many studies (Oliveira et al., 2013; Dyck et al., 2002; and Christian et al., 2014) reported average pre-pregnancy BMI of 28.3 ± 6.0 kg/m^2, 25.3 ± 6.0 kg/m^2, and 26.0 ± 6.0 kg/m^2 respectively, suggesting high number of women in overweight and obese categories. National statistics on the pregnant First Nations women diagnosed with chronic illnesses was 4.7% (PHAC, 2010), conversely this cohort has a notably higher proportion of diagnosed participants. This is a concerning finding as literature unequivocally points to increased risk of pregnancy complications and infant co-morbidities of women experiencing chronic conditions prenatally. About 37% of participants reported smoking cigarettes during the given pregnancy, which is almost identical to the rate reported by Oliveira and colleagues (2013) of 35% among women of the Six Nations Reserve in Ontario. These findings are substantially higher compared to the general Canadian population (7%) (PHAC, 2010). Nineteen (19%) percent of participants reported using illicit drug use such as marijuana (at the time of the data collection marijuana was illicit) and cocaine. This is also markedly higher than the general Canadian average of 1% (PHAC, 2010). There is lack of comparative information available for First Nations pregnant women with regard to rates of illicit drug use during pregnancy. Alcohol use was reported by

40% of participants. Similar to other risk exposures, self-reported alcohol consumption is substantially higher in this sample compared to the prevalence for the general Canadian maternal population (9-10%) (PHAC, 2010; Popova 2017). A meta-analysis on alcohol consumption among pregnant women by Popova and colleagues 2017, reported pooled prevalence of any amount of alcohol consumption during pregnancy among the Indigenous population to be 36.5% (95% CI = 24.7-49.1) in Canada and 42.9% (95% CI = 27.1-59.4) in United States. These estimates align with the sample prevalence of alcohol consumption in our study.

The findings of high rates of risk exposures are concerning, as alcohol, smoking, drug-use, and overall poor health habits are direct provocative factors for FASD (May & Gossage, 2011). There is a growing awareness that drug use, smoking, and alcohol consumption are immediate modulators of macro- and micro-nutrient intake and thus carry special implications for the multifactorial nature of FASD. A high number of reports identify the interactions between nutritional health indicators and prenatal risk exposures (Kuhnert et al., 1987; Carter et al., 2007; McDonald & Watson, 2020; Suzuki et al., 2022; Virji, 1991; Dewan et al., 2003). Alcohol consumption, prenatal smoking, and drug use have been associated with poor maternal weight gain (McDonald & Watson, 2020; Virji, 1991; Suzuki et al., 2022), small for gestational age infant (Al-Sahab et al., 2010; Suzuki et al., 2022), intrauterine growth restriction (Dewan et al., 2003; Feldman et al., 2012), placental abruption (Kaminsky et al., 2007; Aliyu et al., 2011), abnormal levels of plasma nutrients (Kuhnert et al., 1987; Carter et al., 2007; Miller et al., 1983). Several known biological mechanisms have been delineated to explain these phenomena. High concentrations of ethanol-induced systemic and localized ROS, sub-optimal placental angiogenesis, fetal hypoxia, and resulting reduced levels of oxygen and nutrients for the fetus preclude proper development and growth (Memon et al., 2009; Micangeli et al., 2022). Further to that, epigenetic modifications occurring in utero and manifesting through improper DNA methylation and disruption of human growth, neurocrine, and endocrine factors lead to congenital malformations, cognitive impairments, and lifelong implications (Alberry et al., 2021). An important consideration is the accuracy of the present study's self-reported information on the risk exposures. Studies with biological samples where the exposures could be confirmed or approximated through the biological markers are required.

Access to resources

Although this study did not focus on direct food security indicators, several questions have been asked with respect to access and decision-making on finances for food, access to food, and affordability of food. Most participants responded positively to the set of questions on having access to financial resources and having access to food. This finding is inconsistent with the evidence of food insecurity in First Nations communities. The First Nations Regional Health Survey (RHA) reported that just over half (54.2%) of participating households experienced food insecurity in Canada (FNIGC, 2012). Furthermore, in Manitoba, food insecurity increases as the latitude increases, reaching astounding proportions north of the 50th parallel (Thompson et al., 2012), which is an approximate location of OCN community. The positive responses to the food security questions in this community could be attributed to its location near the town of the Pas, where the town's infrastructure, accessibility to local grocery stores, and road access to the city of Winnipeg, Flin Flon, Brandon, and Thompson promote better access and availability of foods. In the context of FASD and maternal risk factors this is a positive finding for this community, as food security is an important protective factor. Qualitative explorations on community development topics, traditional food systems, management of community food insecurity, as well as expansion of the sample size and inclusion of food security indicators is recommended for future studies.

Food group and macronutrient intake

In the present sample, women were not meeting the CFG (2007) recommendations for Vegetable and Fruit and Grain Products. Although little comparative information is available with respect to maternal food group intake, the findings of this study are consistent with the findings of Johnson-Down and Egeland (2013), where FN women of child-bearing age did not meet the recommendations Fruits and Vegetables (>90% of participants), Grain Products (>70% of participants) and Milk and Alternatives (>90% of participants). It is concerning that the sample of expecting women with higher self-reported prevalence of risk exposures and chronic illness has low intakes of nutrient-dense and fiber-rich foods such as fruits, vegetables, dairy and whole grain products. Lower intakes of these food groups could be due to nutrition transition, which is marked by increased consumption of highly processed western foods, experienced by Indigenous people in remote communities (Earle, 2011). Scholars argue that nutrition transition diverted Indigenous people away from traditional foods such as berries, wild greens, and traditional meats

and fish and has led to the adoption of foods from nonindigenous sources, which are higher in calories, added sugar, sodium, and undergo processing (Earle, 2011; Tarasuk et al., 2015).

The median energy and macronutrient intakes (g/day) for all participants in the present study are very similar to the intakes of pregnant women in the study performed by Back and colleagues (2012) who investigated dietary intake and physical activity in Frist Nations pregnant women living in rural and urban settings (total 2259 ± 977 kcals/day, 308.8 ± 121.0 g/day carbohydrates, 83.1 ± 31.8 g/day protein, and 81.2 ± 52.5 g/day fat).

The results of this study showed a relationship between total energy intake and drug exposure, as well as percent fat intake, alcohol consumption, and drug exposure. These findings were not surprising, as they are consistent with the published literature on patterns of food consumption in the presence of appetite modulators such as alcohol and drugs (marijuana) (Schrieks et al., 2015, Hetherington et al., 2001, Addolorato et al, 2006). Alcohol's effect on increased energy intake from food is attributed to the alcohol's impact on CNS' γ-Aminobutyric acid (GABA) and opioids receptors which are involved in appetite control and reward mechanisms (Addolorato et al., 2006). Increased appetite and energy intake from food is also well documented for cannabis use (Farokhina et al., 2020; Ngueta et al., 2015). Little is known about the influences of alcohol and cannabis use with respect to the state of pregnancy.

Micronutrient intake

The results of the present study for micronutrient intakes reveal that OCN pregnant women are meeting and exceeding the DRI recommendations for vitamin A, vitamin C, thiamin, niacin, vitamin B12, choline, calcium and zinc. However, women's median intake of folate, iron and DHA was below the DRI recommendations and were not met by high proportion of participants. Similar micronutrient inadequacies were displayed by other Canadian studies. A report by Berti and colleagues (2008) revealed that pregnant, lactating and women of child-bearing age, residing in Canadian Arctic communities had inadequate intake levels of magnesium, calcium, vitamins A, C, E, and folate as well as infrequent use of nutritional supplement, which was also identified in this cohort. A Manitoban report by Chan and colleagues (2010) corroborated these findings by detecting inadequate intakes for vitamin A, folate, calcium, and iron for women ages 19-50. However, this report did not focus on pregnant and lactating women. This finding is concerning due to the roles of folate and choline in DNA methylation cycle, gene expression, cell

differentiation and proliferation, which occurs at a higher rate in the first 12 weeks of gestation. The deficits of nutrients involved in methylation cycle lead to complex postnatal metabolic abnormalities, tumors, neural tube defects, and neurodegenerative or psychiatric disorders (Blom & Smulders, 2011).

The present study also found associations between niacin, vitamin C, calcium and self-reported alcohol exposure, through the Spearman correlation and hierarchical regression analysis. Although these findings need further testing due to methodological implications of small sample size, data normality, variability, and overall cross-sectional design limitations, these results point toward interactions between risk variables and micronutrient intake. Associations between vitamin C and niacin intake and alcohol consumption raise concern, especially in combination with low intake of food groups which are common sources of these nutrients, as identified by the food group intake. Vitamin C and vitamin B-group are common nutrient deficiencies seen in alcohol consuming individuals (Lieber, 2003), thus adequate intakes of these nutrients are especially critical during gestation.

No known Canadian studies have reported the associations between maternal nutrient intake and risk exposures for First Nations pregnant women living on-reserve. Our laboratory has previously conducted a similarly designed pilot study in urban settings and reported similar findings for pregnant women who are at risk of consuming alcohol during pregnancy (Dyck, 2016). While the study included all Indigenous women, the study did not specifically investigate intakes for First Nations women only, therefore meaning comparative parallels are difficult to execute. Several international studies have investigated dietary intakes in the context of maternal alcohol consumption (May 2014; 2016; Carter et al., 2017; Goathup et al., 2017). May and colleagues 2014, found that women who had children with FASD had lower mean EAR (%) for riboflavin ($p<0.05$), calcium ($p<0.01$), choline ($p<0.05$) compared to healthy controls. The study by the same group of researchers reported opposing results where exposed mothers had significantly higher mean intakes of vitamin D, thiamin, phosphorus ($p<0.05$), selenium and omega-3 fatty acids ($p<0.01$) compared to unexposed controls (May et al., 2016). Unlike the women in our study, the participants (in case and control groups) of both studies by May and colleagues (2014; 2016) were substantially below the recommended intakes (% DRI) for nearly all of the studied nutrients (May et al., 2014; 2016). Due to May studies being done in South

African locality with different ethnic and socio-demographic conditions, relevant comparisons cannot be made. Canadian population-based studies in pregnant First Nations women living on reserves is required.

Taken together, the findings of our study point toward interactions between maternal dietary intake and risk exposures, especially alcohol consumption. The present study could not properly assess the amount of alcohol associated with reduced intake, due to poor response rate to the questions about timing, frequency, and the pattern of consumption and lack of biological sampling. Based on the results obtained from this study, it is evident that more rigorous nutrition and risk exposure association studies, with ascertainment through biological markers, are required to obtain reliable and generalizable findings. Identifying the levels, timing, and frequency of alcohol consumption which lead to dietary deficits is necessary, as experimental studies demonstrate that various levels of consumption are associated with adverse physiological outcomes, including small amounts (Kesmodel et al., 2002). For example, alcohol-related effects which do not meet the criteria FASD, such as spontaneous abortion (Kesmodel et al., 2002), still birth (Kesmodel et al., 2002), and low birth weight baby (Passaro et al., 1996; Addila et al., 2021), were reported in women who consumed low to moderate amounts of alcohol during pregnancy (approx. 1/day or 2 drinks/day).

The overall findings of this study cannot be taken out of context of historic policies of colonization, assimilation, and residential school system (Tait, 2003). Maternal health specifically has been impacted through the eradication of midwifery, whereby traditional prenatal practices were criminalized (Wolfson et al., 2022), enactment of birthing evacuation policy and the medicalization of birth (Kornelsen et al., 2010; Lawford et al., 2018). The overall history of colonization, the present-day policies of evacuation, lack of access to maternal care in the communities affect First Nations women's agency, capacity, and child-rearing practices and perpetuate intergenerational trauma manifesting in various ways, including risk exposures.

Strengths

One particular strength of this project lies in community's involvement in the planning and execution of the project. Extensive community consultations were performed at all stages of the project, as well as throughout the project, through the regular reporting, presentation meetings,

and community event attendance. This greatly contributed to building trust-based relationship with the community residents, BW Health Center staff, and participants.

Another noteworthy strength of the study is the continuous and consistent presence of the researcher (OK) in the community. This allowed for further relationship building with each of the participants, as well as accounting the unique circumstances of each participating woman. Such collaborative research practices prompt respect and recognition of the knowledge and capabilities of the participants and valuing of each individual experience (Hayward et al., 2021). Studies indicate that collaborative and involved approach to research with Indigenous communities increases the probability of the knowledge transfer, which occurs during research process (Sheridan, 1998). Furthermore, collaborative research creates distinctive opportunities for a researcher to take on a more profound perspective of the community and participants, thus more critical evaluation of results and interpretation (Sheridan, 1998).

This study utilized an interactive Food Frequency Questionnaire (FFQ) coupled with 24-hour dietary recall. FFQ enables the evaluation of long-term dietary intakes in a reasonably simple, cost-effective, and time-efficient manner (Feskanich et al., 1993). Noteworthy, that the FFQ used in this study was tailored specifically for the data collection on nutrients important for CNS development as published by Young et al., (2014). Additionally, the questionnaire placed particular emphasis on cultural appropriateness, sensitivity, and competency. This is especially important since dietary characteristics are influenced by socioeconomic, cultural, and environmental attributes of the study population (Teufel, 1997).

Limitations

One of the most critical limitations of this project was a small sample size. Due to OCN's notable distance from the city of Winnipeg, the ability to collect an appropriate number of participants was constricted. Although the present study is a pilot study, the sample size of 37 impeded various forms of sub-analysis. In addition, a small sample size reduced statistical power and generates complications for generalizing these findings to the maternal population of various First Nations communities.

Another limitation of the project is inherent to FFQ and 24-hour dietary recall, which rely solely on self-report. It generates participant's response-bias and complicates accurate assessment and

interpretation. One major criticism of self-reported data is the extent of error in portion size reporting (Prentice, 1996). This error is substantial, as supported by steady findings from comparisons studies of self-reported total energy expenditure (21.5-67.0% of various cohorts underreported and 1.0-6.0% over-reported intakes) (Prentice et al., 2013; Prentice, 1996). The measurement error contributes to the minimization of statistical power and complicating results generalizability and interpretation. To mitigate the impact of this limitation on the study results, food models were used, as well re-affirming and clarifying questions with respect to portion sizes, brand names, and frequency of intake were asked. A similar study with recovery biomarkers would greatly strengthen the results and its applicability.

The collection of sensitive information also induced limitations to transparent data collection. Not all participants answered the questions about alcohol intake, drug use, and smoking during pregnancy. Although re-assured of confidentiality, not all participants decided to disclose their answers. This might have been due to the intrusiveness factor and respondents might have viewed it as an invasion of privacy and inappropriate for the conversation. Another factor contributing to the lack of responses for this section could have involved social desirability, as consumption of alcohol during pregnancy is generally deemed as socially undesirable behavior. However, when probing questions were asked about alcohol consumption prior to finding out about pregnancy, a number of women disclosed that they had alcohol after conception but prior to their knowledge of it. As discussed *in Chapter 3*, the self-reported confirmation of alcohol consumption affects not only individual studies, but the diagnostic field of FASD, due to requirement of the confirmation (Cook et al., 2016). The elucidation of reliable alcohol use biomarker is required.

Another potential limitation is participant recruitment through the maternal program. Although the researcher's strong community engagement is outlined as a strength of this study, the recruitment through the maternal program may be contributing to the increased representation of health-conscious individuals, introducing a higher degree of social desirability and response bias to the data. This limitation was addressed through the application of location flexibility and willingness of the researcher (OK) to meet participants in their own environments.

Lack of extensive validity and reliability testing of the research instrument posed another set of limitations for this project. Although the questions of the survey instrument were derived from

CCHS and content validity was performed through Mothering Project at Mount Carmel clinic (Giesbrecht, 2015), the survey instrument did not undergo reliability tests. Testing for reliability is pivotal as it yields information about congruency and consistency of the instrument items, across the parts of a questionnaire. Despite lack of reliability testing, a pre-test was performed with 10-First Nations women from Point Douglas area in Winnipeg. Women provided oral and written feedback on the readability of the questionnaire, understanding of it, overall content, and visual aspects of the instrument. These methods of validity testing were congruent with those described by Czuber-Dochan and colleagues (2014) and Taherdoost (2016).

During the follow-up participation, the data was gathered only on the dietary information (24-hr recall, FFQ). Although, follow-up data collection was completed, due to an exceptionally small sample size, the analysis on the follow-ups was not performed and not presented in this section.

Conclusion

The preliminary findings presented in this study provide important baseline data on dietary patterns and nutrient intake of micronutrients important for CNS development of First Nations pregnant women residing on a northern Manitoban reserve. The study also identifies differences in dietary patterns and micronutrient intakes between women who are at-risk and non-at-risk of carrying a child with FASD. Future research should focus on exploring in more detail the patterns of maternal health with respect to clinical nutrition markers, alcohol markers and neonatal outcomes. The information presented in this study should be utilized by health professionals, dietitians, clinicians, and public health professionals for the planning of preventative maternal health care delivery on reserve.

Table 5-1. Opaskwayak Cree Nation community profile

Parameters	OCN Census Subdivisions			Division No. 21 (Nor-man region)[1]
	21 E[1]	21 I[2]	21 A[3]	
Total population	2,473	294	210	21,983
Population change since 2011	6.6%	81.5%	16.0%	2.8%
Total no. of households	600	95	70	9,162
Ave. family size	3.5	3.3	2.8	3.0
Ave. persons per household	4.1	3.1	3.0	2.7
Median population age (years)	23.7	33.5	39.0	34.8
Educational attainment (individuals ≥25 years of age):				
Secondary	23.7%	27.3%	31.8%	27.9%
Post-secondary	37.7%	50.0%	40.9%	49.9%
No post-secondary	38.6%	27.2%	27.3%	22.2%
Total median household income	$48,256	$109,312	$50,304	$72,028
Total median individual income	$18,288	$39,808	X	$31,220
Unemployment rate (individuals ≥25 years of age):	18.2%	6.7%	23.8%	9.8%

[1]Census Profile, 2016 Census. Opaskwayak Cree Nation 21E, Indian reserve;
[2]Census Profile, 2016 Census. Opaskwayak Cree Nation 21I, Indian reserve;
[3]Census Profile, 2016 Census. Opaskwayak Cree Nation 21A, Indian reserve.
X, data are not available

Table 5-2. Maternal demographic, health, and pregnancy characteristics of all participants and by self-reported alcohol consumption

Characteristic	All women (n=37)	Non-at-risk (n=22)	At-risk (n=15)	P-value
Age (years)[a]	24.4 ± 7.0	21.5 ± 5.5	28.1 ± 7.4	**0.007**
≤18	9 (24)	7 (32)	2 (13)	
19-30	22 (59)	14 (64)	8 (53)	
31-42	6 (16)	1 (4)	5 (33)	
Education[b]				0.196
Elementary	1 (3)	1 (5)	0	
Junior high	16 (44)	12 (55)	5 (33)	
High school	15 (41)	6 (27)	8 (53)	
Certificate	2 (6)	1 (5)	1 (7)	
University	3 (8)	2 (9)	1 (7)	
Employment[b]				0.237
Unemployed	14 (39)	8 (38)	6 (40)	
Employed part-time	4 (11)	2 (10)	2 (13)	
Employed full-time	6 (16)	4 (19)	2 (13)	
Student	10 (28)	5 (24)	5 (33)	
Maternity Leave	2 (6)	2 (9)	0	
Social Assistance[c]	19 (51)	10 (46)	9 (60)	0.385
# of household residents[b]	4 (3-5)	2 (1-3)	2 (1-4)	0.181
# of Adults (18+ years)	2 (1-3)	2 (2-3)	2 (1-4)	0.280
# of Children (<18 years)	2 (1-3)	2 (1-3)	1 (1-2)	0.360
Pre-pregnancy BMI[a]	26.5 ± 8.8	27.5 ± 8.3	25.9 ± 7.8	0.530
Below	4 (12)	2 (11)	2 (13)	
Normal	11 (33)	6 (33)	5 (33)	
Overweight	7 (21)	3 (17)	4 (27)	
Obese	11 (33)	7 (39)	4 (27)	
Chronic illness[c]:				
Before pregnancy	5 (15)	2 (10)	3 (20)	0.403
During pregnancy	7 (21)	4 (20)	3 (20)	1.000
Medications[c]:				
Prescribed	9 (30)	4 (20)	5 (33)	0.372
Over-the-counter	3 (9)	2 (10)	1 (6)	0.727
Vit & min supplements[c]	31 (94)	18 (90)	13 (87)	0.759
Smoking[c]	11 (37)	7 (35)	6 (40)	0.762
Drugs[c]	7 (19)	4 (19)	3 (20)	0.943
Trimester				
1st	12 (36)	9 (47)	3 (21)	
2nd	4 (12)	3 (16)	1 (7)	
3rd	17 (51)	7 (37)	10 (71)	
Pregnancy outcomes[b]:				
# of pregnancies	2 (1-4)	2 (1-3)	2 (1-5)	0.672
# of miscarriages	0 (0-1)	0 (0-1)	0 (0-1)	0.729
# of stillbirths	0 (0-0)	0 (0-0)	0 (0-1)	0.293
# of abortions	0 (0-0)	0 (0-0)	0 (0-1)	0.620
# of full-term births	1 (0-1)	1 (0-2)	1 (0-3)	0.201
# of pre-term births	0 (0-0)	0 (0-0)	0 (0-1)	0.630
Self-reported health[b]				
Excellent	3 (9)	1 (5)	2 (13)	0.525
Very Good	16 (46)	10 (45)	6 (40)	
Good	6 (17)	2 (9)	4 (27)	
Fair	10 (28)	7 (32)	3 (20)	
Poor	0	2 (9)	0	
Bed rest during pregnancy[c]	6 (18)	5 (25)	1 (7)	0.179

Values are means ± SD, n (percentages), and medians (Q1-Q3). The differences between the risk groups were tested by an independent t-test[a], Wilcoxon rank-sum test[b] or a Chi-square or Fisher's exact tests of independence[c].

Table 5-3. Maternal macronutrient intake of all participants (n=37)

Dietary variables	Median (Q1-Q3)	% Inadequate (95% CI)
Food group (#/day)[†]		
Vegetable and Fruit	5 (3-7)	94 (81-99)
Grain Products	5 (3-7)	89 (74-97)
Milk and Alt.	1 (0-3)	94 (81-99)
Meat and Alt.	2 (2-3)	86 (71-95)
Macronutrient (g/day)		
Protein	81 (64-107)	
Carbohydrate	248 (179-324)	
Fat	66 (52-96)	
Fiber	12 (5-15)	
Sugar	78 (46-113)	
Energy (kcal/day)	1877 (1369-2423)	
Protein	347 (257-430)	
Carbohydrate	990 (719-1287)	
Fat	591 (424-855)	
Sugar	314 (176-409)	
Energy from macronutrients (%)[††]		
Protein	16 (11-19)	18 (8-32)
Carbohydrate[†††]	54 (42-59)	37 (24-50)
Fat	14 (10-17)	50 (34-65)

Data derived from 24-hour dietary recall. [†]Prevalence of inadequacy was assessed using former Health Canada's Eating Well with Canada's Food Guide (2007). [††]Prevalence of inadequacy was assessed using Health Canada's Acceptable Macronutrient Distribution Range (2022). [†††]Carbohydrate includes sugar.

