Intakes of nutrients known for fetal brain development among pregnant women living in Downtown and Point Douglas Winnipeg

by

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Abstract

Optimal maternal nutritional status is required for development of a healthy infant. Drinking during pregnancy puts mothers at risk for nutrient deficiencies, endangering the health of the fetus and increasing the risk of Fetal Alcohol Spectrum Disorder (FASD). The current body of research has focused on interventions using nutrients important to fetal brain development (choline, DHA, folate, vitamin A, zinc) to reduce FASD in animal models. Whether mothers at risk for having a baby with FASD are consuming adequate amounts of these nutrients during pregnancy is unknown, due to a lack of sufficient research data. Therefore, this study aims to identify intake of nutrients important to fetal brain development in pregnant mothers. Through community engagement and partnerships with Mount Carmel Clinic and other prenatal programs located in Point Douglas and Downtown Winnipeg, 56 pregnant women were recruited and interviewed. Findings show that intake of certain nutrients important to fetal brain development are not being consumed in recommended amounts. While most participants met the Dietary Reference Intakes for zinc and vitamin A, only 44.6% met recommendations for folate, 48.2% for choline, and 16.1% for DHA. Dietary intake was not significantly different between women with alcohol exposure during pregnancy and those without. These results are important due to the high rate (46%) of women with prenatal alcohol exposure. Study outcomes may provide future nutrition interventions to enhance the health of mothers consuming alcohol during pregnancy and their infants, potentially reducing the effects of FASD.

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List of Abbreviations

AA	Arachidonic acid, C20: 4n6
ADH	Alcohol dehydrogenase
AI	Adequate Intake
ALA	Alpha-linolenic acid
ALDH	Acetaldehyde dehydrogenase
ALT	Alanine transaminase
AMDR	Acceptable Macronutrient Distribution Range
ARBD	Alcohol-related birth defects
ARND	Alcohol0related neurodevelopmental defects
AST	Aspartate aminotransferase
BAC	Blood alcohol concentration
BMI	Body Mass Index
Ca	Calcium
CCHS	Canadian Community Health Survey
CFG	Canada's Food Guide
CNF	Canadian Nutrient File
CYP2E1	Cytochrome P450 2E1
DHA	Docosahexaenoic Acid, C22:6n-3
DNA	Deoxyribonucleic acid
DRIs	Dietary Reference Intakes
EAR	Estimated Average Requirements
EtG	Ethyl Glucuronide
FAEE	Fatty acid ethyl esters
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorder
Fe	Iron
FFQ	Food frequency questionnaire
GGT	Gamma-glutamyltransferase
IOM	Institute of Medicine
IQR	Inter quartile range
MCV	Mean corpuscular volume
MES	Maternity Experiences Survey
Mg	Magnesium
NAD+	Nicotinamide adenine dinucleotide
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
pFAS	Partial FAS
PFSS	Pregnancy and Family Support Services
PUFA	Polyunsaturated fatty acid
RDA	Recommended Dietary Allowance
ROS	Reactive Oxygen Species
SES	Socioeconomic status
UL	Upper Limit

Chapter I: INTRODUCTION

Infant growth and development is reliant on the mother's nutritional status during pregnancy. Alcohol can interfere with maternal nutrition and contribute to malnutrition. While there is increasing public awareness on the risks of consuming any amount of alcohol during pregnancy, it is still occurring worldwide. This is an issue as the teratogenicity of alcohol creates a risk of developmental disabilities in the infant, which is collectively called Fetal Alcohol Spectrum Disorder (FASD). FASD has also been shown to have a high prevalence of comorbid conditions including conduct disorder, receptive language disorder, and an abnormal peripheral nervous system (Popova et al., 2016). As malnutrition can contribute to the severity of FASD, it is thought that certain neuronal protective nutrients may help mitigate the effects of alcohol on the fetus. However, it is still unknown whether these nutrients are indeed required for mothers during pregnancy due to a lack of sufficient research data. Prior to nutrition provision during pregnancy, it is important to understand the current nutrient intake of pregnant mothers consuming alcohol.

This review aims to further explore how various micronutrients may play a protective role in fetal development, especially during prenatal ethanol exposure. First, general nutritional requirements during pregnancy will be discussed as well as typical deficiencies and supplements taken by pregnant women. Fetal development, specifically brain development, will be reviewed and the effects of alcohol on both mother and fetus explored. As FASD is a more measurable effect of alcohol during pregnancy it will be defined and its prevalence and risk factors discussed. Lastly, this review will look at potential nutrients that may protect the fetus from the effects of alcohol.

Nutritional status during pregnancy

Proper development of the fetus during pregnancy is dependent on maternal nutritional status. The physiological changes during pregnancy lead to an increased need for macro and micronutrients to meet the demands of the mother and fetus, including energy, protein, and folate, among others (Black, 2001). Inadequate intake of these nutrients, as in the case of maternal malnutrition, is predictive of delivering a low birth weight baby, having a pre-term birth, and intra-uterine growth restriction (Abu-Saad & Fraser, 2010; Brough et al., 2010; Imdad & Bhutta, 2012; Keen et al., 2010; Mumbare et al., 2012). These negative outcomes have long-term impacts on the development and quality of life for the child, as well as increased costs for health care (Abu-Saad & Fraser, 2010). For instance, birth weight is highly correlated with infant mortality and morbidity. Newborns who are small for gestational age are at increased risk for long-term health adversity such as hypertension, obesity, glucose intolerance, and cardiovascular disease (Mahan & Escott-Stump, 2008, p 162). It is the mother's nutrient stores, as well as dietary intake that will provide the fetus with the nutrients essential for growth and development (Ramakrishnan et al., 2012).

Nutrient requirements

Dietary Reference Intakes

Dietary Reference Intakes (DRIs) are nutrient reference values developed by the Institute of Medicine (IOM) (National Academy of Science, 2016). These values are specific for age, gender and life-stage and have separate values for pregnant women due to their different nutrient requirements. Some recommendations are based on the Recommended Dietary Allowance (RDA), which is the average dietary intake level each day that would meet the nutrient needs of 97-98% of healthy individuals in this group (Taylor, 2008). Others are based on the Adequate Intake (AI), which are established when there is insufficient evidence to calculate the RDA. It is

believed to cover the needs of all healthy individuals within each population group (Taylor, 2008). Below are the nutrient requirements for pregnant women (summary in Table 1-1).

Macronutrients

Energy requirements vary depending on the stage of pregnancy. The first trimester, while characterized by rapid development, does not require extra energy intake. By the 4th month of pregnancy the uterus, placenta, fetus and mammary glands begin to grow and the mother's metabolic rate increases (Ritchie & King, 2008). By the end of pregnancy, a woman's metabolic rate has increased by 15% (Mahan et al., 2012, p 356). To meet this need an additional 340-360 kcal/day must be consumed in the second trimester and 450 kcal/day in the third (IOM, 2002).

Protein is important for fetal growth, and is required for the synthesis of the placenta, amniotic fluid, and other maternal tissues during pregnancy (Ritchie & King, 2008). Inadequate protein intake may increase the risk of having a low birth weight baby (Ritchie & King, 2008). The recommended intake of protein during pregnancy is 71 grams per day (IOM, 2014). This is a 54% increase up from 46 g/day. Most of the additional protein is required during the last half of gestation.

Vitamins

The RDA increases during pregnancy for a variety of vitamins. For instance, vitamin A, a nutrient important to fetal brain development, increases from 70ug to 770 μg/day (IOM, 2014). For vitamin C, the RDA increases from 75mg/day to 85 mg/day during pregnancy (IOM, 2014). Vitamin C is used to synthesize collagen and is an antioxidant for the body (Mahan et al., 2012, p 358). Folic acid requirements also increase (200 μg/day up to 600 μg/day), as it is needed for the synthesis of nucleic acids and certain amino acids for new cell and tissue production. A deficiency in folic acid can lead to megaloblastic anemia, which may not present until the third trimester (Mahan et al., 2012, p 357). This in turn can lead to spontaneous abortion, preterm delivery and low birth weight, as well as neural tube defects (Ritchie & King, 2008).

Choline is based on AI and increases 25 mg to 450 mg during pregnancy (IOM, 2014). It is involved in many functions including cell membrane integrity, cell signaling, and nerve impulse transmission (Mahan et al., 2012, p 358). Other AIs for vitamins that increase during pregnancy include the B vitamins thiamin, riboflavin, niacin, B6, B12 and pantothenic acid. During pregnancy, vitamin B_6 is needed for the synthesis of heme, erythrocytes, immune proteins, and hormones (Ritchie & King, 2008). Vitamin B_6 is not stored well in the body (Ritchie & King, 2008) and so an additional 0.5 mg/day is required for pregnant women (IOM, 2014).

Minerals

Mineral requirements also increase during pregnancy. The RDA for iodine increases from 70 μg to 220μg/day. Iodine is necessary for the synthesis of thyroid hormones (Ritchie & King, 2008) and even suboptimal intake without deficiency may compromise fetal development, leading to developmental delays (Ohara et al., 2004). Overt deficiencies carry the risk of congenital anomalies, mental retardation, deafness, and cretinism (Ritchie & King, 2008).

Iron is also important during pregnancy and the RDA increases from 18mg to 27 mg/day. During pregnancy the maternal blood supply increases to provide extra blood flow to the uterus and placenta. This allows for the metabolic needs of the fetus to be met and helps the kidneys remove additional metabolic wastes generated throughout the pregnancy (Ritchie & King, 2008). However, red blood cell mass only increases 20-30% during this time and, due to the dilution, creates the relative anemia of pregnancy (Chesnutt, 2004). If the body does not meet the increased iron needs externally it will be taken from maternal liver stores, allowing fetal hemoglobin production to be adequate even if the mother is severely iron deficient (Ritchie & King, 2008). While dietary absorption of non-heme iron is increased during pregnancy, it is difficult to meet the almost 50% increase in requirements, especially when many women enter pregnancy with suboptimal iron stores (Ritchie & King, 2008; Mahan et al., 2012, p359).

The RDA for zinc increases by 38% from 8 to 11 mg/day during pregnancy (IOM, 2014). It is required for gene expression, cell differentiation, and cell replication (Ritchie & King, 2008). If adequate amounts of zinc are not received from the diet, zinc status can decreases rapidly, as bone stores are not readily available (Mahan et al., 2012, p 360). Zinc deficiency is highly teratogenic in rats and can lead to congenital malformations, as well as abnormal brain development in the fetus (Mahan et al., 2012, p 360).

Other increased RDAs for minerals include selenium (55μg/d up to 60 μg/d), molybdenum (45μg/d up to 50 μg/d), magnesium (320mg/d up to 350 mg/d), and copper (900μg/d up to 1000 μg/d). Minerals whose AI increases include chromium (25 μg/d up to 30 μg/d), and manganese (1.8mg up to 2.0 mg/d) (IOM, 2014).

Canada's Food Guide

Canada's Food Guide (CFG) was developed so that intake of the recommended number of servings from each food group at the recommended serving sizes, would meet nutrient standards laid out by the DRI (Health Canada, 2007a). Food groups include vegetables and fruit, grain products, milk and alternatives, and meat and alternatives, and serving sizes are standardized for common food products. Non-pregnant females (19-50 years) are recommended to have 7-8 servings of vegetables and fruits, 6-7 servings of grain products, 2 servings of milk and alternatives, and 2 servings of meat and alternatives (Health Canada, 2007b). Health Canada (2011) suggests that women who are pregnant consume an additional 2-3 servings from any food group during their second and third trimester. This is to help gain a healthy amount of weight during pregnancy and provide additional nutrients.

Table 1-1: Summary of nutrient requirements during pregnancy—females 19-30 years

	Nutrient	Unit	Non-Pregnant Females	Pregnant Females	Percent Increase
Macronutrients					
	Carbohydrates	g/day	130	175	35%
	Protein	g/kg WT/day	0.8	1.1	38%
	Linoleic Acid (n-6)	g/day	12	13	8%
	α-linolenic Acid (n-3)	g/day	1.1	1.4	27%
Vitamins					
	Vitamin A	µg/day	700	770	10%
	Vitamin C	mg/day	75	85	13%
	Thiamin	mg/day	1.1	1.4	27%
	Riboflavin	mg/day	1.1	1.4	27%
	Niacin	mg/day	14	18	29%
	Vitamin B6	mg/day	1.3	1.9	46%
	Folate	µg/day	400	600	50%
	Vitamin B12	µg/day	2.4	2.6	8%
	Pantothenic Acid	mg/day	5	6	20%
	Choline	mg/day	425	450	6%
Minerals					
	Chromium	µg/day	25	30	20%
	Copper	µg/day	900	1000	11%
	Iodine	µg/day	150	220	47%
	Iron	mg/day	18	27	50%
	Magnesium	mg/day	310	350	13%
	Manganese	mg/day	1.8	2.0	11%
	Molybdenum	µg/day	45	50	11%
	Selenium	µg/day	55	60	9%
	Zinc	mg/day	8	11	38%

Source: Institute of Medicine, 1997; 1998; 2000; 2001; 2002.

Typical micronutrient deficiencies in pregnancy

Women may either enter pregnancy with micronutrient deficiencies or develop primary deficiencies. The latter results from increased nutrient requirements compounded with insufficient intake from a variety of dietary food sources. Typical micronutrient deficiencies during pregnancy include iron. This can be seen in the high prevalence of anemia in pregnant women worldwide. In Africa, rates are estimated to be as high as 57.1%, with America and Europe at the next highest prevalence (24.1% and 25% respectively) (World Health Organization, 2008). In a smaller study, data from 63 middle-to-upper class pregnant women showed that usual intake from food did not typically meet the Estimated Average Requirements (EAR) for iron and magnesium. Intake was evaluated through 3-day diet records during each month of pregnancy. This study however, did not evaluate folate intakes, which are important during pregnancy and may also contribute to anemia. In another study following Peruvian women throughout pregnancy (n=78), 6.4% were iron deficient during the first trimester, 44% during the 2nd, and 64% during the 3rd (Horton et al., 2013).

A small population of pregnant Qikiqtarjuaq Inuit women was found to have exceeded the Upper Limit (UL) for iron, when traditional foods made up over 25% of dietary energy (Berti et al., 2008). However, other pregnant women studied in the Canadian arctic have been found to have low intakes of iron as well as folate, zinc, calcium, magnesium, and vitamins E, A and C, as assessed through 24 hour food recall (Berti et al., 2008). Some vitamins, such as vitamin A, can be difficult to measure through 24 hour recalls due to their high availability in some traditional foods, which may lead to inaccurate intake assessments (Berti et al., 2008).