Table 5-4. Maternal micronutrient intake of all participants (n=37)

Dietary intake	Median intake (Q1-Q3)	Median (IQR) %DRI[1]	% Inadequate (95% CI)
Vitamin A (RE) (mcg)	1290 (854-1825)	235 (151-355)	0 (0-0)
Vitamin C (mg)	123 (90-205)	232 (128-292)	16 (6-32)
Thiamin (Vit B1) (mg)	3 (2-4)	228 (165-317)	8 (2-22)
Niacin (Vit B2) (mg)	32 (21-45)	213 (137-307)	5 (0-12)
Folate (Vit B9) (mcg)	425 (309-560)	84 (59-108)	68 (50-82)
Cobalamin (Vit B12) (mcg)	10 (6-14)	441 (286-634)	3 (0-14)
Choline (mg)	487 (350-667)	108 (78-148)	43 (27-61)
Calcium (mg)	813 (656-1288)	107 (81-149)	57 (40-73)
Iron (mg)	19 (14-26)	84 (62-118)	68 (50-82)
Zinc (mcg)	16 (11-21)	163 (114-194)	19 (8-35)
DHA (mcg)	78 (42-105)	38 (20-51)	97 (86-100)

Data derived from FFQ. Nutrient inadequacy was assessed using Health Canada's Dietary Reference Intake, Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50 for vitamins A, B12, C, folate, thiamin, niacin, zinc, calcium, iron; Adequate Intake (AI) for choline. Recommendations for DHA (docosahexaenoic acid, C22:6n-3) were obtained from Global Recommendations for DHA and EPA (eicosapentaenoic acid, C20:5n-3) Intake (2008).

Table 5-5. The effect of individual predictors (Spearman's r) on maternal macronutrient intake (% energy) (n=37).

Variables	Total Energy (kcal)	Protein (%)	Fat (%)	CHO (%)	Fiber (%)†
Risk exposure					
Alcohol (Yes/No)	**0.428***	0.141	**0.551****	0.033	-0.078
Smoking (# of cigarettes/d)	-0.106	-0.224	-0.330	0.630+	0.111
Drugs (Yes/No)	0.276	0.278+	**0.395***	0.188	0.142
Demographic					
Age	0.171	**0.376***	**0.380***	0.170	0.056
Education (level)†	-0.107	0.133	0.208	0.083	0.087
Employment	-0.046	-0.110	**-0.357***	0.117	**0.395***
Soc. Assistance (Yes/No)	-0.297	-0.140	-0.106	0.024	-0.134
Health status					
BMI††	-0.008	0.305+	0.020	-0.094	0.097
Chronic illness (Yes/No)	0.037	0.189	**0.345***	0.000	**-0.381***
Maternal pregnancy					
Trimester	0.294	-0.059	0.163	-0.107	-0.132
# of pregnancies	0.207	0.262	**0.368***	-0.058	-0.126
# of births	-0.121	0.318+	0.107	0.036	0.104

†Fiber - (%) Adequate Intake (AI) (Health Canada, 2010). ††Levels of education included the following variables in ordinal progression: elementary school, middle school, high school, college, university/ professional undergraduate degree, graduate degree. †††Employment included the following variables in ordinal progression unemployed, employed part-time, employed full-time. Pre-pregnancy BMI. Note: *p <0.05, ** p <0.01; +trending toward significance, p<0.1.

Table 5-6. The effect of individual predictors (Spearman's r) on maternal micronutrient intake (% DRI) (n=37).

Variables	Vitamin A	Vitamin C	Thiamin (Vit B1)	Niacin (Vit B2)	Folate (Vit B9)	Cobalamin (Vit B12)	Choline	Calcium	Iron	Zinc	DHA
Risk Exposure											
Alcohol (Yes/No)	0.075	-0.245	-0.108	**-0.210***	-0.180	-0.199	-0.176	-0.203	-0.104	-0.209	0.077
Smoking (# of cigarettes/d)	0.106	-0.037	-0.399	-0.260	0.168	0.087	0.087	0.256	0.131	-0.125	-0.206
Drugs (Yes/No)	0.123	-0.061	0.059	-0.116	0.004	0.110	-0.088	0.016	0.143	-0.063	0.046
Demographic											
Age	0.082	-0.302^{+}	**-0.360***	**0.374***	-0.215	-0.010	-0.099	0.043	-0.188	-0.121	0.268
Education (level)†	-0.030	-0.011	**-0.380***	0.138	-0.155	-0.228	-0.249	-0.176	-0.187	-0.297^{+}	0.263
Employment††	0.057	0.146	0.234	-0.059	0.009	-0.195	-0.079	-0.199	-0.150	-0.114	-0.097
Soc. Assistance (Yes/No)	0.003	0.215	-0.023	0.007	0.154	0.058	-0.096	0.170	0.175	0.033	0.030
Health status											
BMI†††	0.249	-0.194	-0.240	0.120	0.004	**0.361***	-0.075	-0.182	-0.012	-0.151	0.279
Chronic illness (Yes/No)	0.201	-0.055	0.129	0.060	0.129	0.123	0.062	0.084	0.181	-0.057	0.188
Maternal pregnancy											
Trimester	0.135	**-0.440***	-0.037	0.121	-0.055	0.100	-0.003	-0.218	0.086	0.123	0.168
# of pregnancies	0.106	-0.231	-0.180	-0.047	-0.105	0.252	-0.050	-0.030	0.000	-0.069	0.172
# of births	0.128	0.191	-0.192	-0.065	0.129	-0.122	0.167	0.075	-0.047	0.085	0.215
Dietary Intake											
Total energy	-0.055	**0.319***	**0.524****	0.104	-0.163	-0.031	-0.223	**-0.409***	0.005	-0.228	-0.110
V & F†††† (# of servings/d)	-0.107	0.214	0.033	0.078	-0.255	-0.150	-0.065	0.026	**-0.367***	-0.063	-0.070
Grain (# of servings/d)	0.186	-0.135	-0.104	0.073	0.118	0.216	0.082	-0.289	0.272	0.095	0.020
Milk & Alt (# of servings/d)	0.020	-0.019	-0.216	-0.125	-0.288	-0.108	-0.233	0.008	-0.322^{+}	-0.149	-0.194
Meat & Alt (# of servings/d)	0.253	-0.148	0.180	0.096	-0.124	0.066	-0.042	-0.158	0.051	-0.020	-0.040

†Levels of education included the following variables in ordinal progression: elementary school, middle school, high school, college, university/ professional undergraduate degree, graduate degree. ††Employment included the following variables in ordinal progression unemployed, employed part-time, employed full-time. †††Pre-pregnancy BMI. ††††Vegetable & Fruit. Nutrient inadequacy was assessed using Health Canada's Dietary Reference Intake, EAR for pregnant women aged 14-18, 19-30, and 31-50 for vitamins A, B12, C, folate, thiamin, niacin, zinc, calcium, iron; AI for choline. Recommendations for DHA (docosahexaenoic acid, C22:6n-3) were obtained from Global Recommendations for DHA and EPA Intake (2008). Note: *p <0.05, ** p <0.01; $^{+}$trending toward significance, p<0.1.

Table 5-7. Hierarchical linear regression analyzing the predictors of maternal total energy and macronutrient intake (% energy) (n=37)

Block		Model I		Model II		Model III	
		β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
	Total Energy[†] (kcal)						
1	Alcohol (Yes/No)	0.446 (0.238)	0.073[+]	0.384 (0.267)	0.217	0.449 (0.326)	0.219
	Drugs (Yes/No)	0.394 (0.274)	0.163	0.373 (0.285)	0.203	0.358 (0.334)	0.296
2	Age			0.012 (0.028)	0.584	0.016 (0.025)	0.808
	Employment[††]			0.047 (0.098)	0.636	0.062 (0.108)	0.574
3	Trimester					-0.007 (0.152)	0.968
	BMI[†††]					0.010 (0.020)	0.502
	Chronic illness					0.053 (0.405)	0.897
	R^2	0.174		0.191		0.214	
	Protein (%, Energy)						
1	Alcohol (Yes/No)	-0.4 (2.4)	0.863	-2.5 (2.0)	0.431	-2.0 (3.1)	0.594
	Drugs (Yes/No)	3.0 (3.0)	0.381	2.3 (2.6)	0.473	2.8 (3.0)	0.766
2	Age			0.290 (0.200)	0.257	0.237 (0.224)	0.538
	Employment[††]			0.363 (0.960)	0.721	0.234 (1.000)	0.945
3	Trimester					-0.432 (2.012)	0.784
	BMI[†††]					0.142 (0.184)	0.960
	Chronic illness					-3.319 (2.903)	0.686
	R^2	0.029		0.122		0.152	
	Fat (%, Energy)						
1	Alcohol (Yes/No)	0.256 (0.086)	**0.007**	0.275 (0.100)	**0.012**	0.257 (0.111)	**0.032**
	Drugs (Yes/No)	0.125 (0.099)	0.221	0.139 (0.098)	0.171	0.063 (0.110)	0.574
2	Age			-0.004 (0.008)	0.570	-0.005 (0.009)	0.607
	Employment[††]			-0.057 (0.034)	0.108	-0.045 (0.036)	0.223
3	Trimester					-0.015 (0.050)	0.767
	BMI[†††]					-0.007 (0.006)	0.696
	Chronic illness					0.218 (0.134)	0.120
	R^2	0.288		0.365		0.397	
	CHO (%, energy)						
1	Alcohol (Yes/No)	-5.3 (4.2)	0.526	-7.6 (4.3)	0.381	-6.0 (4.5)	0.451
	Drugs (Yes/No)	2.2 (8.4)	0.800	1.5 (9.0)	0.890	-6.6 (9.6)	0.851
2	Age			0.500 (0.647)	0.610	0.454 (0.680)	0.644
	Employment[††]			0.328 (3.059)	0.921	-0.626 (3.180)	0.912
3	Trimester					-1.328 (4.354)	0.506
	BMI[†††]					-0.736 (0.619)	0.248
	Chronic illness					-16.583 (11.920)	0.640
	R^2	0.017		0.041		0.149	

Models description: Model I - risk exposures; Model II – risk exposures and demographic factors; Model III - risk exposures, demographic factors, and maternal health factors. [†]Total energy (kcal) and Fat (%, Energy) are log transformed. [††]Employment included the following variables ordinal progression: unemployed, employed part-time, employed full-time. [†††]Pre-pregnancy BMI; [+]trending toward significance, $p<0.1$.

Table 5-8a. Hierarchical linear regression analyzing the predictors of maternal micronutrient intake (% DRI)

Block		Model I β (SE)	p-value	Model II β (SE)	p-value	Model III β (SE)	p-value	Model IV β (SE)	p-value
	Vitamin A†								
1	Alcohol (Yes/No)	-0.018 (0.252)	0.943	-0.161 (0.306)	0.604	-0.202 (0.344)	0.565	-0.216 (0.355)	0.549
	Drugs (Yes/No)	0.142 (0.291)	0.628	0.121 (0.300)	0.690	0.028 (0.339)	0.934	-0.015 (0.372)	0.967
2	Age			0.020 (0.024)	0.398	0.017 (0.026)	0.513	0.018 (0.027)	0.503
	Employment††			0.010 (0.104)	0.927	0.057 (0.111)	0.614	0.065 (0.116)	0.584
3	Trimester					0.117 (0.155)	0.460	0.111 (0.160)	0.496
	BMI†††					0.013 (0.019)	0.496	0.014 (0.020)	0.490
	Chronic illness					0.265 (0.415)	0.531	0.250 (0.428)	0.565
4	Total Energy (kcal)							$5.953e^{-5}$ (0.000)	0.750
	R^2	0.010		0.042		0.131		0.136	
	Vitamin C								
1	Alcohol (Yes/No)	-120 (56)	**0.040**	-107 (67)	0.127	-78 (71)	0.289	-71 (73)	0.344
	Drugs (Yes/No)	-78 (64)	0.232	-80 (66)	0.235	-87 (70)	0.232	-65 (76)	0.402
2	Age			-1 (5)	0.793	-2 (5)	0.726	-2 (6)	0.668
	Employment††			18 (23)	0.432	8 (23)	0.734	4 (24)	0.862
3	Trimester					-64 (32)	0.061^{+}	-61 (33)	0.077^{+}
	BMI†††					-3 (4)	0.402	-4 (4)	0.371
	Chronic illness					22 (86)	0.803	29 (88)	0.745
4	Total Energy (kcal)							-0.029 (0.038)	0.452
	R^2	0.193		0.224		0.371		0.390	
	Thiamin (Vit B1)†								
1	Alcohol (Yes/No)	-0.041 (0.098)	0.827	0.079 (0.103)	0.452	0.087 (0.120)	0.479	0.056 (0.111)	0.625
	Drugs (Yes/No)	0.025 (0.111)	0.681	0.057 (0.101)	0.576	0.024 (0.119)	0.813	-0.065 (0.117)	0.586
2	Age			-0.018 (0.008)	0.062^{+}	-0.020 (0.009)	0.056^{+}	-0.018 (0.009)	0.064^{+}
	Employment††			-0.073 (0.039)	0.076^{+}	-0.077 (0.043)	0.062^{+}	-0.061 (0.039)	0.113
3	Trimester					-0.030 (0.060)	0.583	-0.041 (0.050)	0.405
	BMI††					-0.002 (0.007)	0.810	-0.001 (0.006)	0.952
	Chronic illness					0.085 (0.160)	0.582	0.054 (0.145)	0.694
4	Total Energy (kcal)							0.008 (0.002)	**0.041**
	R^2	0.061		0.247		0.303		0.462	

Models description: Model I - risk exposures; Model II – risk exposures and demographic factors; Model III - risk exposures, demographic factors, and maternal health factors; Model IV - risk exposures, demographic factors, maternal health factors, and total caloric intake. †Vitamin A and thiamin are log transformed. ††Employment included the following variables ordinal progression: unemployed, employed part-time, employed full-time. †††Pre-pregnancy BMI. $^{+}$trending toward significance, $p<0.1$.

Table 5-8b. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Niacin (Vit B2)								
1	Alcohol (Yes/No)	-90 (43)	**0.045**	-54 (51)	0.293	-51 (59)	0.397	-54 (61)	0.384
	Drugs (Yes/No)	-89 (49)	0.084[+]	-82 (50)	0.113	-73 (58)	0.221	-82 (64)	0.210
2	Age			-5 (4)	0.182	-5 (4)	0.269	-5 (5)	0.302
	Employment[††]			-10 (17)	0.568	-14 (19)	0.458	-13 (20)	0.527
3	Trimester					-11 (27)	0.686	-12 (27)	0.662
	BMI[†††]					-1 (3)	0.690	-1 (3)	0.725
	Chronic illness					-24 (71)	0.738	-27 (73)	0.713
4	Total Energy (kcal)							0.013 (0.031)	0.695
	R^2	0.228		0.288		0.310		0.316	
	Folate (Vit B9)[†]								
1	Alcohol (Yes/No)	-0.141 (0.203)	0.493	-0.066 (0.248)	0.715	-0.090 (0.289)	0.758	-0.011 (0.263)	0.968
	Drugs (Yes/No)	-0.246 (0.234)	0.303	-0.230 (0.243)	0.354	-0.150 (0.285)	0.604	0.085 (0.276)	0.761
2	Age			-0.011 (0.019)	0.554	-0.009 (0.022)	0.681	-0.014 (0.020)	0.478
	Employment[††]			-0.030 (0.084)	0.727	-0.039 (0.093)	0.681	-0.080 (0.076)	0.363
3	Trimester					0.57 (0.130)	0.665	0.088 (0.119)	0.465
	BMI[†††]					$2.887e^{-5}$ (0.016)	0.999	-0.003 (0.015)	0.831
	Chronic illness					-0.234 (0.349)	0.510	-0.155 (0.317)	0.630
4	Total Energy (kcal)							0.033 (0.012)	**0.030**
	R^2	0.058		0.074		0.100		0.302	
	Cobalamin (Vit B12)[†]								
1	Alcohol (Yes/No)	-0.344 (0255)	0.190	-0.494 (0.305)	0.119	-0.459 (0.338)	0.190	-0.488 (0.346)	0.174
	Drugs (Yes/No)	0.044 (0.294)	0.883	0.037 (0.299)	0.902	0.122 (0.333)	0.718	0.035 (0.363)	0.923
2	Age			0.019 (0.023)	0.431	0.012 (0.025)	0.634	0.014 (0.026)	0.590
	Employment[††]			-0.065 (0.103)	0.536	-0.038 (0.109)	0.730	-0.023 (0.113)	0.841
3	Trimester					0.146 (0.153)	0.350	0.134 (0.156)	0.399
	BMI[†††]					0.026 (0.019)	0.183	0.027 (0.019)	0.174
	Chronic illness					-0.238 (0.408)	0.567	-0.266 (0.417)	0.530
4	Total Energy (kcal)							0 (0)	0.520
	R^2	0.069		0.124		0.229		0.246	

[†]Folate and cobalamin are log transformed.

Table 5-8c. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Choline								
1	Alcohol (Yes/No)	-33 (23)	0.171	-39 (28)	0.173	-40 (33)	0.240	-39 (34)	0.264
	Drugs (Yes/No)	-33 (27)	0.222	-33 (28)	0.241	-33 (33)	0.321	-31 (36)	0.399
2	Age			0.805 (2)	0.712	0.954 (3)	0.705	1 (3)	0.729
	Employment††			-5 (10)	0.596	-6 (11)	0.588	-6 (11)	0.581
3	Trimester					-2 (15)	0.875	-2 (15)	0.894
	BMI†††					-0.496 (2)	0.791	-0.526 (2)	0.785
	Chronic illness					6 (40)	0.999	8 (41)	0.985
4	Total Energy (kcal)							-0.033 (0.018)	0.865
	R^2	0.120		0.142		0.147		0.148	
	Calcium								
1	Alcohol (Yes/No)	-48 (23)	0.087+	-56 (26)	**0.042**	-46 (29)	0.075+	-45 (30)	0.149
	Drugs (Yes/No)	-26 (26)	0.339	-28 (25)	0.285	-29 (29)	0.326	-25 (31)	0.444
2	Age			3 (2)	0.111	3 (2)	0.177	3 (2)	0.203
	Employment††			-6 (9)	0.518	-9 (9)	0.362	-10 (10)	0.344
3	Trimester					-19 (13)	0.167	-18 (14)	0.192
	BMI†††					-0.784 (2)	0.634	-0.842 (2)	0.619
	Chronic illness					4 (35)	0.914	5 (36)	0.619
4	Total Energy (kcal)							-0.006 (0.016)	0.715
	R^2	0.097		0.234		0.314		0.319	
	Iron†								
1	Alcohol (Yes/No)	0.013 (0.143)	0.928	0.106 (0.171)	0.542	0.071 (0.198)	0.723	0.032 (0.216)	0.886
	Drugs (Yes/No)	-0.073 (165)	0.602	-0.052 (0.169)	0.685	-0.002 (0.196)	0.918	-0.044 (0.212)	0.836
2	Age			-0.014 (0.013)	0.284	-0.012 (0.015)	0.426	-0.011 (0.015)	0.491
	Employment††			-0.043 (0.058)	0.461	-0.045 (0.064)	0.486	-0.042 (0.066)	0.535
3	Trimester					0.064 (0.089)	0.485	0.058 (0.092)	0.531
	BMI†††					0.000 (0.011)	0.990	0.000 (0.011)	0.970
	Chronic illness					-0.147(0.239)	0.569	-0.151 (0.244)	0.543
4	Total Energy (kcal)							0.000 (0.001)	0.610
	R^2	0.008		0.066		0.110		0.113	

Table 5-8d. Continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Zinc								
1	Alcohol (Yes/No)	-59 (41)	0.162	-66 (50)	0.201	-67 (58)	0.262	-65 (60)	0.579
	Drugs (Yes/No)	-42 (47)	0.385	-40 (49)	0.424	-29 (58)	0.620	-23 (63)	0.698
2	Age			0.403 (4)	0.917	0.883 (4)	0.843	0.746 (5)	0.871
	Employment††			-14 (17)	0.415	-17 (19)	0.373	-18 (20)	0.366
3	Trimester					-0.009 (26)	1.000	0.807 (27)	0.977
	BMI†††					-0.880 (3)	0.790	-0.964 (3)	0.777
	Chronic illness					-32 (71)	0.657	-30 (73)	0.687
4	Total Energy (kcal)							-0.008 (0.031)	0.792
	R^2	0.099		0.130		0.147		0.150	
	DHA†								
1	Alcohol (Yes/No)	-0.020 (0.110)	0.858	-0.090 (0.132)	0.503	-0.152 (0.143)	0.304	-0.082 (0.152)	0.594
	Drugs (Yes/No)	-0.119 (0.127)	0.357	-0.136 (0.119)	0.305	-0.105 (0.142)	0.467	-0.031 (0.151)	0.839
2	Age			0.011 (0.010)	0.283	0.014 (0.011)	0.228	-0.011 (0.011)	0.312
	Employment††			0.035 (0.045)	0.444	0.046 (0.044)	0.333	0.039 (0.046)	0.403
3	Trimester					0.116 (0.065)	0.090+	0.125 (0.064)	0.067+
	BMI†††					0.003 (0.008)	0.718	0.004 (0.008)	0.625
	Chronic illness					-0.095 (0.174)	0.591	-0.088 (0.171)	0.609
4	Total Energy (kcal)							-0.000 (0.007)	0.624
	R^2	0.041		0.090		0.219		0.282	

†DHA is log transformed.