When looking at nutrient status in the first trimester of pregnancy among a low-income, multi-ethnic population in East-London, United Kingdom, it was found that 13% were anemic, 72% had vitamin D deficiency, 12% thiamin deficiency, and 5% folate deficiency (Brough et al., 2010). Therefore, micronutrient deficiencies during pregnancy can vary widely.

Common nutrient supplements during pregnancy

In Canada, several nutrient supplements are recommended during pregnancy. For instance, it is recommended that women planning to conceive take 0.4 mg folic acid prior to conception (IOM, 2013). With the high prevalence of iron deficiency anemia in pregnancy, it is also recommended for pregnant women to take a multivitamin that contains iron (Health Canada, 2012).

Canadian data shows that the majority of pregnant women taking dietary supplements use a prenatal multivitamin. In a small pilot study from Quebec, 175 pregnant women noted their use of dietary supplements, of which 82.9% reported taking a prenatal multivitamin. Of single vitamins, the most commonly taken were folic acid (19.3%), omega-3 fatty acids (11.4%), and calcium (8%). Very small portions of this group were taking single supplements of iron, vitamin C, magnesium, vitamin D, and vitamin B complex (Grigoriadis et al., 2010). Similarly, in a survey of over 2,000 pregnant women in London, Ontario, 66.7% took a prenatal multivitamin and 7.4% took a regular multivitamin (Roy et al., 2012). Single nutrient supplements were less common with 4% using a single-nutrient iron supplement, 0.08% (n=2) using a single zinc supplement, and 14% using a single-nutrient folic acid supplement.

Supplement use may also vary in different Canadian populations. Using data from the Maternity Experiences Survey, Han et al. (2009) collected data on pre-conception folic acid supplement use in Canada. The highest pre-conception folic acid supplement use was seen in Canadian-born mothers (61%). There was lower use in those that had immigrated to Canada in the past 3 years (41% Caribbean and Latin America, 44% sub-Saharan Africa, Northern Africa, and Middle East, 46% South Asia, 47% China and South Pacific) (Han et al., 2009). Other demographic characteristics of pre-pregnancy supplement users are older women, and those with a postgraduate degree (Masih et al., 2015).

Fetal development

Prenatal development may be categorized into three different stages—blastogenic, embryonic, and fetal stage. Development is measured in weeks from first day of the mother's last menstrual cycle (Storck, 2013). This means that during the first 2 weeks the woman's body is simply preparing for gestation by releasing an egg (Storck, 2013). During the third week the blastogenic stage begins as fertilization occurs and a zygote is formed. A blastocyte is formed with an inner and outer group of cells (Storck, 2013). The inner group will become the embryo, eventually developing into the fetus. Most importantly, the blastogenic period is characterized by rapid cell division in which tissues begin to form (Insel et al., 2013).

The embryonic stage takes place from implantation (week 5) to week 10 of pregnancy. This is a time of cell differentiation where all essential organs are forming. These including the central nervous system, ears, eyes, heart, kidneys, gastrointestinal tract, and lungs (Insel et al., 2013). This critical period is when insults to the baby (such as heavy alcohol use, illegal drugs, etc.) could result in birth defects (Storck, 2013).

The fetal stage, which is the longest, lasts from the embryonic stage till birth (week 11 to 40). It begins during what is known as the 2nd trimester. This is a time of rapid growth for the fetus (Insel et al., 2013). In fact, more than 90% of fetal growth occurs in the last half of gestation (King, 2000). Fetal landmarks within this period include the production 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), initiation of rhythmic breathing develops (weeks 31-34), and full development of muscles and bones (weeks 35-37) (Storck, 2013).

Brain development

Brain development begins with a process of cell movement, or gastrulation. Gastrulation creates three germ layers in the embryo—endoderm, mesoderm, and ectoderm (Sunderland, 2001). These layers will develop into every tissue and organ in the body. It is the ectoderm that

will become the central nervous system. However, the mesodermal layer is also important. Mesodermal cells (from the middle germ layer) form the notochord, a cylinder of cells that run the length of the embryo. It is from the notochord that cell differentiation occurs and signals the ectoderm to thicken and form the neural plate (Sunderland, 2001).

The neural plate forms during the third week after fertilization, and ultimately folds in on itself to form the neural groove (O'Rahilly & Muller, 2008). These folds from the neural groove begin to fuse along its length to form the neural tube by "zippering" itself closed around day 30 after ovulation (Bangalore, 2007). This process is called neurulation. Formation of the neural tube is important for brain and spinal cord differentiation during gestation (Semple et al., 2013). Above the neural tube forms the neural crest, whose cells are important for differentiation into various peripheral nervous system cells such as neurons and glial cells (Sunderland, 2001).

By the end of this period, brain structures are beginning to form from the neural tube. At the embryo's head is the prosencephalon, which will become the forebrain, in the middle is the mesencephalon, which will become the midbrain, and rhombencephalon, which will become the hindbrain (Stiles & Jernigan, 2010). By the end of the embryonic stage, these have further subdivided (prosencephalon to telencephalon (future cerebral cortex) and diencephalon (future thalamus and hypothalamus), and rhombencephalon to metencephalon (future cerebellum and pons) and myelencephalon (future medulla) (Stiles & Jernigan, 2010).

Part of neurodevelopment during the fetal period is the process of neural death. This is due to the overproduction of neurons and glial cells during brain development called "synaptic exuberance" (Stiles & Jernigan, 2010). Apoptosis, or intrinsic cell death may be used to remove cells with temporary functions, or remove neural circuits that are ineffective (Stiles & Jernigan, 2010). This systematic reduction can eliminate up to 50% of the neurons produced (Stiles & Jernigan, 2010). This process is also of importance for its role in removing damaged neurons through episodes of necrotic death after insult or injury to the brain (Stiles & Jernigan, 2010).

Effects of alcohol during pregnancy

Effects on nutritional status

Though ethanol provides calories (7.1 kcal per 1 gram) it is void of any other nutritional value. Therefore, excessive amounts of alcohol can compromise nutritional status through primary and secondary malnutrition. Primary malnutrition involves displacing the intake of other nutrients through consumption of large quantities of alcohol. Secondary malnutrition occurs when adequate amounts of nutrients are consumed, however alcohol impairs gastrointestinal function and the absorption of nutrients, making them unavailable for use in the body. These effects have been seen in the general population consuming alcohol. Due to a lack of research, it is unknown whether these effects are the same in pregnant women.

Primary malnutrition

Heavy alcohol consumption, defined as 15 drinks or more per week for men, or 8 for women (Center for Disease Control and Prevention, 2014), affects dietary intake and may lead to primary malnutrition. However, moderate alcohol consumption, when 16% of daily calories come from alcohol, leads to increased dietary intake and has short-term appetite stimulation (Yeomanset et al., 2003). Heavy alcohol intake, or greater than 30% of daily calories from alcohol, produces significantly reduced carbohydrate, protein and fat intake, as well as vitamins A, C, some B vitamins, calcium and iron (Lieber, 2003). Ethanol also delays gastric emptying, which may cause satiety and increases circulating leptin (Nicolás et al., 2001). Leptin is a peptide hormone known for its regulation in appetite and may contribute to the anorexia of alcoholism (Nicolás et al., 2001).