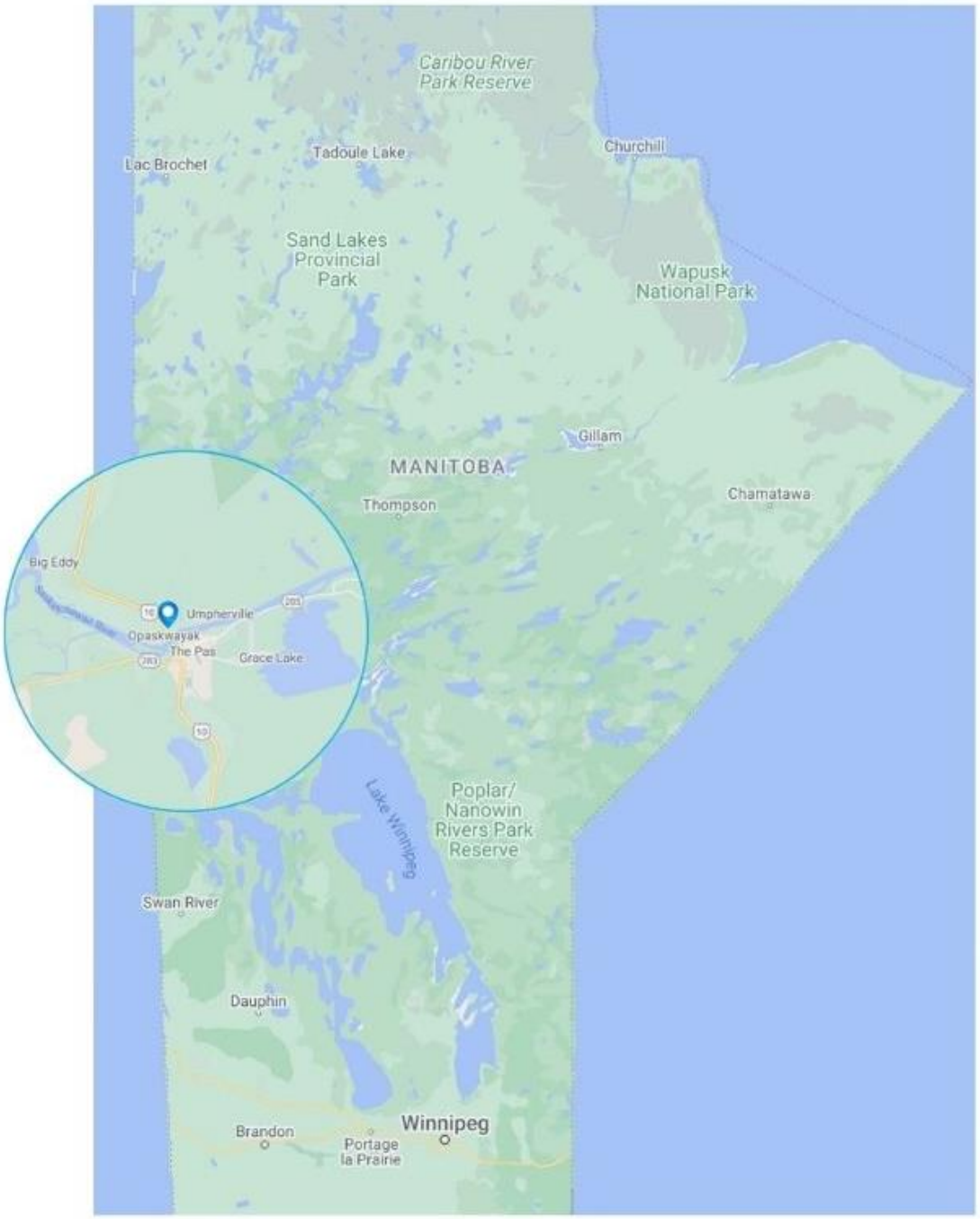


Figure 5-1. Opaskwayak Cree Nation Location.
Adapted from Google Maps.

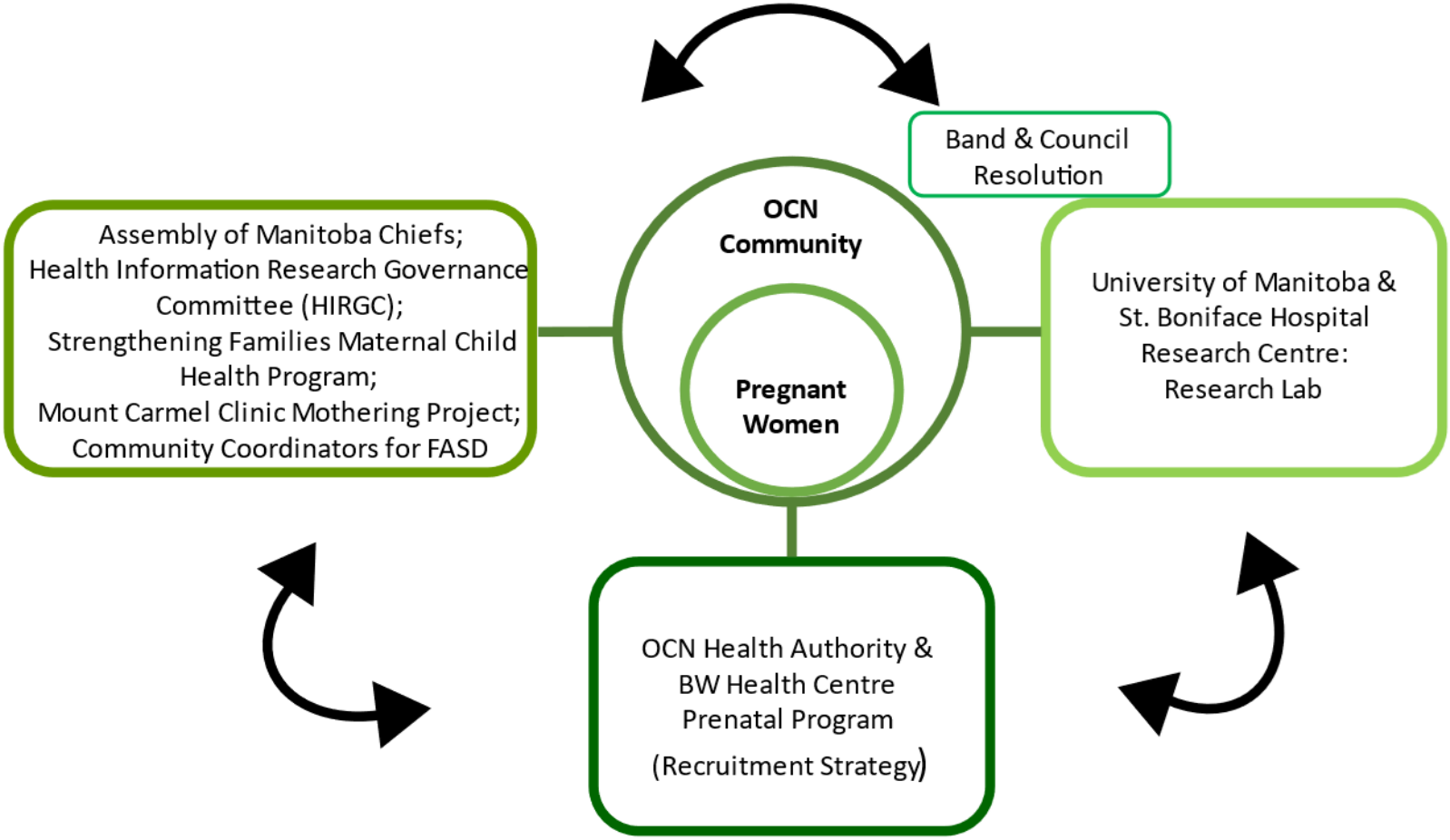
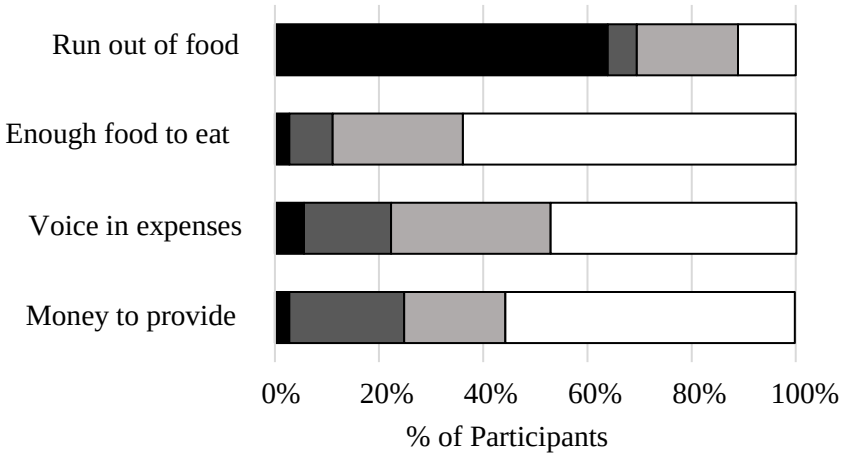


Figure 5-2. Community engagement and maternal program development logic model.

a)



b)

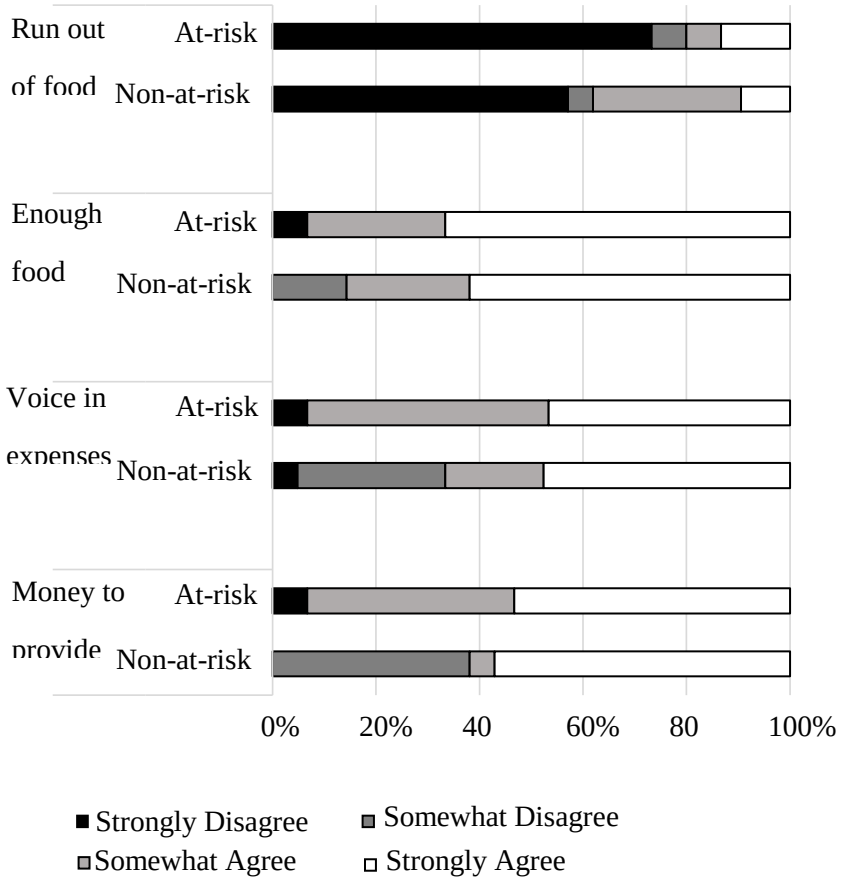


Figure 5-3. Self-reported access to financial resources a) all women (n=37); b) non-at-risk (n=22) and at-risk women (n=15). Data presented in proportion of participants. No significant difference was observed.

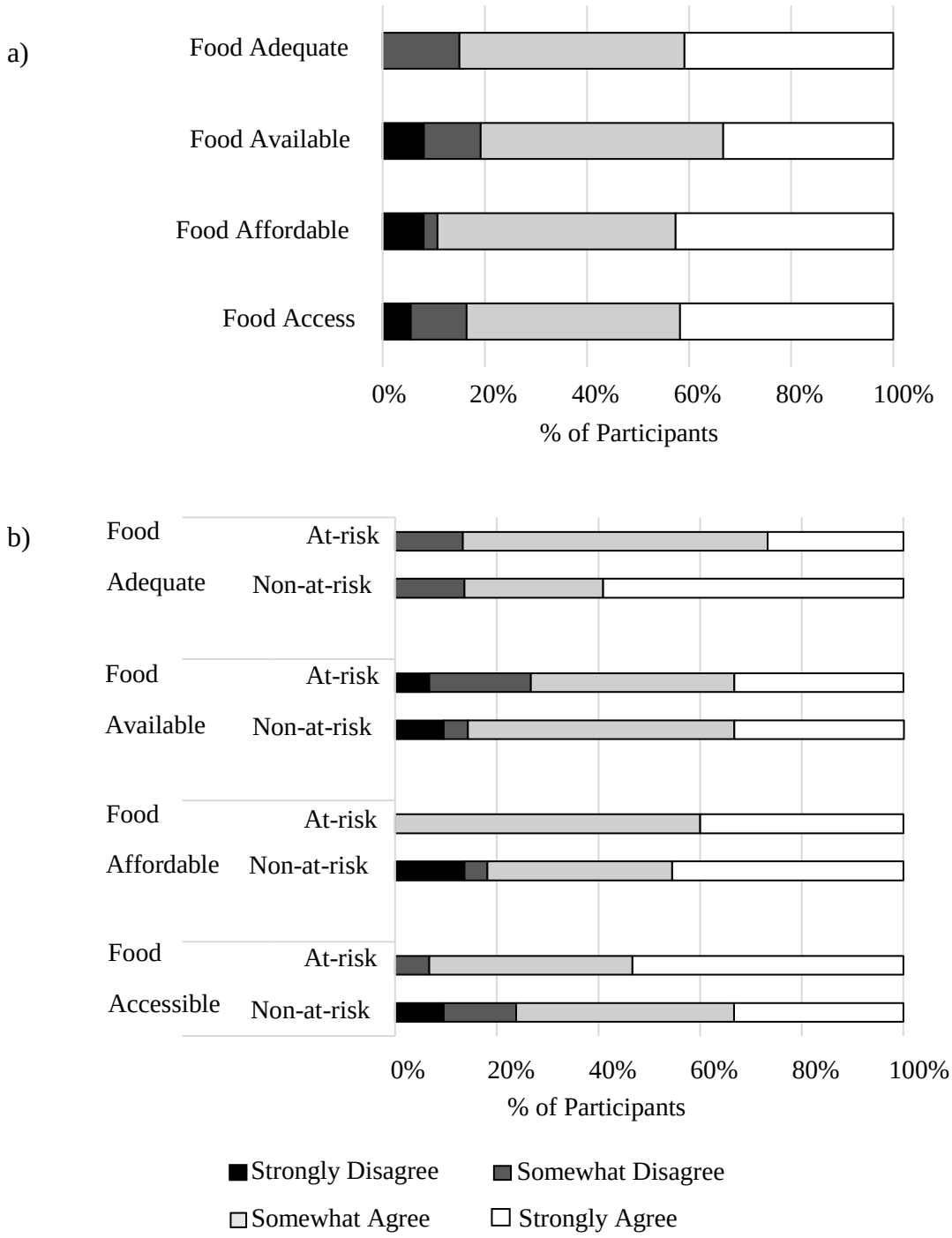


Figure 5-4. Self-reported access to food a) all women (n=37); b) non-at-risk (n=22) and at-risk women (n=15). Data presented in proportion of participants. No significant difference was identified between non-at-risk and at-risk groups.

CHAPTER VI: THE EXPLORATORY STUDY ON NUTRIENT INTAKE AND RISK EXPOSURES OF PREGNANT FIRST NATIONS WOMEN: IMPLICATIONS FOR FASD

*This Chapter presents the findings of two pilot studies conducted in pregnant, status, First Nations women from the Chemawawin Cree Nation (CCN) and Opaskwayak Cree Nation (OCN). Since there was no significant difference in macro- and micro-nutrient intake between these two pilot studies, except for two variables (Meat & Alternative and %fat intake) (data are presented in this chapter), the data were pooled and investigated the influence of alcohol consumption on the dietary intake of nutrients known to be important for fetal central nervous system development. The analytical methodology was based on previously completed two pilot studies.

Abstract

Background: Suboptimal maternal nutrition intake has been implicated in Fetal Alcohol Spectrum Disorder (FASD) outcomes, however, information on the nutrition intake and dietary behaviors in relation to risk exposure during pregnancy is scarce, particularly in hard-to-reach First Nations women living in remote communities. ***Objective:*** This study aimed to determine the macro- and micro-nutrient intakes and identify the relationship between nutrient intake and risk exposures (primarily alcohol consumption) during pregnancy. ***Methods:*** Through partnerships with two Frist Nations communities in Manitoba 59 women, ages 14-4 2 participated in the study. An interactive questionnaire obtained information on participant demographics, anthropometrics dietary intake substance use, and maternal health. Nutrient values were assessed using Acceptable Macronutrient Distribution Range and Dietary Reference Intake measures. Hierarchical linear regression modelling was performed to evaluate the contributions of predictors in blocks (which included i) risk exposures, ii) demographics, iii) health, and iv) total caloric intake as covariates). ***Results:*** A high prevalence of inadequacy existed for the Vegetable and Fruit 94% (95%CI = 84-99), Grain Products 89% (95%CI = 77-96), Milk and Alternatives 94% (95%CI = 84-99), and Meat and Alternatives 77% (95%CI = 64-88) food groups. Similarly, high prevalence of inadequacy was observed for folate 78%, (95%CI = 65-88), iron 75%, (95%CI = 62-85), and DHA 93% (95%CI = 84-98). Self-reported alcohol consumption showed a significant association with total energy intake ($\beta = 0.291$; SE, 0.219; $p < 0.05$) and fat intake (%) ($\beta = 0.290$; SE, 0.122; $p < 0.05$) in the model adjusted for other risk exposures. Inverse relationship was detected between the self-reported alcohol consumption and DRI (%) for niacin across all four regression blocks ($\beta = -0.103$; SE, 0.27; $p < 0.01$), ($\beta = -0.99$; SE, 0.29; $p < 0.01$), ($\beta = -0.96$; SE, 0.31; $p < 0.01$), ($\beta = -0.99$; SE, 0.32; $p < 0.01$); folate ($\beta = -46$; SE, 0.19; $p < 0.01$), ($\beta = -0.42$; SE, 0.20; $p < 0.01$), ($\beta = -0.38$; SE, 0.26; $p < 0.01$), ($\beta = -0.33$; SE, 0.31; $p < 0.01$); choline for the first three (I-III) regression blocks ($\beta = -0.661$; SE, 0.195; $p < 0.01$), ($\beta = -0.548$; SE, 0.228; $p < 0.05$), ($\beta = 0.545$; SE, 0.255; $p < 0.05$); and calcium for the I, III, and IV block ($\beta = -0.556$; SE, 0.223; $p < 0.01$), ($\beta = -0.352$; SE, 0.266; $p < 0.05$), and ($\beta = -0.468$; SE, 0.310; $p < 0.05$). ***Conclusion:*** These study outcomes suggest dietary intake is affected by alcohol consumption. The findings of this study provide useful insight for maternal community programs and information for future research directions for maternal First Nations populations.

Keywords: FASD; Dietary patterns, Nutrient intake, First Nations, Prenatal nutrition.

Introduction

Maternal health, defined by the World Health Organization (WHO) as "women's health during pregnancy, childbirth, and the postpartum period", is a key determinant of infants' developmental and physiological outcomes through adulthood (WHO, 2018). Maternal health among First Nations population – one of the three constitutionally recognized Indigenous groups in Canada, is consistently reported to be poorer compared to the general Canadian maternal population, as measured by maternal and newborn health indicators (Sheppard et al., 2017; Smylie et al., 2010; Luo et al., 2010; Auger et al., 2013). First Nations population in Canada experience substantially higher infant mortality rates (1.7 to over 4 times higher) (Smylie et al., 2010), rate of death from sudden infant death syndrome (SIDS) (7 times higher) (Sheppard et al., 2017), stillbirth (5.7 per 1000 births vs 3.6 per 1000 births) (Auger et al., 2013), and preterm birth (9.0 per 100 births vs 6.6 per 100 births) (Sheppard et al., 2017) compared with non-Indigenous Canadians. Elevations in maternal and infant negative health indicators are attributed to the disparities in proximal and distal determinants of health such as congenital abnormalities and infections (proximal), inadequate access to prenatal care, risk exposures, and overall poor quality of life (distal).

Fetal alcohol spectrum disorder (FASD) – a constellation of congenital abnormalities associated with prenatal alcohol exposure, has also been reported to be higher in First Nations communities. The most cited estimate for FASD prevalence in Canada is 9 in 1,000 births (Government of Canada, 2017). According to a meta-analysis on FASD estimates in Indigenous North American populations, the pooled prevalence of FASD in First Nations Canadian population is 86.8 per 1000 births (95% CI: 0.0-198.7 per 1000) (Popova et al., 2016). Higher estimates of FASD in First Nations communities resulted in identifying FASD as a public health concern among Indigenous Peoples by National Collaborating Centre for Aboriginal Health (NCAH), Truth and Reconciliation Commission (TRC), Government of Canada (NCAH, 2009; TRC, 2015; House of Commons, 2006).

Risk exposures are some of the major attributes of health disparities in Canada's First Nations population. Women in First Nations communities have 10 times higher relative risk of death due to alcohol consumption compared with their non-Indigenous counterparts (Park et al., 2015). First Nations women are also more likely to engage in binge drinking, including during

pregnancy (Lavallée & Bourgault, 2000; MacMillan et al., 2003). Presently, there is a debate whether there is a safe level of alcohol consumption during pregnancy (NCAH, 2009; CanFASD, 2013). While the dose-response relationship between maternal alcohol consumption and alcohol-induced structural and functional effect on a developing fetus is not known, research reveals that various amounts, times of fetal exposure, and patterns of consumption contribute to great variation in fetal malformations and neurodevelopmental outcomes (May et al., 2005; May & Gossage, 2011; Abel & Hannigan, 1995; Young et al., 2014). This variation is attributable to various developmental mechanisms playing distinct roles at different gestational periods (Jones & Smith, 1973; Abel & Hannigan, 1995; May et al., 2013). This evidence lay the foundation for the establishment of low-risk and abstinence guidelines from alcohol consumption during pregnancy in North America (Thomas et a., 2014).