The general reduction in intake during heavy alcohol consumption in conjunction with less varied diets predisposes alcoholics to malnutrition. Other predisposing factors may include irregular meal patterns and disruption of social and family life (Santolaria & González-Reimers, 2012). This is why typical nutrient deficiencies seen in alcoholics include B vitamins, thiamin,

riboflavin, B6, and vitamin C as well as folic acid (Lieber, 2003). Whether similar deficiencies also occur when pregnant women consuming alcohol is unknown.

Secondary malnutrition

Secondary malnutrition occurs when alcohol related diseases lead to decreased intake and poor absorption or digestion of nutrients. For example, complications from alcohol consumption may include chronic alcoholic gastritis accompanied by anorexia and vomiting, as well as chronic diarrhea (Santolaria & González-Reimers, 2012). Typical vitamins affected by malabsorption include B6, B12 and folic acid (World et al., 1985). In addition, muscle breakdown in alcoholics can increase excretion of some minerals including zinc and magnesium (World et al., 1985).

Effects on fetal development

Prenatal alcohol exposure affects fetal development primarily through hypoxia and production of reactive oxygen species. Hypoxia occurs when alcohol restricts placental blood flow to the fetus (Schenker et al., 1990; Randall et al., 1990). A reduction in blood flow limits the nutrients and oxygen transported to the fetus and result in brain cell death (Golan & Huleihel, 2006). Alcohol induced damage may also be due to the formation of oxygen-containing free radicals known as reactive oxygen species (ROS) (Bosco & Diaz, 2012). ROS are created during the process of alcohol metabolism. Alcohol is first converted to acetaldehyde by alcohol dehydrogenase (ADH), then to acetic acid by acetaldehyde dehydrogenase (ALDH). Both of these enzymes require nicotinamide adenine dinucleotide (NAD+) to become oxidized to NADH. In the mitochondria, NADH is reduced back to NAD+, producing ROS (Brocardo et al., 2011). Ethanol can also be converted to acetaldehyde via cytochrome P450 2E1 (CYP2E1), which generates hydroxyl radicals (Brocardo et al., 2011). ROS can damage lipids, proteins and DNA (Wu & Cederbaum, 2003) creating a state of oxidative stress and affecting fetal development (Bosco & Diaz, 2012).

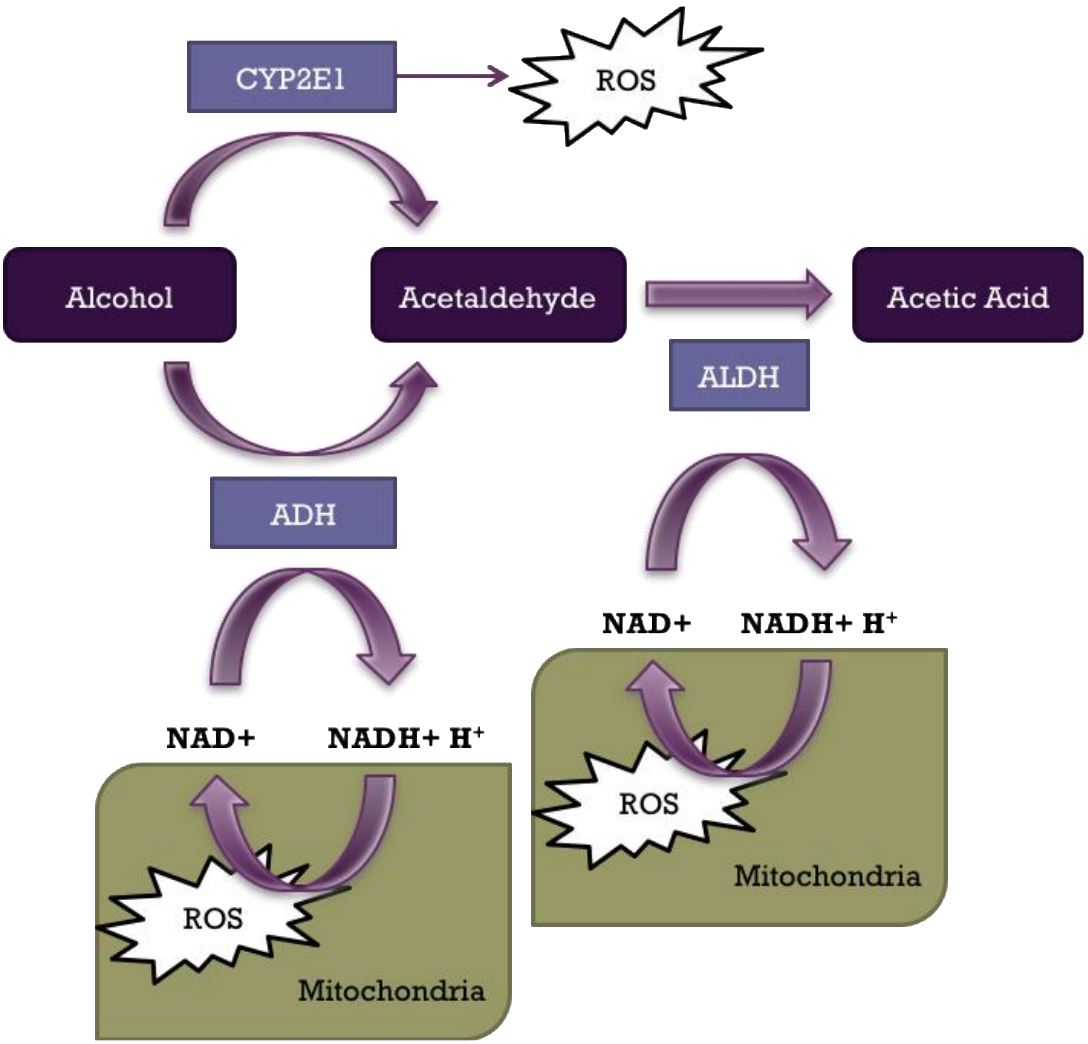


Figure 1-1: Metabolism of alcohol and the creation of Reactive Oxygen Species (ROS)

Adapted from Brain Research Reviews, 67(1), Brocardo, Gil-Mohapel, & Christie, The role of oxidative stress in fetal alcohol spectrum disorders, p 213, Copyright (2011), with permission from Elsevier.

Other factors also influence the effects of alcohol on fetal brain development. These include maternal drinking patterns (timing throughout pregnancy, frequency and amount); the mother's capacity to metabolizes alcohol, variability in genetic susceptibility, and differences in

vulnerability of fetal brain regions (Maier & West, 2001). Some of these factors, including drinking patterns, affect the concentration of alcohol in the blood, known as blood alcohol concentration (BAC). BAC is affected by how quickly alcohol is absorbed, metabolized and removed from the body (Zakhari, 2006). This is important as ethanol can be transported to the fetus via the placenta (Burd et al., 2012). The placenta is able to metabolize some alcohol, but not enough to reduce the ethanol exposure to the fetus (Burd et al., 2012). Therefore, the fetus is exposed to the same BACs as the mother within 2 hours of consuming alcohol (Idänpään-Heikkilä et al., 1972). After 2 months gestation, the fetus has the capabilities to metabolize ethanol. However, it is in so small an amount that maternal ethanol metabolism is still key to its removal (Burd et al., 2012).