Although alcohol consumption during pregnancy is the number one risk factor for FASD, food security, nutrition intake, and status have received significant attention as being the major modifiable, predisposing factors contributing to FASD (May & Gossage, 2011; Ballard et al., 2011; Abel & Hannigan, 1995; Young et al., 2014). Overall, prenatal nutrition, maternal body composition, nutritional stores, and dietary characteristics have significant and enduring consequences on offspring health long-term and disease risk into adulthood (Zhang et al., 2005; Ramakrishnan et al., 2012; Hsu & Tain, 2019; Barker, 1992). The physiological changes associated with pregnancy necessitate increased macro- and micro-nutrient intake to sustain fetal development (Black, 2001). Inadequate intake of macro and micronutrients during pregnancy has been implicated in various negative maternal and infant health outcomes (intrauterine growth restriction, premature birth, and having a low-birth-weight infant) (Abu-Saad & Fraser, 2010; Brough et al., 2010). While the mechanisms of alcohol's impacts on macro- and micro-nutrients have not been comprehensively elucidated, alcohol consumption can lead to malnutrition by displacing energy from macronutrients and interfering with nutrient uptake from the gastrointestinal tract, metabolism cycles, nutrient cellular transport, and cellular utilization (Lieber et al., 2000; Kloss et a., 2022; Young et al., 2014). A body of research demonstrates compounding negative influences of alcohol and nutrient deficiencies on the structure and function of the fetal central nervous system (CNS) (Marrs et al., 2010; Ba, 2011; Yamamoto et al., 1988), cardiac tissue (Ford et al., 2021; Serrano et al., 2010), endocrine system (Huebner et al., 2016), and skeletal system (Moghimi et al., 2017). Moreover, nutrient supplementation

research identifies restorative effects on the structural, functional, behavioral, and neurocognitive anomalies associated with ethanolic damage (Young et al., 2014; Sebstiani et al., 2018). Specifically, prenatal supplementation studies on antioxidants, vitamin A, folate, thiamin choline, docosahexaenoic acid (DHA), and zinc revealed reduction in alcohol induced-oxidative damages (Ojeda et al., 2009; Memon & Pratten, 2009; Cano et al., 2001), improved CNS function (Tveden-Nyborg et al., 2012; Bekdash et al., 2013; Monk et al., 2012), boosted fetal growth (Hewitt et al., 2011), restored heart development (Ford et al., 2021; Linask et al., 2016), and improved cognitive and behavioral processes (Jacobson et al., 2018; Thomas et al., 2004; Thomas et al., 2007).

Studies have shown that First Nations pregnant women and women of childbearing age have inadequate intake of Vegetables and Fruits, Milk and Alternatives, and Grain Products (Back et al., 2012; Chan, 2012). Other studies have shown inadequate consumption of micronutrients, including vitamins A, C, E, D, folate, calcium, iron, and magnesium in women of reproductive age in First Nations communities (Berti et al., 2008; Chan, 2012; Waiters et al., 1998). Furthermore, high rates of low individual and total household income, the primary determinant of food security, are well documented for First Nation individuals, including women (Heaman & Chalmers, 2005; Wenman et al., 2004).

Despite the evidence pointing toward the potential benefits of nutrition intervention as a preventative measure for FASD, currently nutrition is not at the forefront of FASD prevention programming. This is due to a dearth of conclusive information on maternal baseline nutrition status, intake, and overall patterns, especially in Frist Nations maternal population, which has not been actively included in population-based nutrition surveillance programs or general nutrition research. Furthermore, to our knowledge no studies explored the relationships between dietary intake and risk exposures, mainly alcohol consumption for First Nations maternal population, except our previously conducted pilot studies reported in *Chapter 3* and *Chapter 5*. This gap in knowledge restricts evidence-based maternal health program development and maternal public health policy in First Nations communities. Thus, the objective of the present study is to address this knowledge gap by identifying and analyzing the relationship between nutrient intakes and primary FASD risk factor - alcohol consumption, as well as additional risk exposures – smoking and drug use. Since the two pilot studies (*Chapter 3 and 5*) had small sample size with the

participants from the similar population geographically and culturally, this study combined the data to find more generalizable results. This objective is met in collaboration with the First Nations communities of interest, supporting the TRC of Canada Call to Action 33 of recognizing the need FASD prevention, and developing, in collaboration people, FASD preventative programs that can be delivered in a culturally appropriate manner (TRC, 2015).

Methodology and Design

Detailed summaries of the methodology and design (Band and Council Approvals, study setting, community engagements, participants, datal collection tool, the assessment of risk variables, and the quantification of the dietary intake and adequacy) are provided in the *Methodology and Design* sections of *Chapter 3* and *Chapter 5*. This chapter demonstrates pooled findings for both communities, OCN and CCN, which took place between 2015-2019. Study history is presented in **Figure 6.1**. Since these communities are located within 200 km boundary, within the same ecozone, share same tradition as Cree Nations, this Chapter has attempted to pool the data from both communities to allow for higher power, improved generalizability, and interpretation of results. To test whether the community is a factor with respect to dietary and alcohol consumption variables and whether the results of both communities can be pooled, the intakes and risk exposure variables were analyzed by community prior to merging the data (Table 6-1, 6-2).

Sample size determination

The sample size computation approach is described in detail in *Chapters 3*. With sample size of 59, we could detect the partial correlation around 0.37.

Statistical analysis

The statistical methodology employed for this study of two communities is similar to that described in *Chapter 3* and *Chapter 5*. Briefly, the normality testing for variables distributions was performed by the Kolmogorov-Smirnov and Shapiro-Wilks tests. Total energy, (%) energy from protein, fat, and carbohydrates, (%DRI) vitamin A, vitamin C, thiamin, niacin, cobalamin, choline, calcium, iron, zinc, and DHA were not normally distributed.

Sample descriptive statistics (mean, SD, median, and IQR) were calculated for continuous variables and proportions with 95% CIs for categorical variables. Student's t tests and Wilcoxon rank-sum test were used to compare the difference between the two groups for normally and not normally distributed data, respectively. Chi-square (χ^2) test of independence was used to assess differences between categorical variables. Sub-group analysis stratified by community was conducted to identify whether there were any differences between the two communities. Kruskal-Wallis test was used to asses the macro- and micronutrient intake between trimesters. These results are presented in **Appendix 5**.

Similarly to the statistical methodology described in *Chapter 5*, hierarchical regression analysis with forward selection was employed to evaluate the level of contribution for each of the selected predictors on the macro- and micro-nutrient (%) energy and DRI (%) intakes. The dependent variables, independent variables, and the combination of the blocks are described in detail in *Chapter 5*. In addition to the selected variables identified in *Chapter 5*, this analysis of the two communities introduced two new independent variables: cigarette smoking in Block 1/Model 1and indicator for community in Block 2/Model 2. Therefore, the blocks were analyzed in the following order:

- Block 1/Model 1: included risk exposures (alcohol consumption, drug use, cigarette smoking);
- Block 2/Model 2: to the afore-stated risk exposes, the demographic factors (community ("0" for OCN, "1" for CCN), age, employment) were added;
- Block 3/Model 3: maternal health variables (trimester, pre-pregnancy BMI, chronic illness) were added to the risk and demographic factors. Block 3/Model 3 represents an omnibus model for each of the macronutrient variables.
- Block 4/Model 4: total energy (kcal) (applicable to micronutrients). This independent variable was added to identify the relationships between total energy collected from 24-hour dietary recall and FFQ. Block 4/Model 4 represents an omnibus model for each of the micronutrient variables.

The models were tested for multicollinearity problem using Variance Inflation Factor (VIF) (cut-off 2), the assessment of the normality of the residuals using Q-Q plots. To confirm the results of Q-Q plots, the normality of the residuals was tested using Wilks-Shapiro test. The non-normal residual distribution was identified for the following dependent variables: total energy, (%)

energy from protein, fat, and carbohydrates, (DRI%) vitamin A, vitamin C, cobalamin, choline, calcium, iron, zinc, and DHA. These variables were transformed using the natural logarithmic transformation. The follow-up residual testing confirmed normal residual distribution.

Results

Maternal demographic and health status characteristics by community

First, participants' characteristics were separated by community (OCN vs CCN) to discern any community-specific differences in maternal demographic, health status, and pregnancy outcomes (**Table 6-1**) and to identify whether the dietary characteristics data can be combined and assessed by alcohol consumption variable. Alcohol consumption was not a significant discernible factor between participants from OCN and CCN communities (41% vs 50%, respectively, $p = 0.270$). Geographical differences were observed for the percentage of CCN participants who reported smoking, which was significantly higher for CCN community compared to the OCN community (73% vs. 37%, $p<0.01$). In addition, CCN participants also had a significantly higher median number of pregnancies (4 (2-6) vs. 2 (1-4), $p<0.01$) and successful full-term births (2 (0-3) vs. 1 (0-1), $p<0.05$). No statistically significant differences in participants' age or pre-pregnancy BMI were identified between the OCN and CCN groups.

Participants' macro- and micronutrient (%) intakes compared by community are presented in **Table 6-2**. Food group, macronutrient, and micronutrient dietary characteristics did not differ between the OCN and CCN participants groups except for the median intake (#/day) of Meat and Alternatives which was significantly higher in the CCN participants compared with the OCN group (3 (2-4) vs. 2 (1-3), $p < 0.05$) and the median percentage of energy intake from fat, which was significantly higher in the CCN participants compared with the OCN group (36% (31-43) vs. 14% (12-17), $p <0.05$).

Maternal demographic, health, and pregnancy characteristics

Maternal demographic is described in **Table 6-3**, including health, and pregnancy characteristics for all participants and by self-reported alcohol consumption. A total of 59 treaty-status women ages 13-42 have taken part in the completion of the study. Twenty-six women were identified as at-risk, as per the definition described in Chapter 3 (any level of alcohol consumption during the

studied gestational period). All women were residents of the OCN or CCN communities belonging to their respective bands.

The estimated average age for the participants was 25.3 ± 6.7 years of age. The education levels were as follows: 2% of participants had completed elementary school, 41% junior high, 46% high school, and 13% completed post-secondary education (8% - certificate, 5% - university). A high proportion of participants were unemployed during the studied pregnancy (46%); 7% were employed part-time; 18% were full-time; 21% of participants were students (high school, certificate program, or diploma); and 8% of women were on maternity leave. Over half of the participants have reported being on social assistance (56%).

Significant differences ($p<0.05$) between non-at-risk and at-risk groups were noted for age, with women in the at-risk group having higher mean age. No significant differences were detected for other demographic variables.

Maternal pre-pregnancy BMI was overweight 27.6 ± 8.6 kg/m^2 with 14% of participants being underweight, 31%-normal, 21%-overweight, and 29%-obese. Moreover, 18% of participants had a chronic illness before pregnancy and 21% during pregnancy. Major chronic illnesses reported were type 2 diabetes, gestational diabetes, and asthma. The majority of women stated that they were taking a form of vitamins, minerals, and other supplements (76%). Over half (52%) of the participating women were smoking one or more cigarettes per day and 26% of women reported using drugs during the studied pregnancy. The most common drugs reported were marijuana. The studied pregnancies were equally distributed between the three trimesters. No significant differences were detected between non-at-risk and at-risk groups for any of the maternal health variables.

Self-reported access to finances and food

Participants' financial resources for food are presented in **Figure 6-1**. The following four statements were requested to be rated on the Likert scale "I often they run out of food," "I have enough food to eat," "I have a say in the way money is spent," "I have enough money to provide food for myself and my family." Over, 20% of participants disagreed with the first two respective statements and about 80% agreed. A high proportion of participants has agreed with the statement "I have a say in the way money is spent," which equated to about 88%. When asked about having enough money to provide food for themselves and their families 65% of

participating women have disagreed, with 61% of women strongly disagreeing and about 35% agreeing with the statement. No significant differences were identified between at-risk and non-at-risk groups for self-reported access to financial resources (**Figure 6-2**).

Participants' access to food was assessed through four statements about adequacy, availability, affordability, and access to food. Similar to the statements above, participants were agreeing or disagreeing with the following four statements "The food I eat is adequate to keep me healthy", "All the food I want to eat is available from a store near my home", "I can afford to buy all the foods I want to eat". Nearly 80% of pregnant women (41% strongly and 40% somewhat agreed) that the food they eat is adequate to keep them healthy. Approximately 63% (27% strongly agree, 36% somewhat agree) of participants agreed with the food availability statement. With regard to affordability, 83% of women agreed with the statement and 17% disagreed with the statement, with 10% strongly disagreeing. A high proportion of women (73%) have also responded positively to the statement about access to food.

The Wilcoxon rank-sum revealed statistically significant differences between at-risk and non-at-risk groups for two out of the four studied food security variables. A substantially lower mean rank for the at-risk group was identified for the statements "the food I eat is adequate to keep me healthy"; and "all the food I want to eat is available from a store near my home" ($p < 0.05$) (**Figure 6-3**).

Macronutrient dietary characteristics for all participants and by self-reported alcohol consumption

Table 6-4 displays the median intake for Health Canada's CFG food groups, macronutrients, and energy intake for all participants and by self-reported alcohol consumption. The percent of participants with inadequate intakes of Vegetable and Fruit, Grain Products, Milk and Alternatives, and Meat and Alternatives were 94% (95%CI = 84-99), 89% (95%CI = 77-96), 94% (95%CI = 84-99), and 77% (95%CI = 64-88) of the recommended food group servings /day, respectively. No significant differences were detected between at-risk and non-at-risk groups.

The median intake (g/day) of total carbohydrate, protein, fat, and fiber for the entire cohort was 213 g/day (175-301), 80 g/day (63-104), 70 g/day (52-101), and 12 g/day (8-17), respectively. The median intake for sugars was 65 g/day (45-94). No significant difference was observed in

the intake of protein, total carbohydrates, fiber, or added sugar between the two risk groups. However, the median daily fat intake was significantly higher in the at-risk group participants compared with the non-at-risk group (95 g/day (66-108) vs. 56 g/day (38-77), $p < 0.01$).

The most contributing sources of energy were total carbohydrates including sugar, followed by proteins and fat. Of note, the median energy intake from fat was significantly higher in the at-risk group compared to the non-at-risk group (378 kcal/day (263-432) vs. 223 kcal/day (151-308), $p < 0.01$). This yielded statistically significant difference in the energy intake (kcal) from fat between the at-risk and non-at-risk groups (2357 kcal/day (1612-2707) vs. 1665 kcal/day (299-2159), $p < 0.01$). In agreement, the median percentage energy intake from fat was significantly higher for the at-risk group (16% (15-20) vs. 13% (11-16), $p < 0.01$).

Macronutrient dietary characteristics for all participants and by self-reported alcohol consumption

The intakes for micronutrients are reported in **Table 6-5.** The median intake of all micronutrients was within the recommended reference DRIs (%), except it was lower for folate 83% (57-108), iron 82% (59-115), and DHA 38% (20-53); which corresponded with high proportions of participants falling below the recommendations for these nutrients (78%, 95%CI = 65-88; 58%, 95%CI = 44-70; 75%, 95%CI = 62-85; and 93%, 95%CI = 84-98, respectively). A higher prevalence of inadequacy was also detected for calcium (58%, 95%CI = 44-70). Compared with the non-at-risk group, at-risk participants had significantly lower median intakes for the recommended reference DRI (%) for the thiamin ($p<0.05$), niacin ($p<0.05$), folate ($p<0.01$), choline ($p<0.01$), iron ($p<0.01$), and zinc ($p<0.01$).

Effect of combined predictors on macro- and micronutrient (%) intake

Hierarchical linear regression modelling was performed to evaluate the contributions of risk, demographic, and health predictors in blocks **(Tables 6-6)**. The first block of regression analyses examined the impact of three risk variables (alcohol consumption, drug use, cigarette smoking) on maternal energy (%) from each macronutrient intake. Self-reported alcohol consumption precipitated a significant association with increased total energy intake ($\beta = 0.291$; SE, 0.219; $p < 0.05$) and increased percentage energy from fat ($\beta = 0.290$; SE, 0.122; $p < 0.05$). Cigarette smoking (# of cigarettes/day) was significantly associated with percentage energy from carbohydrate ($\beta = 0.044$; SE, 0.018; $p < 0.05$).

The second block of regression analyses examined the combined impact of risk exposure and demographic variables (community, age, employment) on maternal energy (%) from macronutrient intake. Self-reported alcohol consumption lost its significant associations from Model I. On the other hand, employment status presented a significant negative association with percentage energy from fat (β = -0.435; SE, 0.152; p <0.01). Model II also detected significant positive association between cigarette smoking and percentage energy from carbohydrate (β = 0.049; SE, 0.020; p <0.05).

The third block of regression analyses examined the combined impact of risk exposure, demographic variables, and maternal health factors (trimester, pre-pregnancy BMI, chronic illness) on maternal energy (%) from macronutrient intake. The result for percentage energy from fat intake and employment status maintained its significance (β = -0.476; SE, 0.157; p <0.01).

The results of hierarchal linear regressions for % DRI for micronutrients are presented in **Tables 6-7a-f.** Total energy (kcal) was introduced as a fourth Block/Model in the hierarchal sequence. A significant negative association was observed between pregnancy trimester and vitamin C intake in Model III (β = -0.446; SE, 0.202; p <0.05) and persisted in Model IV (β = -0.535; SE, 0.220; p <0.05; **Table 6-7a**). On the other hand, increased cobalamin intake was positively associated with maternal age in Model II (β = 0.100; SE, 0.038; p <0.05), Model III (β = 0.100; SE, 0.044; p <0.05), and Model IV (β = 0.097; SE, 0.045; p <0.05; **Table 6-7c**).

A decrease in niacin was consistently observed with self-reported alcohol consumption (Model I (β = -0.103; SE, 0.27; p <0.01), Model II (β = -0.99; SE, 0.29; p <0.01), Model III (β = -0.96; SE, 0.31; p <0.01), and Model IV (β = -0.99; SE, 0.32; p <0.01; **Table 6-7b**). The same was also true for folate intake in Model I (β = -46; SE, 0.19; p <0.01), Model II (β = -0.42; SE, 0.20; p <0.01), Model III (β = -0.38; SE, 0.26; p <0.01), and Model IV (β = -0.33; SE, 0.31; p <0.01; **Table 6-7c**). Moreover, a decrease in choline intake was significantly associated with alcohol consumption (Model I (β = -0.661; SE, 0.195; p <0.01), Model II (β = -0.548; SE, 0.228; p <0.05), and Model III (β = 0.545; SE, 0.255; p <0.05); **Table 6-7d**).

Furthermore, a decrease in calcium intake was significantly associated with alcohol consumption in Model I (β = -0.556; SE, 0.266; p <0.05) and this negative effect persisted in Model II (β = -

0.352; SE, 0.266; p <0.05), Model IV (β = -0.468; SE, 0.310; p <0.05; **Table 6-7d**). The same table also shows that calcium intake was equally affected with employment status as seen in

Model II (β = -0.880; SE, 0.358; p <0.05) and Model III (β = -0.935; SE, 0.396; p <0.05).

Likewise, employment status was negatively associated with iron intake in Model II (β = -0.759; SE, 0.348; p <0.05), Model III (β = -0.859; SE, 0.383; p <0.05) and Model IV (β = -0.885; SE, 0.416; p <0.05), and with zinc intake in Model II (β = -0.766; SE, 0.325; p <0.05) and Model III (β = -0.843; SE, 0.352; p <0.05; **Table 6-7e**).

Lastly, a decrease in DHA intake was associated with chronic illness in Model III (β = -0.012; SE, 0.855; p <0.05; **Table 6-7f**).

Discussion

The objective of this study was to explore the relationship between macro- and micro-nutrient intake and self-reported alcohol consumption during pregnancy in First Nations women residing in two remote communities in Manitoba. While there is some observational research on the aspects of maternal nutrient intake in relation to alcohol consumption (May et al., 2014, 2016; Flynn et al., 1981; Sowell et al., 2020) and other risk exposures, to our knowledge, this is the first Canadian study to explore these relationships in status, First Nations, pregnant women, living on-reserves. Important findings of this study are associations between risk exposure – alcohol consumption and increased intake of total energy and energy from fat (%), as well as decreased intakes of niacin, folate, choline, and calcium. This suggests that women who report the presence of risk exposures during pregnancy may have poorer overall diet quality.

Community comparisons

The present study demonstrated that there were some similarities and differences in the two communities studied. No statistically significant differences of the community were observed on the intake of micronutrients and risk exposures. Statistically significant differences were observed for Meat and Alternative food group and (%) fat median intakes. This served as the rationale for pooling the data for two localities. Regression analyses on pooled data from two communities show that factors associated with macronutrients and micronutrient assessments are much similar between two communities. Statistically significant observations were identified for

the pregnancy outcomes and smoking, with higher number pregnancies, full-term births and smoking prevalence in CCN community (Community Comparisons discussed in detail in *Chapter 7*).

Demographic characteristics

The findings for studied maternal demographic variables such as age, education, employment and social assistance are consistent with other maternal studies done in First Nations communities (Sheppard et al., 2017; Oliveira et al., 2013), re-confirming lower than the average maternal age, level of education, and higher rate of unemployment compared to the general Canadian population (Statistics Canada, 2018). The burden of poverty falls heavily on First Nations women. Recent research indicates that 44% of the on-reserve population in Canada live in low-income households and estimated rates of poverty for First Nations women are 2-3 higher compared to their non-First Nations counterparts (Townson, 2005; Statistics Canada, 2021). These socio-economic statistics affect maternal ability to meet basic living standards and add to maternal stressors that can generate or worsen health problems, including FASD outcomes.

Health status

Despite younger maternal average age, the prevalence of overweight, obesity, and self-reported chronic illness during the studied pregnancy were 21%, 29%, and 21% respectively. The findings from the present study align with the findings of population-based studies in First Nations maternal populations in Ontario, Saskatchewan, and Alberta (Dyck et al., 2002; Walker et al., 2020; Fuchs et al., 2017); as well as with the findings available from multiple cycles of First Nations Regional Health Survey (RHS) for First Nations on-reserve general population (Elias & LaPlante, 2006; First Nations Health Authority, 2012). This health gap for First Nations women is furthered when compared to non-Indigenous Canadian women (Hosseini et al., 2019; Aljohani et al., 2008; Dyck et al., 2010).

Over half (52%) of the participating women reported smoking cigarettes during a given pregnancy. This prevalence is congruent with previously reported prevalence estimates of smoking in First Nations community among expecting women and women of childbearing age (34.9%-80%) (Elias & LaPlante, 2006; First Nations Health Authority, 2012; Oliveira et al., 2013; Heaman & Chalmers 2005; Mehaffey et al., 2010). These estimates are substantially higher than the identified rates for the general Canadian population, with national statistics of

10.5% (Al-Sahab et al., 2010). Smoking is considered to be the leading preventable cause for IUGR, low birth weight, and preterm birth (Mehaffey et al., 2010; Salihu & Wilson, 2007; Lumley et al., 2009). Furthermore, recent research advances have identified negative impacts of maternal smoking on offspring's weight gain and cardio-metabolic risk factors in childhood and adolescence (Oken et al., 2008; Syme et al., 2010).

Approximately 26% of women reported using drugs, with marijuana being most commonly reported. The results from the latest FNRHS (2015-2016) suggest a similar overall prevalence (30.3%; 95% CI 28.8-31.8%) for First Nations adults living on reserves (FNIGC, 2018). These numbers are markedly higher than the general Canadian estimate of 1% for maternal population (PHAC, 2009). Although, an implementation of the Cannabis Act in October 2018, legalized cannabis for non-medical use across Canada, its effects on developing fetus are largely unknown. While the Act may improve safety due improved control and adulteration of the cannabis supply, cannabis use prenatally is associated with low birth weight (Luke et al., 2019), small for gestational age (Fergusson et al., 2002), premature birth (Mark et al., 2016), still birth (National Institutes of Health (NIH), 2013). A line of evidence also indicated that prenatal cannabis use may also affect infant's neurological development (Zhang et al., 2017; Goldschmidt et al., 2012).

Taken together, increased prevalence of metabolic conditions and risk exposures among our study participants are concerning, as the relationship between maternal metabolic conditions, risk exposures and poor prenatal and birth outcomes have been well documented (Agarwal et al., 2018; Corsi et al., 2020; Fuchs et al., 2017; Lu et al., 2018; Marufu et al., 2015; Oral et al., 2001). Furthermore, similar findings with respect to these disparities were documented for CCN and OCN communities independently. Collectively, these findings may have stronger implications for alcohol affected pregnancies, as alcohol also carries metabolic impacts on the fetus, which lead to growth restriction (Feldman et al., 2012; Fraser et al., 2012), poor neonatal weight gain and low birth weight baby (Passaro et al., 1996; Virji et al., 1991). The analysis of the present study identified marginally significant ($p<0.1$) elevations in chronic illness before pregnancy for the at-risk group. While the evidence on the concomitant risk behaviors in maternal populations is scarce, one report found the link between behaviors such as consuming an unhealthy diet, which are well-founded risk factors for chronic illness, and exceeding the recommended alcohol consumption limits (Goathup et a., 2017). This inverse relationship

between dietary quality and higher levels of alcohol consumption is better documented in the general population (Ruidavets et al., 2004; Nelson et al., 2009; Valencia-Martin et al., 2011). Therefore, this finding on the presentation of several risk factors during pregnancy need to be urgently addressed through maternal health programming and clinical advice.

Access to resources

Although this study did not directly estimate household or individual food security level, several food access variables were assessed. High proportion of women in the study reported disagreement with the statements about having food accessibility and availability (28% and 38%, respectively). These findings are consistent with the other on-reserve studies, which report on food security. According to the First Nation RHS (2008-2010), 54.2% of households were classified as food insecure, with over 14% as severely food insecure (FNIGC, 2012). In more remote and isolated communities, household food insecurity ranges from 45-69%, depending on the region (Chan, 2012).

Food insecurity presents not only a social problem but is a situational factor that may contribute to alcohol use during pregnancy. While this information is limited for pregnant women a few international reports (Eaton et al., 2014; May et al., 2005), identify the link between food insecurity and this risk exposure. Eaton and colleagues (2014) studied food security in relation to FASD using similar indicators as presented in this study. The authors reported that 63% of women who consumed alcohol during the studied pregnancy stated being unable to afford to purchase a balanced meal, akin to the observations identified in the present study for the at-risk group. Given an already existing burden of chronic conditions and risk-exposures in this cohort the poor access to food may increase the vulnerability of women to further negative health outcomes.

Noteworthy, the disparities in First Nations women's maternal health status, when compared to that of general Canadian women, can only be understood and interpreted in the context of the effects of historic colonial policies (land dispossessions, Residential Schools, prohibition of cultural practices), which greatly impacted the state of the determinants of health for the population. The substandard state of determinants of health such as socioeconomic status, education, housing conditions, access to care, healthcare infrastructure, among others make First Nations women vulnerable to poor health across the life course (Tait, 2000; 2003). The lack of

reliable access to care, healthcare infrastructure, continuity of care, and high medical professional turnover rate in remote communities has especially detrimental implications for expecting First Nations women (Heaman et al., 2005; Lemchuck-Favel & Jock, 2004).

Food group and macronutrient intake

The results of the present study demonstrate that women were below the recommendations for all four food groups and a high proportion of participating women were not meeting the recommendations for all four CFG food groups. These findings were consisted with on-reserve studies for maternal (Back et al., 2003) and women of child-bearing age (Chan et al., 2019; Johnson-Down & Egeland 2012) populations. The present study reports nearly identical results to that of a study conducted in 7 Cree communities in Northern Quebec, which revealed that over 90% of women of childbearing age were not meeting the recommendations for Vegetable and Fruit and Milk and Alternatives, and over 70% did not meet the recommendations for Grain Product food groups (Johnson-Down & Egeland 2012). The use of same instrument (24-hour dietary recall) in the study by Johnson-Down & Egeland, makes the results quite comparable.

Noteworthy, the present study results for food group intake were assessed utilizing former CFG for general population (Health Canada, 2007). While the four food groups and the recommendations for the daily number of serving sizes between CFG for general population and CFG for Canadian Indigenous Populations are the same, the types of recommended foods differ. The CFG for Canadian Indigenous Populations recommends intakes of traditional sources of protein, berries, and greens (Health Canada, 2007). The participants had reported very low intake of traditional food items. Traditional food intake and harvesting practices are identified as a health promoting factor (Sheehy et al., 2015). In the presence of chronic illness, FASD context, and risk exposures these findings raise concerns and require further assessment to ascertain these findings.

A significant difference was detected between at-risk and non-at-risk groups in this study, with an at-risk group having higher intake of dietary fat (99 ± 53 vs. 60 ± 29 g/day, $p<0.05$). This positive relationship held up in the hierarchical regression analysis after cigarette smoking and drug use were added as covariates ($p<0.05$). Furthermore, marginal significance ($p<0.1$) was observed through the entire three models with demographic and health factors covariates. Similar observations were made for the caloric intake, likely due to greater fat intake among individuals

who reported alcohol consumption. Interestingly, the influence of alcohol consumption on fat intake was observed for the community of OCN and CCN independently (*Chapter 3* and *Chapter 5*). It is difficult to compare this finding with other Canadian studies, as the dietary data on pregnant First Nations women living on reserves is limited. A couple of international studies (May et al., 2014; 2016) documented the intake of macronutrients for women who reported alcohol consumption during pregnancy using 24-hour dietary recall. The authors found no relationship between various levels of alcohol consumption and any of the macronutrient intake. However, a noteworthy limitation of these studies was that the dietary data was collected 7 years after birth, which substantially impacts the accuracy of recall making it difficult to ascertain the validity and generatability of the study results.

Although the information on the relationship between alcohol consumption and food intake is scarce for maternal population, evidence from investigations in general populations exists (Ruidavets et al., 2004; Nelson et al., 2009; Valencia-Martin et al., 2011). The studies reported a link between high quantities of alcohol consumption per occasion and diets higher in the intakes of processed foods, red meats, and lower high-fiber fruit and vegetable ingestion (Ruidavets et al., 2004; Nelson et al., 2009; Valencia-Martin et al., 2011), which may be translated into higher intakes of fat macronutrient.

To date, a higher volume of research, which explores the role of diet in relation to FASD, is dedicated to the investigation of micronutrients in preclinical, clinical, and observational studies (Young et al., 2014; Kloss et al., 2014; Sebastiani et al., 2018). Although this research delivers valuable insights into the associations between risk exposures and nutritional health, the examination of all dietary aspects in the FASD context is paramount. Therefore, it is the recommendation of the present study to build on the results of this research and perform a large-scale dietary intake patterns study in First Nations maternal populations with and without risk exposures to derive evidence on macronutrient intake, food group consumption, and overall food intake patterns.

Micronutrient intake

The present study identified high prevalence of inadequacy for folate, calcium, and iron. Although the information on micronutrient intake for pregnant First Nations women is scarce, the higher prevalence of inadequacy for folate and calcium are consistently reported for pregnant

(Berti et al., 2008) and childbearing age (Chan et al., 2019; Delormier & Kuhnlein, 1998; Johnson-Down & Egeland, 2013), First Nations women who reside on reserves. Interestingly, the nutrients stated above had similar prevalence of inadequacy in both communities, independently. Furthermore, these findings align with national and international reports, as these are some of the most commonly micronutrient deficiencies reported globally (Gernant et al., 2016). These observations are concerning as the adequate periconceptional and prenatal levels of folate are important for neural tube closure (Blom & Smulders, 2011), proper heart and urinary tract development (Blom & Smulders, 2011), and proper growth and limb development (Chrisman et al., 2004). Likewise, iron plays a role in fetal growth (Rufer et a., 2012; Carter et al., 2007), hemoglobin concentration and oxygen transport (Connor et al., 2019), and brain development (Miller et al., 1995; Moos et al., 2018).

Especially concerning prevalence of inadequacy was observed for DHA. While these findings are consistent with the findings of Denomme and colleagues (2005) (82 ± 33 mg/day), Friesen and Innis (2010) (146 ± 161 mg/day), and Dyck 2016 (97.0 (81.7) mg/day) prolonged under-consumption of DHA is problematic. The DHA consumption was even lower among women who reported alcohol consumption at any point during pregnancy in this study (65 ± 43 mg/day). The accretion of DHA in the fetal CNS during the end of 2nd and the entire 3rd trimesters, provides evidence supporting the notion of a unique role of DHA in CNS membrane structure and functionality. A strong line of evidence indicating a causal relationship between the low dietary omega-3 intake and reduced DHA concentration in cerebral cortex (Neuringer et al., 1986; Yamamoto et al., 1988). Restricted accumulation of DHA in the brain leads to impediments in proper neuron myelination, phospholipid layer formation, cell signalling regulation (Stillwell et al., 2005), hormonal functioning, and others. Thus, DHA is a conditionally essential nutrient in early life stages and brain DHA content rapidly increases at key stages of neurodevelopment, including the last trimester of pregnancy and the first few months of postnatal life (Martinez, 1992).

Alcohol consumption during pregnancy was associated with reduced intake of niacin, folate, choline, and calcium. The relationships between the DRI (%) niacin, folate, choline and self-reported alcohol consumptions upheld its significance even after the adjustments for other risk exposures, demographic, and health factors ($p<0.05$). These findings are concerning, as folate

and choline are key co-enzymes involved in DNA methylation, cell differentiation, and proliferation. The ingestion of alcohol has imminent impacts on enteric folate uptake and plasma folate and choline levels (Medici & Halsted, 2013; Thomas et al., 2009). An experimental report, which measured the effects of ethanol on folate, revealed that folate levels substantially decreased after eight hours of ethanol introduction (Eichner & Hillman, 1973). This evidence on the methylation cycle nutrients and ethanol-induced aberrations in the metabolism of these nutrients has been corroborated by several additional reports (Ballard et al., 2012; Ford et al., 2021). Thus, low folate intake in combination with alcohol consumption during pregnancy may have compounding negative impacts increasing fetal and maternal susceptibility to DNA damage, impaired growth, and CNS development (Ballard et al., 2012).

An inverse relationship between alcohol and niacin and calcium intakes was detected not only in the present analysis, but for both communities independently (*Chapter 3* and *Chapter 5*). There is great lack of comparative studies on the influence of risk variables in maternal population in Canada. Two international case-control studies by May and colleagues (2014; 2016), reported information on intake micronutrients for alcohol consuming women. Whereas the 2014 study demonstrated lower mean intakes for riboflavin, calcium, choline, n-3 docosapentaenoic acid (DPA) ($p<0.05$) for women exposed to alcohol during pregnancy, the 2016 study reported higher mean intakes for vitamin D, thiamin, phosphorus ($p<0.05$), and eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and DPA ($p<0.01$). The strength of these studies was confirmed alcohol exposure, as the selected participants were the women who birthed children with FASD diagnosis. An impactful limitation of both studies was the dietary data collection commenced 5-7 years after the FASD-indexed pregnancy (May et al., 2014; 2016). Noteworthy, the sample of women with and without FASD pregnancies were completely different in both studies conducted by May and colleagues (2014, 2016). Similar findings were reported by Carter and colleagues (2017), who detected positive relationships between micronutrient intake (phosphorus, choline, vitamin B12, and vitamin D) and various patterns of alcohol consumption in Cape Coloured pregnant women. Although these studies populations differ from ours, all three studies employed similar methodological approaches in data collection, DRI (%) estimations and application, therefore, the results from these three studies were deemed as the best comparable evidence available to date.

There appears to be great variation in findings among the authors investigating dietary intake in the presence of prenatal alcohol consumption. Likewise, the variation in findings was also noted among the two pilot studies conducted, as the results somewhat differed between the CCN and OCN communities. Whereas the inverse relationship between alcohol consumption and DRI (%) for thiamin, niacin, folate, choline, calcium, iron, and zinc for the CCN community (*Chapter 3*), only three nutrients were affected by consumption for OCN community (vitamin C, niacin, calcium) (*Chapter 5*). Although these findings could be due to the methodological techniques employed, such as different types of linear regression and the number of covariates used in the models, these community differences were supported through Spearman correlation independently for both communities. Furthermore, for the OCN community, the Spearman correlation analysis did not detect any influence of alcohol on the DRI (%) for micronutrients studied with the exception of niacin (*Chapter 5*). These findings indicate that self-reported alcohol consumption alone does not explain the variations in DRI (%) intake for micronutrients. Interestingly, the earlier studies (May et al., 2014, 2016) also observed great variation in intake and identified similar methodological challenges faced by the present study, these are discussed further in the *Limitations* section.

Although the populations in our study and the studies examined by May and colleagues (2014; 2016) and Carter and colleagues (2017) are different, similarly to our study the women with confirmed exposure experienced higher levels of poverty and homelessness, lower incomes and education attainment. First Nations women experience increasing levels poverty, social marginalization, food insecurity, psychological distress, lower education levels, and exposure to partner's violence, thus making them at higher risk of alcohol consumption during pregnancy (Heaman et al., 2012). These disparities among maternal populations point to the need of a comprehensive maternal health policy targeting aspects of proximal and distal determinants of health and addressing systemic barriers faced by First Nations on-reserve, maternal populations.

The translatable impacts of the present work identify the need for the mobilization of all-sector efforts to address the state of the determinants of health for First Nations women, including pregnant women. The emergence of epidemiological overlap of chronic illness, mental health disorders, FASD, prenatal risk exposures in First Nations maternal populations clearly points toward a need for the enhancement and better co-ordination of health services, policy, and public

health sectors in order to reverse this trend (Smylie et al., 2010; Kornelsen et al., 2010). It is important to recognize that the efforts to reverse the trend must include the amelioration of the medical infrastructure in remote communities, restoring maternal traditional birthing practices, addressing the impacts of birth evacuation policy, and working in partnerships with communities to develop appropriate policy and programming (Lawford et al., 2018; Townson, 2005; Smylie et al., 2010; Kornelsen et al., 2010).

Strengths

The strengths and limitations of this Chapter are similar to those discussed in previous *Chapter 3* and *Chapter 5*, as this Chapter combined data from both communities to increase sample size and the power of the study. The increased sample size improved the ability to study the impacts of maternal alcohol consumption in conjunction with other predictors. The increased sample size improves generalizability and applicability of these results in the field of nutrition and FASD prevention programming for First Nations women residing on reserves.

As indicated in previous Chapters, particulars strengths of the two pilot studies include strong community engagements, community consultations and regular reporting, and consistent presence of the same researcher (OK) in both communities. These strong collaborative research practices contributed to trusting relationships between the researcher and the clinical teams and community members, and participating mothers.

This study utilized an in-person, interactive FFQ, as well as 24-hour dietary recall. The benefits of the application of these tools and their performance in the field of nutrition is well documented (Feskanich et al., 1993; Karvetti & Knuts, 1985; Gibson & Ferguson, 2008; Gleason et al., 2010). Further to that, the research instrument has placed particular emphasis on cultural appropriateness and competency. This is a particular strength of the instrument as dietary habits are influenced by environments and socio-cultural factors (Teufel, 1997). Additionally, the questionnaire has undergone face and content validity testing in a similar population (Giesbrecht, 2015; Dyck 2016).

One of the most noteworthy strengths of this study is the collection of dietary and risk-exposure information of status, First Nations women, residing on-reserves. The dearth of data on the First Nations maternal population, due to the exclusion from national surveillance programs and

general lack of research is a long-standing impediment for maternal on-reserve programming, evidence-based nutrition advice, and maternal public health policy. To our knowledge, this is the first attempt, which reported on the influence of the self-reported risk exposure and the intake of macro- and micro-nutrients. The findings of this report could be utilized by the respective communities to affect change in grass-roots programming.

Limitations

As identified in previous Chapters, the most critical limitations of these pilot projects include suboptimal sample size, the use of self-reports, lack of repeated measures throughout the trimesters, absence of control for seasonality, and lack of extensive validity and reliability testing of the instrument.

The sample size of 59, which was produced as a result of combining of the two communities, is only sufficient to produce medium to large effect size (for partial correlation, 0.37) which are results in less conclusive results (Aloe, 2014). Small sample obstructed various forms of sub-analysis, created risk of hierarchical regression model overfitting, and largely contributed to the great variations in observed dietary intakes. These complications, consequently, generate obstructions in finding interpretation, generalization, and application to other Manitoban First Nations communities. Larger sample size might improve the parametric distributions for variables under investigation, reduce the influence of suspected outliers, and overall improve the generatability of the results.

The collection of information through self-reports posed serious limitation for nearly all studied variables. As stated in *Chapter 3*, these limitations affect the field of FASD to a great extend as the self-reported alcohol consumption is central to the offspring's diagnosis and clinical treatment plan (Cook et al., 2016; Chudley et al., 2005; Chudley et al., 2018). Reliance on the self-reported confirmation of prenatal alcohol exposure is necessary for the diagnosis of FASD forms (pFAS, ARND, ARBD) where there is no presentation of the three facial features (reduced palpebral fissure length, smooth philtrum, thin upper lip) (Cook et al., 2016; Chudley et al., 2018). Furthermore, the presentation of less than three facial features also requires confirmation of exposure, as there is less diagnostic specificity (Cook et al., 2016). The need for elucidation of an objective marker and/or other confirmatory criteria has been clearly highlighted in FASD

literature (Astley & Clarren et al., 1997; Cook et al., 2016; Chudley et al., 2018; CanFASD, 2020).

Additional limitation of the self-reported consumption is lack of validated data collection instruments for this purpose (Stevens et al., 2020), which was the limitation greatly affecting the present study. Self-reported prenatal alcohol consumption is greatly affected by underreporting due to embarrassment or stigma (Tait, 2003; Stevens et al., 2020). The experience of stigma associated with substance use has been well documented for First Nations populations (Tait, 2000, 2003; NCAH, 2009; TRC, 2015). High proportion of women in both communities refused to answer in-depth questions about the level, timing, and quantity of consumption. Some women provided reasons for the lack of response which included complicated family situations, involvement with child and family services and justice systems.

The limitations inherent to the FFQ, 24-dietary recall, and overall self-reported have impacted the study's results. Measurement error is constitutionally present in all dietary data collection instruments, which rely on retrospective self-report (Prentice, 1996). Dietary measurement error creates serious challenges to the validity and reliably of the results (Gleason et al., 2010; Prentice, 1996). Further to that, this limitation particularly impacted the results of hierarchical linear regression which used unattenuated DRI (%) intakes as dependent variables. Due to the previously discussed limitation of the lack of validation the use of attenuation factors was not possible (Gleason et al., 2010). Therefore, it is a major recommendation to perform a validations study as the next step of this research.

Conclusion

This study evaluated dietary intake in First Nations pregnant women living in two northern communities in Manitoba – OCN and CCN. High prevalence of inadequacy was detected for intake of all four food groups and for folate, iron, and DHA. Self-reported alcohol consumption was shown to have impacts on the intakes of total energy intake and fat intake (%), as well as on the DRI (%) for niacin, folate, choline, and calcium. These findings are aimed at assisting with the formulation of maternal and child health programming, public health policy, and FASD interventions. The health of First Nations women and babies is negatively impacted by food insecurity, substance use, and lack of access to resources, which have been identified in this

study. As this study provides preliminary data, further investigations should focus on examining larger samples of Indigenous women, including Inuit and Metis populations with larger sub-groups and at-risk analysis.

Table 6-1. Maternal demographic, health, and pregnancy characteristics by community

Characteristic	OCN (n=37)	CHEM (n=22)	P-value
Age[a]	24.4 ± 7.0	27.2 ± 5.9	0.168
≤18	10 (27)	1 (5)	
19-30	21 (57)	15 (68)	
31-42	6 (16)	6 (27)	
Pre-pregnancy BMI[a]	26.5 ± 8.8	25.9 ± 7.8	0.345
Below	4 (12)	4 (18)	
Normal	11 (33)	7 (32)	
Overweight	7 (21)	5 (23)	
Obese	11 (33)	6 (27)	
Chronic illness:			
Before pregnancy[b]	5 (15)	5 (23)	0.298
During pregnancy[b]	7 (21)	7 (32)	0.347
Alcohol[c]	15 (41)	11 (50)	0.270
Smoking[b]	11 (37)	16 (73)	**0.005**
Drugs[c]	7 (19)	8 (36)	0.272
Trimester			0.578
1st	12 (36)	6 (27)	
2nd	4 (12)	13 (59)	
3rd	17 (52)	3 (14)	
Pregnancy outcomes:			
# of pregnancies[c]	2 (1-4)	4 (2-6)	**0.003**
# of full-term births[c]	1 (0-1)	2 (0-3)	**0.012**

Values are means ± SD, n (percentages), and medians (Q1-Q3). The differences between the communities were tested by an independent t-test[a], Wilcoxon rank-sum test[b] or a Chi-square tests of independence[c]. Opaskwayak Cree Nation (OCN), Chemawawin Cree Nation (CCN).

Table 6-2. Maternal dietary characteristics by community

	OCN (n=37) Median (Q1-Q3)	CHEM (n=22) Median (Q1-Q3)	P-value
Macronutrients			
Food group (#/day)			
Vegetable and Fruit	5 (3-7)	3 (3-5)	0.385
Grain Products	5 (3-7)	4 (2-6)	0.552
Milk and Alt.	1 (0-3)	1 (0-2)	0.671
Meat and Alt.	2 (1-3)	3 (2-4)	**0.031**
Energy from macronutrients (%)			
Protein	17 (14-20)	16 (13-20)	0.699
Carbohydrate	52 (46-55)	49 (39-53)	0.434
Fat	14 (12-17)	36 (31-43)	**0.033**
Micronutrient (%DRI)[†]			
Vitamin A (RE)	235 (151-355)	255 (145-346)	0.372
Vitamin C	202 (128-292)	200 (135-252)	0.693
Thiamin (Vit B1)	228 (165-317)	201 (156-302)	0.680
Niacin (Vit B2)	213 (137-307)	206 (168-304)	0.744
Folate (Vit B9)	84 (59-108)	78 (57-122)	0.797
Cobalamin (Vit B12)	441 (286-634)	476 (249-592)	0.643
Choline	108 (78-148)	108 (81-205)	0.414
Calcium	87 (81-149)	134 (74-190)	0.257
Iron	84 (62-118)	79 (58-126)	0.744
Zinc	163 (114-194)	165 (105-248)	0.381
DHA	38 (20-51)	36 (21-77)	0.612

Values are medians (Q1-Q3). [†]Carbohydrate includes total nutrient components: fiber, sugar, starch. [††]Nutrient intake was assessed using Health Canada's Dietary Reference Intake; Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50 for vitamins A, B12, C, folate, thiamin, niacin, zinc, calcium, iron; Adequate Intake (AI) for choline. Recommendations for DHA (docosahexaenoic acid, C22:6n-3) were obtained from Global Recommendations for DHA and EPA (eicosapentaenoic acid, C20:5n-3) Intake (2008). The differences between the communities tested by the t-test of independence for normally distributed data and Wilcoxon's rank-sum test for not normally distributed data. Opaskwayak Cree Nation (OCN), Chemawawin Cree Nation (CCN).