Timing

Various regions of the brain are vulnerable to alcohol during different periods of development. For instance, late nutrition insults affect differentiation, while early nutrition insults would primarily affect proliferation or number of cells formed (Georgieff, 2007). *In vitro***,** animal, and human studies have all tried to understand whether the teratogenic effects of alcohol are more potent during various points in pregnancy.

During *in vitro* studies, when embryonic rat hippocampal neurons were exposed to ethanol on day 6, high neuronal loss occurred, with three quarters of neurons lost by day 14 (Lindsley et al., 2002). Since this period resembles rapid dendrite and synapses formation in the third trimester of rapid human brain growth (Beblo et al., 2005), these results imply that prenatal ethanol exposure during the third trimester leads to extensive neuronal loss.

In an animal study by Livy et al. (2003), rat dams were divided into groups of ethanol exposure during different stages of pregnancy (correlated to human pregnancy) including second trimester only, first plus second trimester, third trimester, and all trimesters, as well as the period of hippocampal pyramidal cell neurogenesis in the rat. They found an increased vulnerability to the fetal brain during the third trimester. These results should be extrapolated cautiously as brain

development seen in humans during the third trimester occurs in post-natal rats from days 1-12 (Dobbing & Sands, 1979).

The ethics surrounding prenatal alcohol consumption in human studies often results in observational data. For instance, Feldman et al. (2012) looked at the risk of developing facial features and growth patterns associated with FASD, based on when and how much alcohol the mother consumed. Results showed that during the second half of the first trimester (weeks 7-12) an increase in the average number of drinks consumed resulted in an increased risk of dysmorphed physical features such as thin vermillion and smooth philtrum as well as microencephalopathy, reduced birth weight, and birth length. Only modest effects were found for second and third trimester, and only with certain drinking patterns.

Frequency

It is thought that drinking alcohol throughout pregnancy is necessary for the effects to be significant enough to diagnose FASD (May & Gossage, 2011). A retrospective study compared the drinking patterns of mothers with children diagnosed as FASD, to mothers of controls in South Africa. It was found that FASD was 12 times more likely in mothers only drinking during the first trimester, compared to mothers who did not drink at all (95% CI 4.1-25.8). For mothers who drank in the 1st and 2nd trimester, the odds of giving birth to a child with FASD increased to 61x that of a non-drinking mother (95% CI, 13-291). Finally, this was increased to 65x if the mothers drank throughout all trimesters of pregnancy (May et al., 2013a). This shows that drinking throughout pregnancy leads to the greatest risk of alcohol related effects. However, it is important to note that this study was completed in a population where binge drinking is prominent. Binge drinking with frequency throughout pregnancy, produces consistently high BAC levels that affect the fetus.

Quantity

Binge drinking during pregnancy is thought to cause the most damage to the fetus (Maier & West, 2001). This is when an amount of alcohol is consumed within a short period of time and

the time in-between each drink may be short and inconsistent (Maier & West, 2001). This type of intake pattern produces higher peak levels of BAC than when similar amounts of alcohol are consumed in a non-binging pattern (Able & Hannigan, 1995). Higher BACs lead to prolonged alcohol exposure as alcohol is metabolized in the body at the same rate, regardless of how much is consumed (Maier & West, 2001). Maier and West (2001) also suggest that binge drinking exposes the fetus to periods of withdrawal, and that multiple withdrawal periods may put the fetus at risk for brain injury.

Animal studies have also proven the detrimental effects of binge drinking. Pierce and West (1986) found that a dose of 6.6g/kg of ethanol delivered over 24 hours in 12 fractions produced a lower BAC (46.6 mg/dl) compared to the same dose concentrated into 6 fractions over 12 hours (BAC 270.2mg/dl). The alcohol exposure took place during the 3rd trimester equivalent in humans and the pattern associated with binging had a significant reduction in brain growth. Similarly, in a study by Bonthius et al. (1988), rat pups artificially reared postnatal days 4-10, were given 6.6g/kg/day of alcohol in varying concentrations (5% ethanol in 12 feedings, 7.5% ethanol in 4 feedings, and 15% ethanol in 2 feeding). BAC was higher in each of the groups respectively. Results showed that brain weight was inversely associated with BAC (Bonthius et al., 1988).

In humans, less data is available on the effects of binge drinking on fetal development. One large study has shown that light to moderate maternal alcohol consumption of 1-3 drinks per week in mid-to-late pregnancy did not have any effects on fetal growth such as head circumference, abdominal circumference, and femur length (Bakker et al., 2010). However, data was collected through mailed questionnaires, which may have led to underreporting. Also, data only indicated the number of drinks consumed per week, not the pattern of drinking (Bakker et al., 2010).

Barr and Streissguth (2001) found that mothers that reported binge drinking 5 or more alcoholic drinks per month were more likely to have a child with FASD (n=73, 38.4%) than

mothers reporting daily drinking without binging (n=99, 8.1%). Another study assessed black pregnant women on their prenatal alcohol intake then followed-up with their children at age 7. Those children exposed to binge drinking, despite the amount of alcohol consumed throughout pregnancy, had lower verbal IQ score, and higher levels of delinquent behavior reported by teachers at school (Bailey et al., 2004).

Prenatal alcohol consumption rates

Collecting data on prenatal alcohol consumption can be done through various methodologies. These include self-report questionnaires or interviews, community screening tools, and biomarkers (Dell & Roberts, 2005). However, most data is self-reported from questionnaires or interviews meant for research purposes.

Canada

The Canadian Community Health Survey (CCHS, 2007-2008) and the Maternity Experience Survey (MES) provide data on the alcohol consumption of pregnant women in Canada. Analysis of results from the CCHS (2007-2008) revealed that the incidence of drinking during pregnancy in Canada is 5.8%. Rates for two provinces were calculated separately—Ontario and British Columbia (5.4% and 7.2% respectively) (Thanh, 2010).

Another analysis based on the Maternity Experience Survey found that 10.8% of Canadian women had consumed alcohol while pregnant, mostly at low to moderate consumption (95.8%). Heavy drinkers were classified as >1 drink per day (1.7%). The incidence across Canada was 13.8% in Eastern-Central provinces, 7.8% in Western Provinces (British Columbia), 4.1% in Eastern-Atlantic provinces and 4.0% in Western-Prairie Provinces (Walker et al., 2011).

In a smaller, more local study, Muhajarine et al. (1997) found that of the 605 pregnant women surveyed in Saskatoon, 46% reported drinking alcohol during their first trimester of pregnancy. Of these, 75% had less than 2 drinks per week on average. However, the women sampled were accessing prenatal classes or an outreach program for high-risk pregnant women

from the Saskatoon Community Health Unit, which may have increased incidence. Using similar methods, 3.1% of women seen in a Toronto clinic reported binge drinking during pregnancy (Gladstone et al., 1997). This data would be improved by sampling outside of a high-risk population accessing services.

In various populations, maternal consumption of alcohol is seen to be even higher. In a sample of 247 Inuit women living in Northern Quebec, 60.5% drank alcohol while pregnant. Of the women that reported prenatal drinking, 62% noted at least one episode of binge drinking. Consumption patterns showed up to 9 binge episodes during pregnancy with an average of 10 standard drinks per episode (Muckle et al, 2011).