Table 6-3. Maternal demographic, health, and pregnancy characteristics of all participants and by self-reported alcohol consumption

Characteristic	All women (n=59)	Non-at-risk (n=33)	At-risk (n=26)	P-value
Age[a]	25.3 ± 6.7	23.5 ± 6.6	27.7 ± 6.3	**0.018**
≤18	11 (19)	9 (27)	2 (8)	
19-30	36 (61)	19 (58)	17 (65)	
31-42	12 (20)	5 (15)	7 (27)	
Education[b]				0.053
Elementary	1 (2)	1 (3)	0	
Junior high	24 (41)	17 (52)	7 (27)	
High school	27 (46)	12 (36)	15 (57)	
Certificate	4 (8)	1 (3)	3 (12)	
University	3 (5)	2 (6)	1 (4)	
Employment[b]				0.921
Unemployed	27 (46)	16 (50)	11 (42)	
Employed part-time	4 (7)	2 (6)	2 (8)	
Employed full-time	10 (18)	4 (13)	6 (23)	
Student	12 (21)	6 (19)	6 (23)	
Maternity Leave	5 (8)	4 (12)	1 (4)	
Social Assistance[c]	33 (56)	19 (57)	14 (54)	0.775
# of household residents[b]	4 (3-6)	4 (3-6)	4 (2-6)	0.232
# of Adults (18+)	2 (1-3)	2 (1-3)	2 (1-4)	0.280
# of Children (<18)	2 (1-3)	2 (1-3)	2 (1-3)	0.360
Pre-pregnancy BMI[a]	27.6 ± 8.6	24.6 ± 9.5	26.7 ± 8.4	0.383
Below	8 (14)	4 (12)	4 (15)	
Normal	18 (31)	9 (27)	9 (35)	
Overweight	12 (21)	6 (18)	6 (23)	
Obese	17 (29)	10 (30)	7 (27)	
Chronic illness[c]:				
Before pregnancy	10 (18)	4 (13)	6 (23)	0.093
During pregnancy	12 (21)	6 (18)	6 (23)	0.731
Medications[c]:				
Prescribed	11 (19)	5 (15)	6 (23)	0.508
Over-the-counter	9 (15)	6 (17)	3 (12)	0.420
Vit & min supplements[c]	45 (76)	23 (64)	22 (85)	0.336
Smoking[c]	29 (52)	14 (45)	15 (58)	0.164
Drugs[c]	15 (26)	8 (25)	7 (27)	0.868
Trimester				
1st	18 (33)	11 (37)	7 (28)	
2nd	17 (31)	9 (30)	8 (32)	
3rd	20 (37)	10 (33)	10 (40)	
Pregnancy outcomes[b]:				
# of pregnancies	3 (2-5)	3 (2-5)	3 (2-6)	0.612
# of miscarriages	0 (0-1)	0 (0-1)	0 (0-1)	0.705
# of stillbirths	0 (0-0)	0 (0-0)	0 (0-0)	0.440
# of abortions	0 (0-0)	0 (0-0)	0 (0-0)	0.536
# of full-term births	1 (0-2)	1 (0-2)	1 (0-2)	0.418
# of pre-term births	0 (0-1)	0 (0-1)	0 (0-0)	0.948
Self-reported health[b]				
Excellent	5 (9)	3 (10)	2 (8)	
Very Good	20 (35)	11 (36)	9 (35)	
Good	17 (30)	7 (23)	10 (39)	
Fair	14 (25)	9 (29)	5 (19)	
Poor	1 (2)	1 (3)	0	
Bed rest[c]	8 (14)	6 (18)	2 (8)	0.241

Values are means ± SD, n (percentages), and medians (Q1-Q3). The difference between the groups were tested by an independent t-test[a], Wilcoxon rank-sum test[b] or a Chi-square or Fisher's exact tests of independence[c].

Table 6-4. Maternal macronutrient intake for all participants and by self-reported alcohol consumption

Dietary variables	All women (n=59)		Non-at-risk (n=33)		At-risk (n=26)		P-value
	Median (Q1-Q3)	% Inadequate (95% CI)	Median (Q1-Q3)	% Inadequate (95% CI)	Median (Q1-Q3)	% Inadequate (95% CI)	
Food group (#/day)[†]							
Vegetable and Fruit	4 (3-6)	94 (84-99)	3 (2-6)	96 (82-100)	4 (3-6)	92 (74-99)	0.741
Grain Products	5 (3-7)	89 (77-96)	5 (3-6)	86 (67-96)	5 (3-7)	92 (74-99)	0.922
Milk and Alt.	1 (0-3)	94 (84-99)	1 (1-2)	93 (77-99)	1 (0-3)	96 (80-100)	0.864
Meat and Alt.	3 (2-4)	77 (64-88)	2 (1-3)	79 (59-92)	3 (2-4)	76 (55-91)	0.151
Macronutrient (g/day)							
Protein	80 (63-104)		78 (62-93)		89 (74-105)		0.193
Carbohydrate[††]	213 (175-301)		200 (172-273)		256 (181-317)		0.133
Fat	70 (52-101)		56 (38-77)		95 (66-108)		**0.001**
Fiber	12 (8-19)		14 (8-21)		12 (8-18)		0.710
Sugar	65 (45-94)		58 (44-93)		76 (48-102)		0.465
Energy (kcal/day)	1951 (1519-2466)		1665 (1299-2159)		2357 (1612-2707)		0.015
Protein	320 (253-417)		311 (250-373)		354 (297-420)		0.426
Carbohydrate[††]	851 (701-1202)		801 (687-1090)		1023 (723-1267)		0.165
Fat	281 (208-406)		223 (151-308)		378 (263-432)		**0.001**
Sugar	259 (180-378)		231 (175-372)		302 (193-409)		0.442
Energy from macronutrients (%)[†††]							
Protein	17 (14-20)	6 (1-16)	17 (15-20)	7 (1-24)	16 (13-17)	4 (0-20)	0.783
Carbohydrate[††]	50 (41-54)	37 (24-51)	51 (42-56)	33 (17-54)	47 (41-53)	40 (21-61)	0.637
Fat	15 (13-19)	87 (74-94)	13 (11-16)	96 (81-100)	16 (15-20)	76 (55-91)	**0.001**

Data derived from 24-hour dietary recall. [†]Prevalence of inadequacy was assessed using former Health Canada's Eating Well with Canada's Food Guide (2007). [††]Carbohydrate includes sugar. [†††]Prevalence of inadequacy was assessed using Health Canada's Acceptable Macronutrient Distribution Range (2022). P -values indicated are for differences between the groups assessed using the t-test of independence for normally distributed data and Wilcoxon's rank-sum test for not normally distributed data.

Table 6-5. Maternal micronutrient intake for all participants and by self-reported alcohol consumption

Nutrient	All women (n=59)			Non-at-risk (n=33)			At-risk (n=26)			P-value
	Median intake (Q1-Q3)	Median (IQR) %DRI[1]	% Inadequate (95% CI)	Median intake (Q1-Q3)	Median (IQR) %DRI[1]	% Inadequate (95% CI)	Median intake (Q1-Q3)	Median (IQR) %DRI[1]	% Inadequate (95% CI)	
Vitamin A (RE) (mcg)	1346 (835-2047)	245 (155-367)	20 (11-33)	1655 (879-2108)	246 (178-389)	12 (3-28)	1131 (639-1675)	236 (124-359)	31 (14-52)	0.619
Vitamin C (mg)	136 (87-187)	198 (130-286)	25 (15-38)	166 (103-313)	242 (153-433)	12 (3-28)	119 (65-140)	184 (101-229)	42 (23-63)	0.125
Thiamin (Vit B1) (mg)	3 (2-4)	225 (160-303)	22 (12-35)	3 (3-4)	247 (206-340)	12 (3-28)	2 (1-3)	189 (138-242)	35 (17-56)	**0.037**
Niacin (Vit B2) (mg)	32 (19-45)	214 (159-306)	22 (12-35)	37 (28-53)	261 (197-375)	12 (3-28)	23 (17-33)	183 (135-224)	35 (17-56)	**0.011**
Folate (Vit B9) (mcg)	425 (282-567)	83 (57-108)	78 (65-88)	549 (371-682)	100 (70-127)	64 (45-80)	332 (237-423)	69 (51-84)	96 (80-100)	**0.009**
Cobalamin (Vit B12) (mcg)	10 (6-15)	458 (286-625)	3 (0-12)	12 (8-17)	540 (377-724)	3 (0-16)	6 (4-11)	352 (183-482)	4 (0-20)	0.096
Choline (mg)	485 (350-676)	108 (78-150)	41 (28-54)	565 (463-911)	125 (103-203)	24 (11-42)	375 (329-534)	83 (73-119)	62 (41-80)	**0.001**
Calcium (mg)	859 (650-1433)	102 (81-159)	58 (44-70)	1274 (700-1561)	139 (86-182)	42 (26-61)	675 (511-877)	87 (69-125)	77 (56-91)	0.059
Iron (mg)	19 (13-28)	82 (59-115)	75 (62-85)	23 (18-33)	98 (81-140)	61 (42-77)	15 (10-17)	70 (49-81)	92 (75-99)	**0.008**
Zinc (mcg)	16 (10-22)	163 (110-230)	27 (16-40)	20 (14-28)	198 (148-268)	15 (1-32)	13 (9-16)	135 (101-164)	42 (23-63)	**0.012**
DHA (mcg)	75 (40-106)	38 (20-53)	93 (84-98)	94 (45-146)	47 (23-73)	91 (76-98)	62 (35-83)	31 (18-41)	96 (80-100)	0.058

Data derived from FFQ. Nutrient inadequacy was assessed using Health Canada's Dietary Reference Intake, Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50 for vitamins A, B12, C, folate, thiamin, niacin, zinc, calcium, iron; Adequate Intake (AI) for choline. Recommendations for DHA (docosahexaenoic acid, C22:6n-3) were obtained from Global Recommendations for DHA and EPA (eicosapentaenoic acid, C20:5n-3) Intake (2008). The differences between the risk groups were tested by the t-test of independence for normally distributed data and Wilcoxon's rank-sum test for not normally distributed data.

Table 6-6. Hierarchical linear regression analyzing the predictors of maternal total energy and individual macronutrient intake (% energy) (n=59)

Block		Model I β (SE)	p-value	Model II β (SE)	p-value	Model III β (SE)	P-value
	Total Energy (kcal)[†]						
1	Alcohol (Yes/No)	0.291 (0.129)	**0.036**	0.275 (0.140)	0.066[+]	0.205 (0.143)	0.103
	Drugs (Yes/No)	-0.024 (0.131)	0.854	0.087 (0.141)	0.543	-0.003 (0.151)	0.986
	Cigarettes	0.005 (0.031)	0.868	-0.014 (0.031)	0.667	0.016 (0.037)	0.662
2	Community[††]			-0.161 (0.131)	0.234	-0.040 (0.155)	0.799
	Age			0.004 (0.014)	0.781	-0.007 (0.015)	0.642
	Employment[†††]			-0.360 (0.175)	0.055	-0.304 (0.183)	0.117
3	Trimester					0.132 (0.093)	0.175
	BMI[††††]					0.011 (0.013)	0.416
	Chronic Illness (Yes/No)					0.118 (0.151)	0.447
	R^2	0.097		0.202		0.344	
	% Energy Protein						
1	Alcohol (Yes/No)	-0.063 (0.108)	0.568	-0.113 (0.128)	0.387	-0.149 (0.130)	0.271
	Drugs (Yes/No)	-0.025 (0.110)	0.823	-0.076 (0.128)	0.563	-0.096 (0.137)	0.494
	Cigarettes	-0.034 (0.026)	0.207	-0.026 (0.028)	0.373	0 (0.033)	0.995
2	Community			0.004 (0.119)	0.971	0.122 (0.141)	0.400
	Age			-0.005 (0.012)	0.716	-0.009 (0.013)	0.488
	Employment[†††]			0.207 (0.160)	0.211	0.209 (0.166)	0.228
3	Trimester					0.011 (0.084)	0.897
	BMI[††††]					0.020 (0.012)	0.176
	Chronic Illness (Yes/No)					-0.195 (0.138)	0.176
	R^2	0.095		0.174		0.347	
	% Energy Fat[†]						
1	Alcohol (Yes/No)	0.290 (0.122)	**0.047**	0.236 (0.122)	0.069[+]	0.211 (0.128)	0.095[+]
	Drugs (Yes/No)	-0.110 (0.124)	0.383	0.041 (0.122)	0.742	0.059 (0.129)	0.652
	Cigarettes	-0.028 (0.030)	0.365	-0.048 (0.027)	0.093	-0.062 (0.031)	0.069
2	Community[††]			-0.095 (0.114)	0.416	-0.093 (0.132)	0.492
	Age			0.019 (0.012)	0.132	0.018 (0.012)	0.175
	Employment[†††]			-0.435 (0.152)	**0.010**	-0.476 (0.157)	**0.008**
3	Trimester					-0.017 (0.079)	0.831
	BMI[††††]					-0.004 (0.011)	0.741
	Chronic Illness (Yes/No)					0.272 (0.129)	0.053[+]
	R^2	0.135		0.435		0.566	
	% Energy CHO						
1	Alcohol (Yes/No)	-0.094 (0.075)	0.225	-0.126 (0.089)	0.172	-0.062 (0.075)	0.421
	Drugs (Yes/No)	0.136 (0.076)	0.089	0.105 (0.089)	0.253	0.135 (0.079)	0.111
	Cigarettes	0.044 (0.018)	**0.025**	0.049 (0.020)	**0.022**	0.039 (0.019)	0.063[+]
2	Community[††]			-0.016 (0.083)	0.846	-0.113 (0.082)	0.186
	Age			-0.004 (0.009)	0.669	0.003 (0.008)	0.655
	Employment[†††]			0.135 (0.111)	0.239	0.147 (0.096)	0.150
3	Trimester					-0.044 (0.049)	0.380
	BMI[††††]					-0.009 (0.007)	0.205
	Chronic Illness (Yes/No)					-0.212 (0.080)	**0.018**
	R^2	0.323		0.381		0.660	

Models description: Model I - risk exposures; Model II – risk exposures and demographic factors; Model III - risk exposures, demographic factors, and maternal health factors. [†]Total Energy (kcal) and Fat (% energy) are log transformed. [††]Community: Opaskwayak Cree Nation (OCN), Chemawawin Cree Nation (CCN). [†††]Employment included the following variables ordinal progression: unemployed, employed part-time, employed full-time. [††††]Pre-pregnancy BMI; [+]trending toward significance, $p<0.1$.

Table 6-7a. Hierarchical linear regression analyzing the predictors of maternal micronutrient intake (% DRI) (n = 59)

		Model I		Model II		Model III		Model IV	
Block		β (SE)	P-value	β (SE)	P-value	β (SE)	P-value	β (SE)	P-value
	Vitamin A[†]								
1	Alcohol (Yes/No)	0.072 (0.417)	0.864	0.151 (0.464)	0.749	0.150 (0.515)	0.774	0.372 (0.587)	0.536
	Drugs (Yes/No)	0.190 (0.423)	0.657	0.596 (0.458)	0.209	0.652 (0.534)	0.240	0.639 (0.540)	0.256
	Cigarettes[††]	0.046 (0.105)	0.665	-0.003 (0.106)	0.981	-0.024 (0.137)	0.865	-0.028 (0.139)	0.843
2	Community			-0.272 (0.445)	0.548	-0.208 (0.560)	0.715	-0.329 (0.585)	0.582
	Age			0.073 (0.046)	0.130	0.071 (0.054)	0.207	0.065 (0.055)	0.253
	Employment[†††]			-1.000 (0.594)	0.108	-0.977 (0.671)	0.111	-0.980 (0.713)	0.085[+]
3	Trimester					-0.160 (0.331)	0.636	-0.039 (0.366)	0.917
	BMI[††††]					0.013 (0.042)	0.755	0.009 (0.043)	0.839
	Chronic Illness (Yes/No)					0.165 (0.557)	0.771	0.321 (0.594)	0.597
4	Total Energy (kcal)							0 (0)	0.426
	R^2	0.020		0.213		0.238		0.271	
	Vitamin C[†]								
1	Alcohol (Yes/No)	-0.323 (0.293)	0.282	-0.114 (0.345)	0.745	0.119 (0.314)	0.709	-0.044 (0.354)	0.902
	Drugs (Yes/No)	-0.333 (0.298)	0.275	-0.332 (0.341)	0.343	-0.088 (0.326)	0.789	-0.078 (0.326)	0.813
	Cigarettes	0.043 (0.074)	0.570	0.030 (0.079)	0.708	-0.046 (0.084)	0.590	-0.043 (0.084)	0.616
2	Community[††]			0.279 (0.331)	0.410	-0.003 (0.341)	0.994	0.087 (0.353)	0.809
	Age			-0.004 (0.034)	0.901	0.024 (0.033)	0.468	0.029 (0.033)	0.402
	Employment[†††]			-0.478 (0.442)	0.293	-0.657 (0.409)	0.127	-0.523 (0.430)	0.242
3	Trimester					-0.446 (0.202)	**0.042**	-0.535 (0.220)	**0.028**
	BMI[††††]					-0.014 (0.026)	0.587	-0.011 (0.026)	0.680
	Chronic Illness (Yes/No)					-0.599 (0.339)	0.097[+]	-0.714 (0.358)	0.065[+]
4	Total Energy (kcal)							0 (0)	0.332
	R^2	0.113		0.203		0.482		0.515	

Models description: Model I - risk exposures; Model II – risk exposures and demographic factors; Model III - risk exposures, demographic factors, and maternal health factors; Model IV - risk exposures, demographic factors, maternal health factors, and total caloric intake. [†]Vitamin A and vitamin C are log transformed. [††]Community: Opaskwayak Cree Nation (OCN), Chemawawin Cree Nation (CCN). [†††]Employment included the following variables ordinal progression: unemployed, employed part-time, employed full-time. [††††]Pre-pregnancy BMI; [+]trending toward significance, $p<0.1$.

Table 6-7b. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Thiamin								
1	Alcohol (Yes/No)	-54 (29)	0.384	-50 (35)	0.750	-44 (37)	0.721	-43 (37)	0.332
	Drugs (Yes/No)	46 (54)	0.401	72 (53)	0.177	69 (57)	0.232	51 (55)	0.360
	Cigarettes	2 (11)	0.858	5 (11)	0.652	7 (13)	0.603	1 (13)	0.920
2	Community††			82 (53)	0.128	86 (56)	0.131	75 (54)	0.167
	Age			-2 (4)	0.683	-5 (7)	0.480	-8 (6)	0.251
	Employment†††			-28 (17)	0.109	-24 (18)	0.189	-23 (17)	0.191
3	Trimester					26 (24)	0.297	9 (24)	0.716
	BMI††††					-0.222 (3.004)	0.939	-0.091 (3.023)	0.947
	Chronic illness (Yes/No)					128 (159)	0.363	149 (157)	0.397
4	Total Energy (kcal)							0.072 (0.030)	0.052+
	R^2	0.027		0.172		0.202		0.291	
	Niacin								
1	Alcohol (Yes/No)	-103 (27)	**0.000**	-99 (29)	**0.001**	-96 (31)	**0.003**	-99 (32)	**0.004**
	Drugs (Yes/No)	-32 (29)	0.267	-28 (30)	0.363	-25 (32)	0.444	-26 (33)	0.425
	Cigarettes	7 (6)	0.243	6 (6)	0.340	6 (7)	0.435	5 (8)	0.486
2	Community††			2 (30)	0.944	7 (31)	0.825	6 (32)	0.851
	Age			1 (2)	0.774	-3 (4)	0.356	-4 (4)	0.336
	Employment†††			-6 (10)	0.558	-5 (10)	0.632	-5 (10)	0.642
3	Trimester					10 (14)	0.460	8 (14)	0.550
	BMI††††					-0.103 (2.023)	0.949	-0.092 (2.041)	0.956
	Chronic illness (Yes/No)					-33 (33)	0.329	-34 (34)	0.314
4	Total Energy (kcal)							0.006 (0.018)	0.726
	R^2	0.332		0.354		0.401		0.403	

Table 6-7c. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Folate (Vit B9)								
1	Alcohol (Yes/No)	-46 (19)	**0.000**	-42 (20)	**0.000**	-38 (26)	**0.000**	-33 (31)	**0.001**
	Drugs (Yes/No)	1 (10)	0.883	3 (10)	0.771	4 (11)	0.710	4 (11)	0.693
	Cigarettes	3 (2)	0.111	3 (2)	0.165	3 (3)	0.304	3 (3)	0.299
2	Community††			2 (10)	0.861	4 (11)	0.726	4 (11)	0.716
	Age			0.419 (0.761)	0.585	-1 (1)	0.341	-1 (1)	0.375
	Employment (Yes/No)			-0.634 (3.015)	0.848	-0.529 (3.421)	0.879	-0.552 (4.001)	0.876
	Trimester					1 (5)	0.763	2 (5)	0.728
	BMI†††					0.085 (0.548)	0.877	0.083 (0.554)	0.882
	Chronic illness (Yes/No)					-11 (11)	0.322	-11 (11)	0.348
4	Total Energy (kcal)							-0.001 (0.006)	0.829
	R^2	0.300		0.316		0.362		0.363	
	Cobalamin (Vit B12)†								
1	Alcohol (Yes/No)	-0.200 (0.376)	0.600	-0.298 (0.384)	0.447	-0.247 (0.415)	0.561	-0.132 (0.479)	0.787
	Drugs (Yes/No)	0.294 (0.381)	0.449	0.779 (0.380)	0.054+	0.824 (0.431)	0.074+	0.817 (0.441)	0.084+
	Cigarettes	-0.025 (0.095)	0.797	-0.070 (0.087)	0.431	-0.081 (0.111)	0.476	-0.083 (0.113)	0.475
2	Community††			-0.470 (0.369)	0.218	-0.389 (0.452)	0.402	-0.451 (0.478)	0.360
	Age			0.100 (0.038)	**0.016**	0.100 (0.044)	**0.036**	0.097 (0.045)	**0.048**
	Employment (Yes/No)			-0.799 (0.492)	0.121	-0.921 (0.541)	0.108	-0.998 (0.582)	0.102
3	Trimester					-0.211 (0.267)	0.441	-0.149 (0.299)	0.626
	BMI†††					0.025 (0.034)	0.476	0.022 (0.035)	0.532
	Chronic Illness (Yes/No)					-0.204 (0.449)	0.655	-0.123 (0.485)	0.803
4	Total Energy (kcal)							0 (0)	0.612
	R^2	0.098		0.310		0.334		0.390	

Models description: Model I - risk exposures; Model II – risk exposures and demographic factors; Model III - risk exposures, demographic factors, and maternal health factors; Model IV - risk exposures, demographic factors, maternal health factors, and total caloric intake. †Cobalamin is log transformed. ††Community: Opaskwayak Cree Nation (OCN), Chemawawin Cree Nation (CCN). †††Pre-pregnancy BMI; +trending toward significance, $p<0.1$.

Table 6-7d. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Choline†								
1	Alcohol (Yes/No)	-0.661 (0.195)	**0.003**	-0.548 (0.228)	**0.027**	-0.545 (0.255)	**0.048**	-0.464 (0.293)	0.134
	Drugs (Yes/No)	-0.048 (0.198)	0.812	0.046 (0.226)	0.842	0.006 (0.264)	0.983	0.001 (0.270)	0.998
	Cigarettes	0.008 (0.049)	0.874	-0.009 (0.052)	0.857	0.007 (0.068)	0.917	0.006 (0.069)	0.937
2	Community††			0.021 (0.219)	0.926	0.039 (0.277)	0.890	-0.006 (0.292)	0.985
	Age			0.011 (0.023)	0.625	0.009 (0.027)	0.728	0.007 (0.028)	0.792
	Employment†††			-0.429 (0.292)	0.158	-0.379 (0.331)	0.269	-0.446 (0.356)	0.229
3	Trimester					0.054 (0.164)	0.745	0.099 (0.183)	0.597
	BMI††††					0.003 (0.021)	0.876	0.002 (0.021)	0.940
	Chronic Illness (Yes/No)					-0.153 (0.275)	0.587	-0.095 (0.297)	0.753
4	Total Energy (kcal)							0.012 (0.009)	0.555
	R^2	0.346		0.387		0.431		0.445	
	Calcium†								
1	Alcohol (Yes/No)	-0.556 (0.223)	**0.009**	-0.352 (0.266)	**0.023**	-0.296 (0.294)	0.069+	-0.468 (0.310)	**0.036**
	Drugs (Yes/No)	-0.023 (0.270)	0.933	0.177 (0.276)	0.530	0.241 (0.316)	0.457	0.252 (0.311)	0.430
	Cigarettes	0.004 (0.067)	0.997	-0.034 (0.064)	0.600	-0.052 (0.081)	0.532	-0.048 (0.080)	0.556
2	Community††			0.123 (0.268)	0.651	0.063 (0.331)	0.852	0.168 (0.337)	0.626
	Age			0.035 (0.028)	0.220	0.043 (0.032)	0.201	0.048 (0.032)	0.155
	Employment†††			-0.880 (0.358)	**0.024**	-0.935 (0.396)	**0.031**	-0.777 (0.410)	0.078+
3	Trimester					-0.145 (0.196)	0.070+	-0.250 (0.210)	0.153
	BMI††††					0.001 (0.025)	0.961	0.005 (0.025)	0.837
	Chronic Illness (Yes/No)					-0.257 (0.329)	0.446	-0.393 (0.342)	0.268
4	Total Energy (kcal)							0 (0)	0.235
	R^2	0.166		0.404		0.446		0.497	

†Choline and calcium are log transformed.

Table 6-7e. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	Iron†								
1	Alcohol (Yes/No)	-0.366 (0.262)	0.176	-0.309 (0.272)	0.270	-0.245 (0.294)	0.417	-0.213 (0.342)	0.544
	Drugs (Yes/No)	0.155 (0.266)	0.566	0.456 (0.269)	0.106	0.531 (0.305)	0.101	0.529 (0.315)	0.114
	Cigarettes	0.020 (0.066)	0.761	-0.015 (0.062)	0.806	-0.038 (0.078)	0.633	-0.039 (0.081)	0.638
2	Community††			-0.155 (0.261)	0.559	-0.174 (0.320)	0.594	-0.192 (0.341)	0.582
	Age			0.058 (0.027)	0.094+	0.063 (0.031)	0.077+	0.063 (0.032)	0.071+
	Employment†††			-0.759 (0.348)	**0.042**	-0.859 (0.383)	**0.040**	-0.885 (0.416)	**0.050**
3	Trimester					-0.188 (0.189)	0.334	-0.171 (0.213)	0.436
	BMI††††					0.007 (0.024)	0.758	0.007 (0.025)	0.787
	Chronic Illness					-0.154 (0.318)	0.634	-0.132 (0.347)	0.710
4	Total Energy (kcal)							$-4.59e^{-5}$ (0)	0.840
	R^2	0.095		0.365		0.416		0.418	
	Zinc†								
1	Alcohol (Yes/No)	-0.436 (0.241)	0.085+	-0.316 (0.254)	0.229	-0.221 (0.270)	0.425	-0.296 (0.312)	0.356
	Drugs (Yes/No)	0.068 (0.245)	0.784	0.309 (0.251)	0.233	0.405 (0.280)	0.168	0.410 (0.287)	0.174
	Cigarettes	-0.028 (0.061)	0.655	-0.061 (0.058)	0.305	-0.090 (0.072)	0.227	-0.089 (0.074)	0.246
2	Community††			-0.046 (0.243)	0.851	-0.149 (0.294)	0.619	-0.108 (0.311)	0.734
	Age			0.043 (0.025)	0.104	0.054 (0.028)	0.075+	0.056 (0.029)	0.076+
	Employment†††			-0.766 (0.325)	**0.029**	-0.843 (0.352)	**0.029**	-0.761 (0.379)	0.057+
3	Trimester					-0.187 (0.174)	0.298	-0.228 (0.194)	0.259
	BMI††††					-0.004 (0.022)	0.875	-0.002 (0.023)	0.931
	Chronic Illness					-0.254 (0.292)	0.396	-0.308 (0.315)	0.345
4	Total Energy (kcal)							0.003 (0.001)	0.608
	R^2	0.140		0.382		0.451		0.461	

†Iron and zinc are log transformed.

Table 6-7f. continued

Block		Model I β (SE)	Model I p-value	Model II β (SE)	Model II p-value	Model III β (SE)	Model III p-value	Model IV β (SE)	Model IV p-value
	DHA †								
1	Alcohol (Yes/No)	-0.120 (0.665)	0.859	-0.168 (0.820)	0.840	0.069 (0.791)	0.931	0.317 (0.911)	0.732
	Drugs (Yes/No)	0.513 (0.675)	0.455	0.629 (0.810)	0.447	0.471 (0.821)	0.575	0.455 (0.839)	0.595
	Cigarettes	0.083 (0.168)	0.627	0.071 (0.187)	0.708	0.157 (0.211)	0.468	0.152 (0.215)	0.492
2	Community††			-0.374 (0.786)	0.639	-0.621 (0.861)	0.481	-0.756 (0.908)	0.418
	Age			0.006 (0.081)	0.941	0.023 (0.083)	0.786	0.017 (0.086)	0.849
	Employment†††			-0.058 (1.021)	0.956	0.363 (1.229)	0.729	0.160 (1.082)	0.887
3	Trimester					0.262 (0.509)	0.614	0.397 (0.568)	0.495
	BMI††††					-0.014 (0.064)	0.825	-0.019 (0.066)	0.773
	Chronic Illness					-0.012 (0.855)	**0.039**	-0.320 (0.922)	0.077+
4	Total Energy (kcal)							0.002 (0.001)	0.564
	R^2	0.038		0.049		0.305		0.321	

†DHA is log transformed.

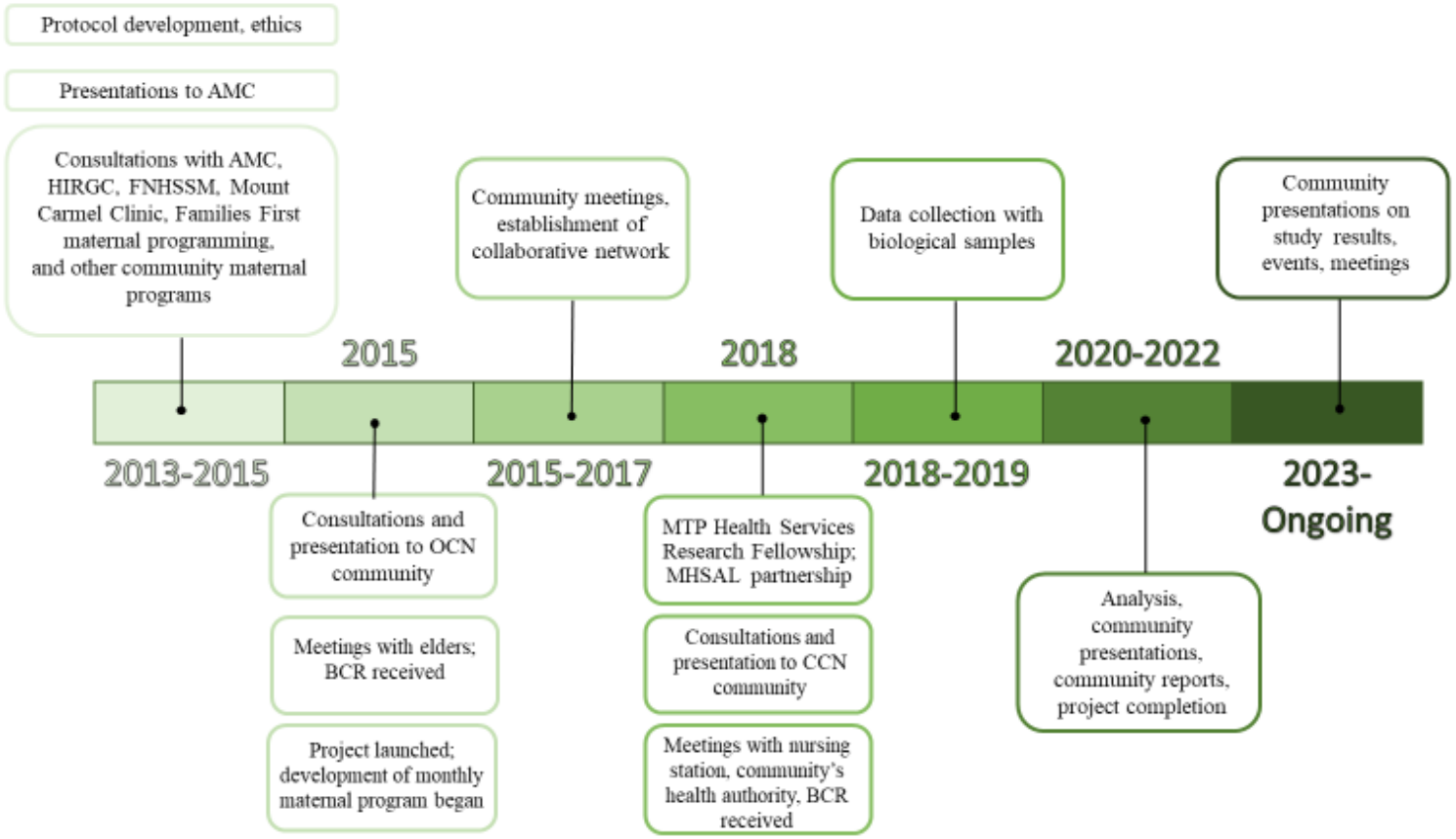
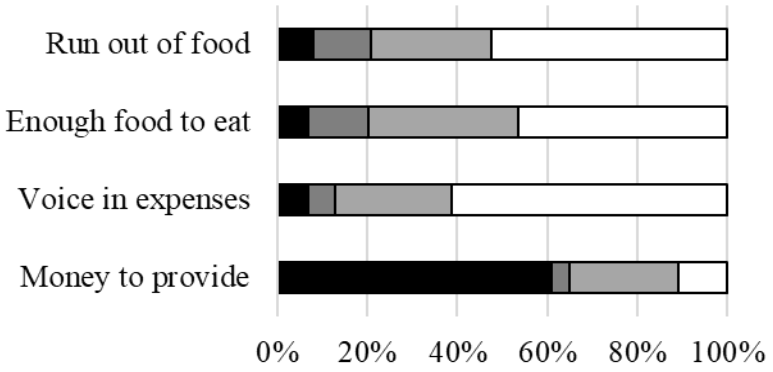


Figure 6-1. Study timeline

AMC-Assembly of Manitoba Chiefs; HIRIG- Health Information Research Governance Committee; FNHSSM-First Nations Health Social Secretariat Manitoba; OCN-Opaskwayak Cree Nation; BCR-Band and Council Resolution; MTP-Manitoba Training Program for Health Services Research; MHSAL-Manitoba Health Seniors and Active Living; CCN-Chemawawin Cree Nation.

a)



b)

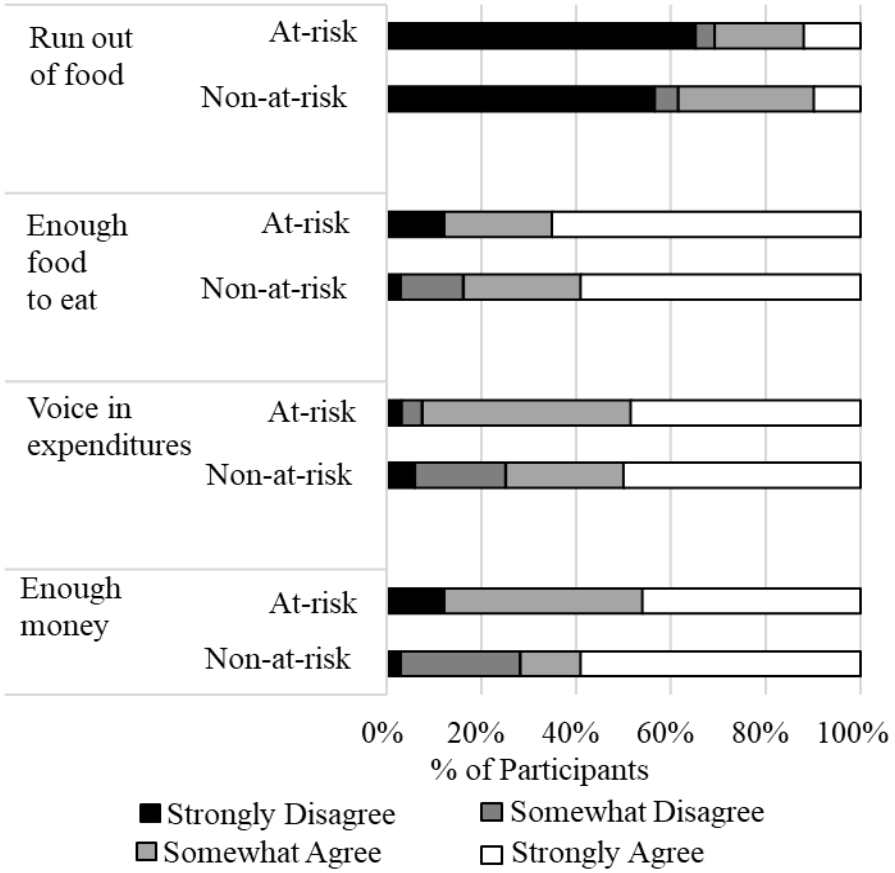
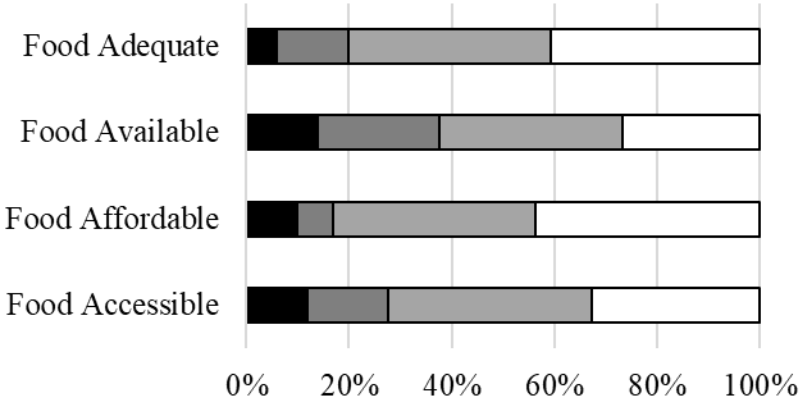


Figure 6-2. Self-reported access to financial resources a) all women (n=59); b) non-at-risk (n=33) and at-risk women (n=26). Data presented in proportion of participants. No significant difference was identified between non-risk and at-risk groups.

a)



b)

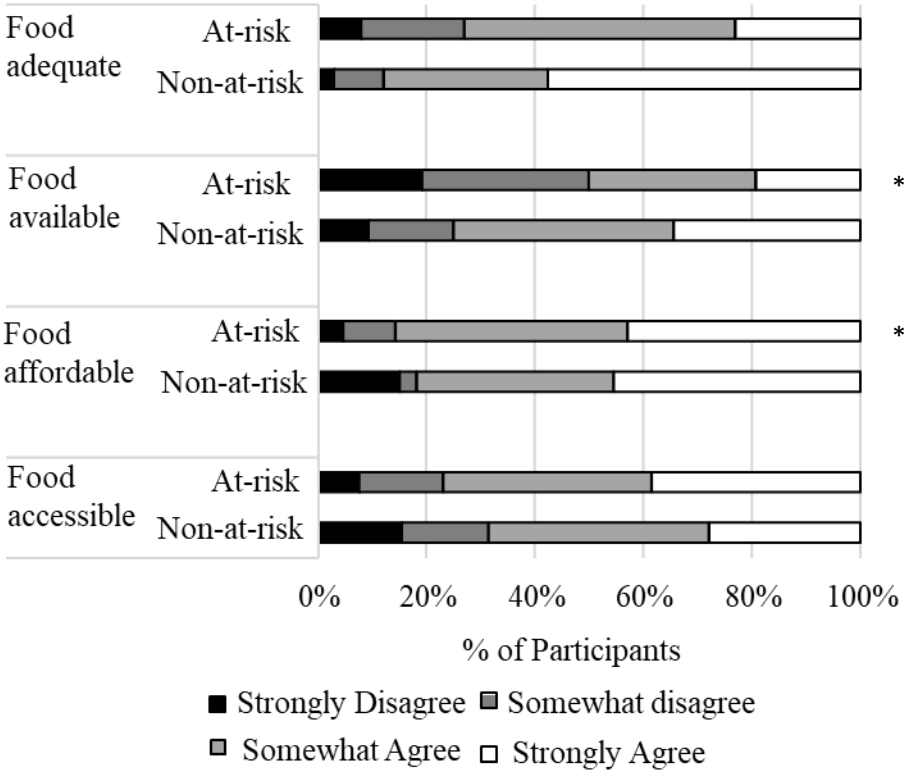


Figure 6-3. Self-reported access to food a) all women (n=59); b) non-at-risk (n=33) and at-risk women (n=26). Data presented in proportion of participants. *significant difference ($p<0.05$) between non-at-risk and at-risk groups.

CHAPTER VII: OVERALL DISCUSSION AND FUTURE DIRECTIONS

The objective of this study was to investigate the dietary intake and to explore the relationship between macro- and micro-nutrient intake and risk factors, specifically alcohol consumption, of pregnant First Nations women residing in Manitoba Northern First Nation communities. The results demonstrate that alcohol consumption and other risk exposure variables (smoking and drugs) are factors that are influencing intakes of macro- and micro-nutrients. In summary, the findings of this study demonstrated that pregnant women in both communities had/showed:

- high prevalence of inadequacy for former Health Canada's food groups (2007); high prevalence of inadequacy collectively for folate, iron, and DHA Additionally, the community of OCN had high prevalence of inadequacy for calcium
- the positive relationship on total energy intake and the energy from fat (%); as well as when the data from two communities was amalgamated and adjusted for other risk factor covariates ($p < 0.05$), respectively.
- inverse associations on DRI (%) intake observed for thiamin, niacin, folate, choline, calcium, iron, and zinc, after controlling for the effects of trimester and BMI in CCN. Likewise, for OCN community, inverse relationships were identified for vitamin C and niacin after adjustments for drug exposures; and calcium after adjustments for drug exposures and demographic variables. Inverse relationships between self-reported alcohol consumption and DRI (%) intake was detected for for niacin and folate for both communities. These findings were statistically significant across all four regression blocks, which included adjustments for risk exposures, demographics, health, and total caloric intake ($p<0.05$). Choline and calcium also demonstrated inverse relationships with alcohol consumption for both communities. Choline for the first three regression blocks ($p < 0.05$); and calcium for the first, second, and fourth block ($p < 0.05$), with marginal significance ($p<0.1$) for the third block.
- The relationship detected between several biomarkers. A significant positive relationship was identified for plasma glucose with BMI and age as covariates in the model; and negative for erythrocyte C18:2n6, C20:4n6, plasma IL-10 after adjustments for trimester and BMI.

Of concerns were the findings regarding high prevalence of inadequacy for folate, iron, and

DHA during the studied pregnancy. As discussed throughout the Chapters these nutrients are critical for the structural and physiological development of the fetal central and peripheral nervous systems, neurogenesis, rapid cell growth, increased cardiac output and blood volume. These nutritional inadequacies are prevalent for women in resource-poor settings, nationally and globally (Gernand et al., 2016). However, the implications of these nutritional deficiencies in First Nations maternal population, a population affected by various systemic and jurisdictional disparities with an already existing burned of illness, might be more serious. Special attention should be placed on maternal intakes of these nutrients in future research and FASD programming.

Taken together the findings of the present study identify an interaction between alcohol consumption and intakes of several macro- and micro-nutrients. Although interpretation with caution is advised due to study limitations, increase power exhibited in combined results provided a higher degree of confidence for the findings that alcohol consumption may influence nutrient intakes crucial for fetal development such as folate, choline, and iron. While no Canadian comparative literature was identified, several international studies (May et al., 2014; 2016; Carter et al., 2017) had similar overall designs and findings. Similar study design and findings for biological markers were reported by Sowell and colleagues (2018; 2020) and Keen and colleagues (2010) who also studied the influence of alcohol on nutritional and inflammatory markers, cytokines and minerals.