Globally

In the United States, approximately 7.3% of pregnant women surveyed in the Behavioral Risk Factor Surveillance System reported drinking in the previous month, with 1.4% reporting binge drinking bahaviours (Marchetta et al., 2012). In an earlier survey from the United States, 30.3% of all women surveyed reported drinking alcohol at some time during pregnancy. Of these, 8.3% reported binge drinking more than four drinks on one occasion. After the first month of pregnancy, rates dropped off to 22.5%. However, 7.9% of women reported drinking in the third trimester and 2.7% reported drinking throughout pregnancy (Ethen et al., 2009).

A survey of Australian women in 2011 reported 34.8% of women consumed alcohol during pregnancy. This declined to 25.8% after the first trimester (Cameron, 2013). This is much higher than the rate reported by Swedish women, of whom 6% reported alcohol consumption at least once after pregnancy recognition (Skagerström et al., 2013).

From a Korean survey of women receiving prenatal care at the department of Obstetrics and Gynecology at a university hospital, 16.4% of participants reported using alcohol during their pregnancy. Of the population sampled, 12.4% drank alcohol less than once a month, 3.6% drank 2-4 times per month, and 0.3% drank 2-3 times per week. Most (12.7%), consumed only 1-2 drinks per occasion (Lee et al., 2010).

Biomarkers for alcohol consumption during pregnancy

Self-reports are often used to assess drinking patterns in pregnant women. This is limited by the stigma around prenatal alcohol consumption (Bearer et al., 2004). To counter underreporting of alcohol consumption, biomarkers of alcohol may be used. However, alcohol and the by-products of alcohol metabolize quickly in the body. Actual ethanol can be measured directly through the breath and blood, but only for very recent drinking (Joya et al., 2012; Bearer et al., 2004). Other direct and indirect biomarkers may help indicate drinking over a longer period.

Direct maternal biomarkers

Direct biomarkers are ethanol or products of ethanol metabolism that may be found in a drinking mother's body or through biological samples from the infant (Joya et al., 2012). Many biomarkers related to alcohol would not change below a threshold of 2 drinks (or 20 g) per day including gamma-glutamyltransferase (GGT), alanine transaminase (ALT), aspartate aminotransferase (AST), urate and ferritin (Whitfield et al., 2013).

Fatty acid ethyl esters (FAEE) are a by-product of ethanol metabolism. They can be found in the liver, pancreas, and adipose tissues up to 24 hours after alcohol is consumed (Peterson, 2004). It can also be found in hair, where the body is unable to remove the FAEE and therefore they build up over time, which can indicate chronic drinking (Peterson, 2004). The meconium, or the infant's first fecal material, may also be used to detect FAEE, as well as their hair and the cord blood at birth (Bearer et al., 2004). Up to 75% of the meconium develops during the last 8 weeks of pregnancy, so it can be an indication of alcohol intake during the last trimester (Bakdash et al., 2010). The limitations with FAEE include that its accumulation in the meconium may vary over time and may depend on the variations in alcohol metabolism due to genetic variance (Bearer et al., 2004). Ethyl oleate's (an FAEE) concentrations in meconium correlated most strongly with self-reported drinking by mothers in a study by Bearer et al. (2003). This was especially true during the second and third trimesters (Bearer et al., 2003).

Ethyl Glucuronide (EtG) is a metabolite formed from the detoxifying of drugs like alcohol into water-soluble compounds by glucuronic acid. It can be found in urine for up to 5 days after heavy alcohol use or up to 36 hours in blood (Peterson, 2004). It is also found in the meconium and can be used to determine maternal alcohol consumption (Pichini et al., 2009). It has also been noted that by analyzing meconium for FAEE as well as EtG increases the accuracy of monitoring maternal alcohol intake (Bakdash et al., 2010).

Indirect maternal biomarkers

Gamma-glutamyltransferase (GGT) is a glycoprotein found in liver cells and other cells that produce bile. Elevated levels may indicate liver damage from long-term alcohol use. However, pancreatitis and other diseases such as obesity and diabetes can also raise GGT (Peterson, 2004). Mostly it has been used to assess liver damage, but serum levels may increase when there is exposure to alcohol (Joya et al., 2012). However, GGT has poor sensitivity in screening for alcohol abuse in pregnant women as it may give false positive results (Joya et al., 2012).

Mean corpuscular volume (MCV), or the volume of a person's red blood cells, is often higher in heavy chronic drinkers (Joya et al., 2012). Due to the half-life of red blood cells, changes in drinking patterns may take time to be reflected in these lab values (Joya et al., 2012). One study did show that it is effective for use as a biomarker to detect heavy drinking in pregnant women (Sarkola et al., 2000).

Fetal alcohol spectrum disorder

Lemoine and colleagues first reported the expression of Fetal Alcohol Syndrome in 1968 from case reports in France showing birth defects associated with alcohol exposure in the womb (Clarke & Gibbard, 2003). Jones et al. (1973) also reported this in 1973 in eight case studies of children born to mothers with chronic alcoholism in the United States. Since then, much research has gone into studying FASD and determining diagnostics, prevalence, and risk factors.

Definition

Fetal alcohol spectrum disorder is an umbrella term for a number of effects related to maternal consumption of alcohol while pregnant (Cook et al., 2015; First Nations and Inuit Health Committee et al., 2002). Fetal alcohol syndrome (FAS), the most severe form of FASD, can be diagnosed based on a history of prenatal alcohol consumption by the mother, along with infant characteristics such as certain facial features, poor growth, and neurological abnormalities. Partial FAS (pFAS) has the same central nervous system impairments as FAS, but may be without the facial characteristics or growth impairments as FAS (Pacey, 2009). Other diagnoses occur if the effects are physical (alcohol-related birth defects, or ARBD), or related to developmental dysfunction (alcohol-related neurodevelopmental defects, or ARND). The effects of prenatal alcohol exposure vary widely from case to case. Distinct facial features include a smooth philtrum, a thin upper lip, and short palpebral fissures (Chudley et al., 2005). On neurodevelopment, microcephaly can occur affecting intelligence, activity and attention, learning and memory, language and motor abilities, and behavior (First Nations and Inuit Health Committee et al., 2002).

Prevalence

The prevalence of FAS and FASD is difficult to determine because diagnosis often requires confirmation of prenatal alcohol exposure (except where alcohol exposure is unknown but the three sentinel facial feature are present) (Cook et al., 2015). The stigma associated with prenatal alcohol exposure may limit diagnosis, especially in ARND (Sampson et al., 1997). An alternative method of determining prenatal alcohol exposure is through measuring fatty acid ethyl esters (FAEE) in the meconium of the infant. Ethanol crossing the placenta is metabolized by the fetus resulting in FAEE biomarkers (Gareri et al. 2008). The FAEE test was used in a sample of 682 Canadian women. It was found that 2.5% of mothers had significant prenatal ethanol exposure, which was higher than rates reported in postpartum questionnaire (0.5%) (Gareri et al., 2008). In this same population, 30% of women with high-risk pregnancies were

testing positive for FAEE (Goh et al., 2010). However, with the meconium only developing at week 12, early prenatal drinking would not be captured by this method.

The main approaches for determining prevalence include reviewing records from a geographical area, clinic-based studies, and active case ascertainment. Epidemiological studies often look at the most severe cases of FASD, FAS and pFAS (May et al., 2009) Records are often limited to diagnoses made at birth, and rely on the completeness of the data set. Also, only the most severe cases of FASD can be diagnosed during infancy (May et al., 2009). This made lead to underreporting in prevalence. Clinical studies are advantageous in their rigorous collection of maternal and infant data; however, data often reflects the prevalence of FASD that is treated, versus that of the population (May et al., 2009). Active case ascertainment uses searches within certain populations for children with FASD. This process is time consuming but is more likely to find unrecognized cases of FASD (May et al., 2009). Active case ascertainment has been seen in some First Nations communities in Canada.

Prevalence in Canada

There is very little data on the prevalence of FASD in Canada. Of the studies done in this area, most have focused on small First Nations communities. For instance, in Northeastern Manitoba, an incidence of about 7.2 per 1000 live births was found (Williams, 1999). In another Manitoba study in a First Nations community (Square, 1997) the prevalence of FAS and pFAS was estimated to be 55–101 per 1000. In 1987, a survey of a First Nations community in British Colombia had a prevalence rate of 190 per 1000 children age 18 or younger (Robinson et al., 1987). It is important to note that studies focusing on communities that are at higher-risk may perpetuate the idea of a higher prevalence of FASD in Indigenous populations (Pacey, 2009). Finally, a study that looked at the incidence of FAS only in Saskatchewan between 1988 and 1992 found it to be 0.59 per 1000 births (Habbick et al., 1996). These results were collected from those who presented themselves to health care professionals able to identify the condition. This

may lead to an underestimate of incidence. Due to the specific populations targeted in these studies, the results are difficult to generalize for the Canadian population.

There have also been studies on the economic impact of FASD in Canada. On an individual level, the annual cost for a person with FASD ages 0-53 is $21,642 (Stade et al., 2009). This includes direct costs such as medical care, education services, social services, and indirect costs such as productivity losses. For all of Canada, the estimated costs in 2013 were $1.8 billion dollars, based on the direct cost of health care, law enforcement, and other direct and indirect costs (Popova et al., 2015). The largest contributing cost was found to be productivity losses due to disability. Thus, the prevention of FASD is urgently required.

Prevalence globally

Abel (1995) used 29 prospective international studies published between 1997 and 1994 to determine the worldwide prevalence of FASD. He determined 0.97 cases per 1000 live births, with the prevalence being 4.3 per 1000 for heavy drinkers. This number is difficult to determine accurately for the reasons listed above, as well as the small sample sizes and different study designs from each country (May et al., 2013b). This study also did not diagnose cases of ARBD. Another literature review and meta-analysis found a global FASD prevalence rate of 22.8 per 1000 births (Roozen et al., 2016).

Some of the highest rates of FASD have come out of South Africa. In a study of grade 1 children in a South African community, it was found that the overall rate of FASD (including FAS, pFAS, and ARND) was 135.1-207.5 per 1000 births (13.6 to 20.9 %). Limitations of this type of study include participation rates based on the number of parents that provide consent. Also, the first tier screening included children with smaller heights, weights and head circumferences, which would lead the researchers to catch more cases of FAS and pFAS (May et al., 2013b). A meta-analysis has fond similar results of 113.2 per 1000 live births (Roozen et al., 2016).

Other in-school studies have been done globally to determine prevalence. In Italy, a similar design was used for grade 1 students, where FASD was a rate of 23.1 to 62.6 per 1000 children (2.3 to 6.3%) (May et al., 2011). In Croatia, the rate was found to be 40.8 per 1000 (FAS and pFAS) (Petkovic & Barisic, 2010). In the US, the rate of FAS was calculated at 3.1 per 1000 students (Clarren et al., 2001), while FASD rates were 33.5 per 1000 births (Roozen et al., 2016).

Risk factors

Not all women who consume alcohol while pregnant have a child born with FASD. The variability in outcomes related to drinking while pregnant suggests that certain risk factors may increase chances of FASD. Able and Hannigan (1995) suggested that there are both permissive and provocative factors. Permissive factors (alcohol intake patterns, socioeconomic status, smoking,) create increased vulnerability to the teratogenic effects of alcohol. These permissive factors contribute to provocative factors (or biological factors) such as higher blood alcohol concentrations, under-nutrition and tobacco smoke constitutes (Able & Hannigan, 1995). Provocative factors contribute to the formation of free radicals as well as hypoxia, which contributes to cell damage in the fetus, increasing the likelihood of FASD. The relationship between these factors is shown in Figure 1-2.

Studies of school children also allow for the collection of maternal risk factors, where mothers of children with suspected FASD are interviewed for demographic data, social issues, alcohol and drug use etc. (May et al., 2009). Various studies have found that increased prenatal alcohol intake was associated with gestational age, higher maternal age, lower education level, prenatal exposure to cocaine and smoking, socioeconomic status (Sood et al., 2001) and maternal under-nutrition (Jones, 2011). While genetics and epigenetics may play a role in the variations in effect of alcohol on the fetus, as seen through the array of diagnoses under FASD, examining other maternal traits and behaviors may be useful in determining their contribution (May & Gossage, 2011).