Community comparisons

The results from both communities demonstrated that there were some similarities and differences in the two communities studied. As identified in *Chapter 6,* statistically significant difference was noted for the (%) fat intake and Meat and Alternatives food group with higher intake in CCN community. This may be a result of seasonality and community infrastructure (with respect to grocery stores). Geographically, the communities are located within the same Boreal Plains ecozone, 203 km away from each other. The ecozone is characterized by high number of freshwater lakes, valleys, and forests (Ecological Framework of Canada, 2023; Chan, 2012). These localities allow for traditional ways of food acquisition such as hunting, fishing, herb, and berry picking. Surprisingly, although the information on traditional food intake was collected, only 6 individuals from both communities reported traditional food consumption (lake

fish, game meat). Harvesting and consumption of traditional food items has been identified as a health promoting factor due to the high nutrition quality of traditional food items, fostering of the preservation of the cultural practice, and improved food security (Thompson et al., 2012; Willow et al., 2009; Chan et al., 2019). Traditional food systems have historically been a fundamental part of First Nations Peoples sustenance (Earle, 2011). Therefore, the lack of traditional food item intake, especially for the population with increased chronic illness and risk exposures, raises concerns. Although these findings could be observed due to the state of pregnancy, which is known to affect normal dietary habits and the seasonal impacts, these findings could also be a consequence of nutrition transition (Johnson-Down & Egeland, 2012). Nutrition transition is characterized by increased presence of western food items and diminished practices of harvesting and consumption of traditional food items in Indigenous populations (Johnson-Down & Egeland, 2012). There is scarcity of information on nutrition transition for women identified as at-risk. A qualitative research study aimed at identifying the environmental influences of pregnant women's food choices, especially in the context of risk exposure and FASD, in these communities is warranted to provide insights into this topic of interest.

Although both communities are within the same ecozone and general geographic location, one notable difference exists with respect to the level of overall community and medical infrastructure. While the community of OCN (~700 km) is located further away from the capital of Manitoba – Winnipeg, it is located near The Pas. As identified in the *Community Profile* section of *Chapter 5*, the town has well developed and reliable infrastructure, including medical and education facilities, grocery stores, various businesses, information, and communications technology services. The community of CCN is located closer (~400 km) to the city of Winnipeg, however, the location of the community is remote. The community is not supplied with infrastructure, therefore the residents of the community experience a lack of access to healthcare services. This difference in location has special implications for maternal population of the CCN community. Whereas OCN women may rely on the access to reproductive care and birth services from nearly located NRHA medical facilities, CCN women have no access to maternal medical care. This increased the prevalence of CCN's births affected by the birth evacuation policy. The policy is characterized by medical evacuation of a pregnant women to urban medical center and confinement starting at 36-38 week until birth. As discussed in *Chapter 3*, this policy has negative impacts of maternal and child health (Lawford et al., 2018), it delays

clinical recognition of pregnancy, contributes to the engagement system involvement (Justice and Child and Family Services) (Silver et al., 2022), and contributes to poorer birth outcomes (Kornelsen et al., 2010). Therefore, it is possible that the effects of evacuation birthing policy might have impacted the study with respect to the sample size (women might have been more hesitant to confirm their pregnancy), responses on risk exposures (especially FASD-associated study) and increased social desirability bias on dietary responses.

Overall Strengths

This project has several strengths. First, the project has been led and conducted in close partnerships with both communities. Study planning and facilitation were guided by community members, nursing staff, community workers, elders, and participants. This assisted in establishing trust and a collaborative relationship between the researcher and the participants.

Second, the face-to face data collection has been performed by the same researcher throughout the entire project. This allowed for a standardized data collection approach and minimizing measurement bias (Freedman et al., 2011). Furthermore, interactive, face-to-face data collection set basis for an involved approach to data harvesting. Studies indicate that collaborative research practices, specifically with Indigenous communities improve knowledge transfer, communication, and information sharing which occurs during research process (Sheridan, 1998)

Third, the study utilized FFQ and 24-hour dietary recall tools, which have previously undergone content validity testing in the First Nations prenatal population (Gisbrecht, 2015; Dyck, 2016). Although the survey was not subjected to an extensive and rigorous validity and reliability testing, prior data collection with the tool at OCN community and Point Douglas, Winnipeg, allowed for the evaluation of the survey tool and its content. The written feedback and evaluation from the mothers were collected at the end of each data collection trip in both communities. The feedback was incorporated into the data collection methods. Additionally, the strength inherent to the FFQ and 24-hr dietary recall include simplicity, inexpensiveness, and time-efficiency (Feskanich et al., 1993).

The combined sample size is another strength of the project which allowed for an increase in statistical power and a higher level of confidence in the findings described in *Chapter 6*. The study contributed crucial information on maternal dietary intake and its association with various

risk exposure, especially alcohol consumption, which is a primary risk factor for FASD. This is the first study, to our knowledge, examined dietary and risk exposure variables in two northern communities in Manitoba.

An additional strength is the collection of biological samples in CCN community. Plasma low density cholesterol (LDL), high density lipoprotein (HDL), triglycerides, and total cholesterol are predictive biomarkers for the long- and short-term intake of dietary fats (Bingham et al. 2003; Jenab et al., 2009). Although larger sample is required to assess meaningful relationships between intake and the plasma status of these indicators, the CCN pilot study provided the first step and important information toward basic biochemistry and plasma nutrition indicators assessment in First Nations maternal population.

Overall Limitations

The study has several limitations that need to be taken into consideration when interpreting the outcomes. Similarly to the limitations outlined in *Chapters 3-6,* the major limitation of this project are a small sample sizes, including a pooled sample size, the use of self-reported unvalidated data collection tool, the lack of recovery biomarkers for the studied macro- and micro-nutrients as well as consumption markers.

As discussed in previous Chapters, the small sample size has greatly affected the power of the study, precluded various forms of sub-analysis, and limited the addition of covariates (to avoid models overfitting) (Kwam & Vidakovic, 2007). Neither of pilots had sufficient sample size to achieve the desirable small effect size (0.1). This limitation negatively impacts the interpretation, generalization, and application of these results to other Manitoban First Nations maternal populations. It is strongly recommended to increase sample size to the number identified in this study's power analysis (n of 783) (*Chapter 3*) to increase power and improve an overall generatability of the results.

The second impactful limitation of this study is the use of self-reported information on dietary intake. The self-reported design creates participant response bias, leading to over- and under-reporting, precluding error-free analysis and interpretation (Prentice, 1996). Dietary intake studies demonstrate that approximately 21.5-67.0% of various cohorts underreport and 1.0-6.0% over-report intakes (Poslusna et al., 2009). Dietary measurement error presents significant

challenges to the reliability of findings in nutritional cohort studies (Freedman et al., 2011). The measurement error greatly contributes to the diminishing of statistical power for the detection of the relationship between the diet and the disease, in this case risk exposures (Freedman et al., 2011). Although the utilization of the food models attempted to minimize the limitation of the measurement error, it did not eradicate it completely. It is recommended to increase sample size and introduce repeated measures through the entire pregnancy to minimize the measurement error. Additional barriers to accurate self-reporting included factors such as literacy skills, motivation, and time investment required, which are responsible for the lack of precision, even with the validation of self-reports (Poslusna et al., 2009).

Likewise, use of self-report also was a serious limitation for the accurate assessment of the self-reported alcohol consumption. This limitation is pertinent to many risk-exposure and FASD-associated studies and diagnosis, as confirmation in the form of self-report is required (Cook et al., 2016; Joya et al., 2013; Stevens et al., 2020). Presently, there is a lack of validated tools for the assessment of maternal alcohol consumption and identification of women at-risk of carrying a child with the potential diagnosis of FASD (Stevens et al. 2020; Joya et al., 2013). Although the elucidation of an objective biomarker of long- and short-term alcohol consumption is required, reducing the stigma associated with consumption during pregnancy is identified as an important approach to data collection on consumption, especially in First Nations populations (Stevens et al., 2020; Tait, 2000). As stated in previous chapters, stigma affected the response to the questions on alcohol in the present study. When asked the questions on the patterns and timing of consumption during the studied pregnancy women had identified the feeling of discomfort due to reasons of systems involvement, complex family situations, and overall negative perceptions of consumption during pregnancy. This limitation affected not only the alcohol consumption section but the *Substance Use* sub-section of the questionnaire.

Another limitation of the study is the lack of various biological markers, including recovery biomarkers. Although some biological data was collected, plasma chemistry has limitations, due to the variability in genetics, metabolism, and disease status. It is recommended to incorporate recovery biomarkers such as doubly labeled water for energy intake assessment, urinary nitrogen for protein intake assessment, and urinary sucrose and fructose for sugar intake assessment (Jenab et al., 2009) to confirm nutrient intake acquired through the 24-hour dietary recall.

The lack of repeated measures throughout pregnancy is another limitation, which did not allow for the full characterization of dietary intake and its association with risk exposures throughout pregnancy. One of the greatest drawbacks of a cross-sectional design is the inability to assess and form associations between exposure and outcome in temporal sequence (Carlson & Morrison, 2008), this limitation has impacted our study.

As previously discussed in *Chapters 3-6*, lack of the research instrument validation in the population studied is another limitation. This limitation is being addressed, presently, as this is the next step of the project.

Methodological Considerations

The major goal of this pilot study was to evaluate the baseline dietary intake of macro- and-micronutrients important for fetal CNS development and assess the contribution of self-reported risk exposure predictors, specifically alcohol, to the %AMDR and %DRI nutrient intake. The findings of this pilot contribute to identifying whether maternal nutrition programming and/or intervention during pregnancy is necessary. To assist with this determination more studies are required, especially large prospective and clinical studies which employ established and validated methodologies with recovery biomarkers and long-term nutrition status indicators. This research is important in First Nations on-reserve populations, as maternal nutritional status has not been assessed regularly to capture the baseline dietary intake, which limits the development of nutrition strategies for the maternal program with and without FASD risks.

Several methodological considerations of this study warrant a mention. The study has assessed the influence of risk-exposures on the percent recommended intake (%DRI and %AMDR for micro- and macro-nutrients, respectively). This methodological approach was taken to align with the set objectives and similar studies published in the field (May et al., 2014,; 2016; Dyck., 2016; Carter et al., 2017); as well as to translate the findings into practical recommendations for the communities. The assessment of risk exposure influences on energy adjusted intake of micronutrients is not reported in this study, however, it presently being computed to identify analytical differences between various approaches.

The study has not utilized the validated research instrument. Although psychometric properties of FFQ and 24-hour dietary recall are well established, to ensure the precision and data quality the use of a previously validated questionnaire is paramount (Cade et al., 2002). It is the goal of

the research team to include validity testing as the next step. Specifically, convergent validity, inter-item reliability, internal validity, test–retest or inter-rater reliability have been planned as the next research step.

The present study has utilized former Eating Well with Canada's Food Guide (CFG) (Health Canada, 2007) for the analysis of food groups median intake and adequacy. The data collection and a part of the analysis occurred prior to the release of the new food guide. The use of former CFG may present limitation to the interpretation and application of the study results in light of new recommendations. The complication may arise with respect to the application of these results when comparing Meat and Alternative and Milk and Alternative foods groups. These food groups have been amalgamated to formulate a new food group titled Protein Foods (Health Canada, 2019). Further to that, the number of daily servings recommendations has been replaced with the representation of a plate which identifies recommended proportions of Protein, Whole Grains, Vegetables and Fruit. Similarly to the previous Health Canada recommendations (2007), the recommended intake proportion for the new Vegetables and Fruit food group is high. The new CFG has received criticism with respect to relevance for Indigenous population, as it does not include some of foundational traditional food items, which were present in the Indigenous CFG (2007) (Brake, 2019). Although the new CFG (2019) substantially differs from the older version (2007), the recommended dietary behaviours remain similar, which include increased intake of nutrient-dense foods such as vegetables and fruits, whole grains, healthy proteins and oils. Therefore, the two communities studied can still rely on the food group findings.

Implications and Future Direction

This study has implications for FASD nutrition strategies, maternal and child health programming, and First Nations health policy. The study advances our knowledge on prenatal maternal dietary intake and its association with alcohol consumption prenatally in hard-to-reach First Nations communities. Policy and program approaches are required to enhance First Nations women's access to culturally appropriate, nutritious, and economically accessible foods. The findings point to a low intake of fruits and vegetables, dairy, and healthy grains, which are nutrient-dense products. Provided that fresh fruits and vegetables are difficult to access due to financial or accessibility reasons, as the findings of the present study, reveal, women in food-insecure households may be compelled to rely on inexpensive, processed, and high glycemic

load foods that foster satiety, but do not contribute to appropriate nutrition status and thus lead to disturbances in energy metabolisms and health status (Council of Canadian Academies, 2018). Consumption of nutritious foods and overconsumption of processed foods, especially in pregnant women at risk who identify themselves as at-risk of carrying a child with FASD, may further exacerbate maternal and child health outcomes.

The historical legacy of assimilation and colonization policies evoked multiple disadvantages for Indigenous women. The residential schooling system, appropriation of Indigenous land and resettlements, eradication of language and culture, and multiple forms of discrimination have elicited substantial disruptions to Indigenous economies, communities' livelihoods, and health (Regan, 2010). These events greatly affected Indigenous matriarchal realities, shifting Indigenous women's identities, autonomy, feminine capacity, and agency exposing women to a higher level of adverse consequences (Heart, 2003).

Nutrition intervention for individuals at-risk of carrying a child with FASD is a novel approach and requires special attention. Nutrition research should focus on all aspects of thorough data, including dietary intake, biological, clinical, and administrative data. Strengthening nutrition research methodologies for the premise of FASD programming will positively impact all not only maternal and child health outcomes, but will also build awareness around the significance of nutrition as a determinant of maternal and child health. Future research undertakings should continue the focus on Indigenous communities, including Metis and Inuit populations, and aim at identifying nutrition intake and status correlating the variables for further understanding of the relationships between the risk factors and nutrition. Furthermore, studies should further investigate the relationships between the timing of pregnancy, the amount and timing of alcohol consumption during gestation, and the duration of ethanol exposure. The studies should also focus on identifying the nutrition status of infants with FASD and infants without FASD diagnosis who were born to at-risk women.

In conclusion, the identification of dietary intake and status of pregnant women at-risk of carrying a child with FASD is crucial for proper programming, policy, intervention, and overall community health. The findings of this study suggest that nutrient intake and risk exposures are associated. Although the ultimate prevention for FASD is abstaining from alcohol consumption during gestation, research indicates that the prevalence of FASD continues to be high, therefore

exploring other preventative approaches, especially in populations that have limited data, is paramount. This study provides some baseline information for future nutrition research and grass-roots community maternal programming that assists in attenuating the FASD outcomes, prevalence, and long-term health care costs associated with the disorder.

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Appendix 1. Maternal micronutrient intake (%DRI) for CCN participants from both the diet supplements.

Micronutrients	All participants (n = 22)	Non-at-risk (n =11)	At-risk (n = 11)	P-value
Vitamin A (mcg)	304 (171-505)	440 (363-661)	188 (151-250)	**0.067**
Vitamin C	265 (207-413)	355 (209-525)	242 (212-382)	0.332
Thiamin (Vit B1)	281 (211-346)	327 (277-478)	230 (184-282)	**0.040**
Niacin (Vit B2)	298 (230-370)	347 (268-482)	254 (201-306)	0.116
Folate (Vit B9)	229 (107-274)	129 (102-310)	237 (217-269)	0.847
Cobalamin (Vit B12)	465 (297-873)	922 (694-1197)	331 (239-405)	0.013
Calcium	155 (93-205)	210 (168-261)	100 (71-139)	**0.008**
Iron	167 (119-205)	171 (111-268)	167 (151-196)	0.652
Zinc	224 (165-286)	290 (216-438)	195 (143-236)	0.056

Values are medians (Q1-Q3). Data derived from FFQ and supplement reported. Nutrient %DRI was assessed using Health Canada's Dietary Reference Intake, Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50. The differences between the risk groups were tested by the t-test of independence for normally distributed data and Wilcoxon's rank-sum test for not normally distributed data.

Appendix 2. Birth and infant parameters from the participants in CCN

Participant	Infant Sex (F, M)[1]	Gestational age at birth	Birth Weight (g)	Apgar score	Delivery method	Discharge summary notes
CHEM-1	F	38 weeks	2,621	N/A	C-section	N/A
CHEM 2	M	36 weeks 1 day	2,940	N/A	Vaginal	Hospitalized for newborn jaundice
CHEM-3	F	35 weeks 5 days	2,726	1 min: 7 5 min: 9	Vaginal	Hypoglycemia
CHEM-4	F	38 weeks	3,592	N/A	N/A	Unremarkable
CHEM-5	F	39 weeks	3,764	1 min: 7 5 min: 10	Vaginal	Unremarkable
CHEM-6	M	37 weeks 3 days	3,580	1 min: 8 5 min: 9	Vaginal with induction	Neonatal hypoglycemia secondary to maternal diabetes
CHEM-7	M	38 weeks	3,532	NA	Vaginal	Unremarkable
CHEM-8	F	40 weeks	3,312	1min: 2 5 min: N/A	N/A	Baby resuscitated at birth. High risk
CHEM-9	N/A	38 weeks 4 days	N/A	1 min: 9 5 min: 10	Vaginal	Unremarkable
CHEM-10	F	39 weeks	3,231	N/A	Vaginal	Unremarkable
CHEM-11	F	37 weeks 4 days	2,512	1 min: 9 5 min: 10	Vaginal	Unremarkable
CHEM-12	F	38 weeks 4 days	3,236	1 min: 9 5 min: 9	Vaginal	Unremarkable
CHEM-13	F	36 weeks	3,180	1 min: 7 5 min: 9	C-section	Late pre-term infant. Ruptured membranes at C-section; baby cyanotic at birth, NICU[2], on CPAP[3] for 24 hours.
CHEM-14	F	39 weeks 4 days	3,006	1 min: 9 5 min: 10	Vaginal	Systolic heart murmur
CHEM-15	M	29 weeks 3days	1,430	1 min: 6 5 min: 6	Spontaneous vaginal	Pre-term infant. Rupture of membranes. Respiratory distress syndrome, ventilated ACVG[4]-CPAP.
CHEM-16	N/A	N/A	N/A	N/A	N/A	N/A
CHEM-17	F	39 weeks	3,212	1 min: 9 5 min: 10	Vaginal	High risk due to poor prenatal care. The mother had anti-K antibodies, an infant was DAT[5] positive.
CHEM-18	N/A	N/A	N/A	N/A	N/A	N/A
CHEM-19	M	38 weeks 6 days	3,012	1 min: 6 5 min: 9	Vaginal	Unremarkable
CHEM-20	M	38 weeks	3,316	N/A	N/A	Unremarkable
CHEM-21	M	40 weeks 3 days	3,900	1 min: 6 5 min: 8	Vacuum assisted vaginal	Born with acrocyanosis
CHEM-22	N/A	N/A	N/A	N/A	N/A	N/A

[1]F, M: Female, Male; [2]NICU, Newborn intensive care unit; [3]CPAP, Continuous positive airway pressure; [4]ACVG, Assist control volume guarantee ventilation; [5]DAT, Direct antibody test. N/A, data not available.

Appendix 3. Maternal trace metal levels for all CCN participants and by self-reported alcohol consumption

Metal	All women (n=22)	Non-at-risk (n=11)	At-risk (n=11)	P-value
Vanadium	0.28 ± 0.09	0.29 ± 0.08	0.25 ± 0.09	0.750
Cobalt	0.44 ± 0.31	0.47 ± 0.41	0.41 ± 0.18	0.669
Nickel	0.73 ± 1.0	0.47 ± 0.14	1.00 ± 1.43	0.098
Arsenic	0.76 ± 0.25	0.73 ± 0.28	0.79 ± 0.23	0.846
Barium	0.34 ± 0.13	0.34 ± 0.14	0.34 ± 0.12	0.990
Lead	0.09 ± 0.04	0.09 ± 0.04	0.08 ± 0.05	0.943

Values are expressed as means ± SD. The significant differences between the risk groups were tested by an Independent t-test and Wilcoxon rank-sum test for not non-normally distributed data.

Appendix 4. Maternal micronutrient intake (%DRI) for OCN participants from the diet and supplements

Micronutrient	All participants (n = 37)	Non-at-risk (n = 22)	At-risk (n = 15)	P-value
Vitamin A (mcg)	264 (186-366)	284 (194-375)	254 (179-359)	0.680
Vitamin C (mg)	337 (201-433)	354 (269-484)	279 (186-352)	0.374
Thiamin (Vit B1) (mg)	320 (232-364)	323 (262-438)	278 (229-330)	0.465
Niacin (Vit B2) (mg)	314 (254-399)	325 (261-448)	297 (255-332)	0.568
Folate (Vit B9) (mcg)	263 (100-298)	276 (244-302)	252 (77-272)	0.265
Cobalamin (Vit B12) (mcg)	574 (408-668)	617 (492-758)	524 (293-606)	0.505
Calcium (mg)	119 (86-169)	121 (113-174)	100 (81-154)	0.634
Iron (mg)	199 (115-218)	207 (168-231)	180 (73-212)	0.465
Zinc (mcg)	227 (172-255)	230 (207-266)	205 (135-250)	0.724

Values are medians (Q1-Q3). Data derived from FFQ and supplement reported. Nutrient %DRI was assessed using Health Canada's Dietary Reference Intake, Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50. The differences between the risk groups were tested by the t-test of independence for normally distributed data and Wilcoxon's rank-sum test for not normally distributed data.

Appendix 5. Maternal nutrient intake across trimesters for all participants (n=59)

Macronutrients	Trimester 1 (n =19)	Trimester 2 (n = 15)	Trimester 3 (n =20)	P-value
Kcal	1688 (1297-2357)	1868 (1460-2501)	2148 (1657-2491)	**0.024**
% Protein	17 (13-20)	16 (14-19)	17 (15-20)	0.864
% Fat	16 (13-29)	45 (35-53)	17 (14-20)	0.875
% Carbohydrate	49 (42-53)	40 (32-46)	48 (40-53)	0.711
Micronutrients				
Vitamin A	197 (122-350)	272 (166-413)	247 (183-411)	0.638
Vitamin C	231(157-306)	214 (155-266)	174 (109-248)	0.201
Thiamin (Vit B1)	220 (141-303)	210 (164-268)	238 (198-332)	0.622
Niacin (Vit B2)	205 (147-299)	195 (165-233)	239 (185-338)	0.616
Folate	71 (53-108)	85 (57-107)	84 (65-107)	0.957
Cobalamin (Vit B12)	373 (285-593)	421 (348-534)	503 (425-692)	0.309
Choline	108 (83-127)	108 (82-169)	127 (85-162)	0.622
Calcium	95 (69-153)	146 (84-192)	90 (83-142)	0.433
Iron	86 (51-100)	75 (61-100)	83 (72-131)	0.571
Zinc	153 (98-192)	156 (115-251)	171 (139-248)	0.381
DHA	36 (21-48)	42 (23-80)	35 (20-58)	0.182

Values are medians (Q1-Q3). Data derived from FFQ. Nutrient %DRI was assessed using Health Canada's Dietary Reference Intake, Estimated Average Requirement (EAR) for pregnant women aged 14-18, 19-30, and 31-50. The differences between the trimesters were tested by the Kruskal-Wallis test.