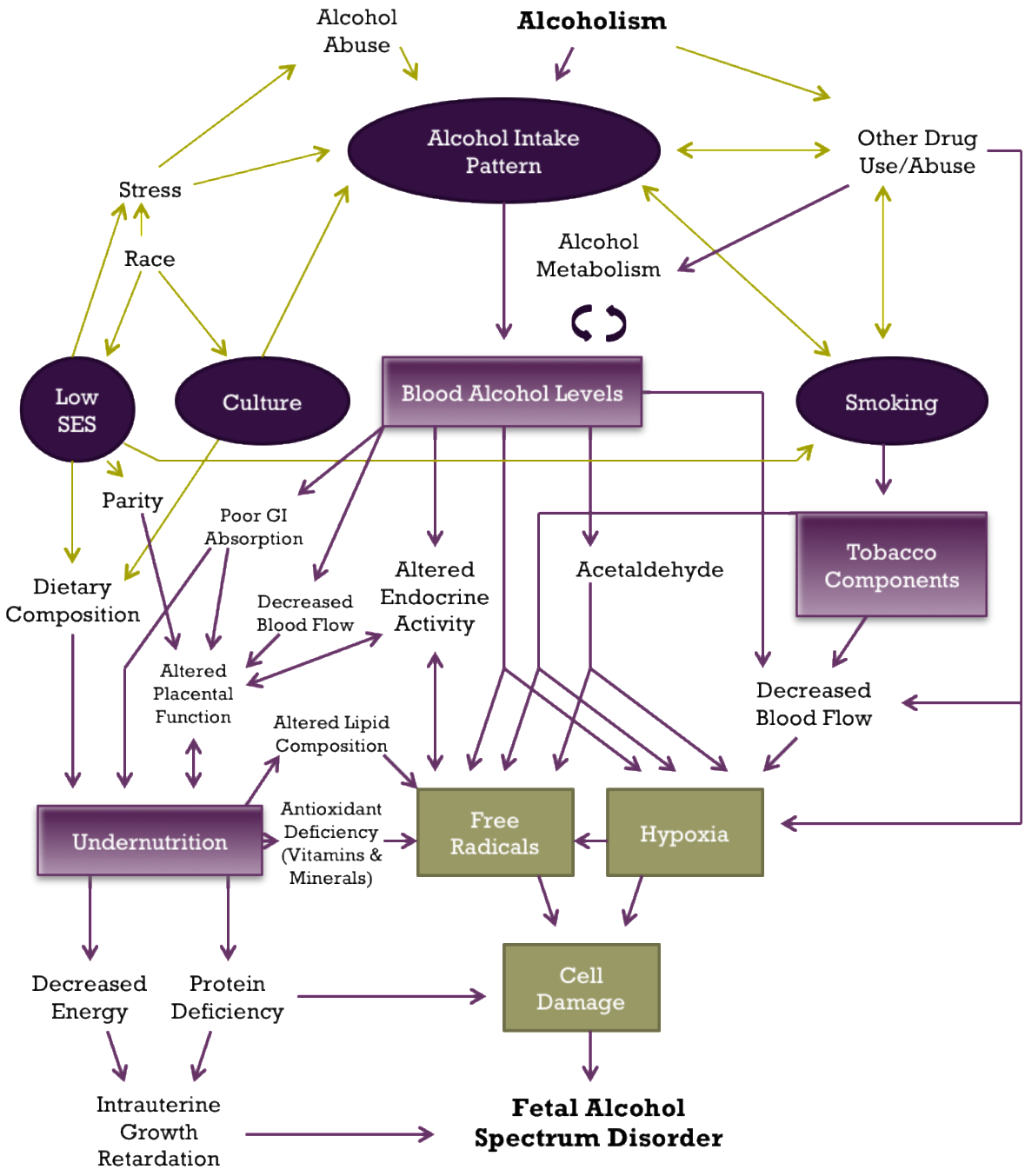


Figure 1-2: Relationships between permissive and provocative factors in FASD. Permissive factors are in dark purple circles while provocative factors are shown in light purple squares. Green lines indicate associations among demographic and behavioural variables. Purple lines indicate physiological pathways.

Adapted from Neruotoxicology and Teratology, 17(4), Able & Hannigan, Maternal risk factors in fetal alcohol syndrome: Provocative and permissive influences, p 450, Copyright (1995), with permission from Elsevier.

Socioeconomic status

While once related to race, this theory has been dismissed as a closer examination reveals an association between FASD and low socioeconomic status (SES) (Abel & Hannigan, 1995). FASD can be diagnosed to women of every socioeconomic status, however, the diagnosis of FAS and pFAS are more frequent in those of lower SES (May & Gossage, 2011). This is true in various countries such as South Africa, where FASD rates are highest among women living on poor rural farms, in bad living conditions, and with poor nutrition (May & Gossage, 2011). Abel and Hannigan (1995) suggest that low SES contributes to poor nutrition, higher stress levels, higher parity, and smoking. As noted in Figure 1-2, these factors contribute both directly and indirectly to a biological state promoting cell damage, and increasing the risk for FASD outcomes.

Maternal age, gravidity, and parity

Studies have found that gravidity, the number of previous pregnancies, and parity, the number of previous births, may be a risk factor for having a child with FASD (Jacobson et al., 1996; May et al., 2005, 2008). This means that of women with similar drinking patterns, the one with more pregnancies and births has an increased risk of having more severe FASD in her child.

FASD has also been associated with mothers of higher maternal age. The idea that maternal age may influence the frequency and severity of FASD comes from Sanchis et al. (1987) when rats with more liver damage from alcohol had more severely affected infants. In another study on rats, alcohol administered during gestation produced higher blood alcohol concentrations, as well as prolongation of alcohol within the blood with increased maternal age (Church, 1990). In humans, Jacobson et al. (1996) notes that this could be due to physiological changes during aging. For instance, as the percent body fat to water increases in older females, BAC's may increase per dose of alcohol consumed (Jacobson et al., 1996). They also suggest that physiological changes of aging may affect alcohol absorption and metabolism (Jacobson et

al., 1996). Other theories propose that, for habitual drinkers, being older may mean more years of drinking, leading it to be more ingrained in the woman's life (Skagerström et al., 2011).

Smoking

While maternal smoking is a risk factor for low birth weight in infants, it is also correlated to alcohol consumption during pregnancy (Abel and Hannigan, 1995; Lange et al., 2015; Skagerström et al., 2013). Able and Hannigan (1995) suggest that those with low SES tend to also smoke, confounding factors related to low-birth weight in pregnant mothers drinking alcohol. A study by May et al. (2008) found that of 78% of mothers with children diagnosed with FAS, 81% of mothers with pFAS children, and 17% of controls drank alcohol and smoked during pregnancy. Cigarette smokers may be more likely to consume alcohol and other substances that contribute to micronutrient deficiencies (Cogswell et al., 2003).

Impaired nutritional status

Mothers with impaired nutritional status are low in the nutrients needed for fetal development. Mothers drinking during pregnancy are less likely to be well nourished and have inadequate diets (May et al., 2008). Under-nutrition can lead to low pre-pregnancy weight and poor pregnancy weight gain, both risk factors for adverse pregnancy outcomes (Shankar et al., 2006). In animal studies, diets containing ethanol and inadequate calories led to impaired ethanol metabolism (Shankar et al., 2006). Consequently, this would increase BAC and expose the fetus to more ethanol. In fact, BACs are often higher in malnourished subjects compared to those with better diets and the same alcohol intake (Keen et al., 2010). Under-nutrition is also related to antioxidant deficiency. This may cause free radicals to build up, increasing the chances of cell damage and therefore traits of FASD (Abel & Hannigan, 1995; May & Gossage, 2011).

Neuronal protective nutrients

Various nutrients are key for fetal and neonatal brain development. Besides energy from macronutrients, various vitamins and minerals are also imperative (Georgieff, 2007). This review

will focus on vitamin A, choline, docosahexaenoic acid (DHA), folate, zinc, and antioxidants, which are commonly studied for brain function. Results are summarized in Table 1-2.

Vitamin A

There are various forms of vitamin A including retinol (alcohol), retinal (aldehyde), and retinoic acid (carboxylic acid). Retinal, in the form of all-transretinal, is required for both vision and cellular signaling (Ballard et al. 2012). It is converted to retinoic acid through retinal dehydrogenase for regulation and expression of many genes (Ballard, 2012). Retinoic acid has also been found to be important in the developing embryo as a signaling molecule (Deltour, 1996). Retinoic acid can be produced from retinol through 2 steps. First it is converted to retinal by retinol dehydrogenase (an enzyme from the alcohol dehydrogenase family) then converted to retinoic acid via retinal dehydrogenase (Delour, 1996).

Similarly, ethanol is oxidized to an aldehyde (acetaldehyde) by alcohol dehydrogenase then to a carboxylic acid (acetic acid) (Deltour, 1996). This means that alcohol can decrease production of retinoic acid through inhibition of retinal dehydrogenase (Ballard, 2012). In vitro, these two alcohol compounds compete for oxidation with alcohol dehydrogenase (Leo, 1999). During intoxication, ADH activity will be used for ethanol oxidation, making it unavailable for retinol oxidation due to its higher Km value (1.0 for ethanol and 0.028 for retinol) (Duester, 1991). This may lead to a deficiency in retinoic acid in various tissues (Duester, 1991). It is also believed that ethanol competitively inhibits retinaldehyde dehydrogenase activity (specifically RALDH2), which is the limiting step in retinoic acid signaling during gastrulation (Kot-Leibovich & Fainsod 2009).

Vitamin A is not usually required as a supplement for pregnant women, as mothers' stores typically provide adequate amounts for fetal development (Mahan et al., 2012, p358). Studies have also shown that excess vitamin A, leading to excess retinol and retinoic acid, have teratogenic effects similar to that of FASD (Duester, 1991). This indicates that there is a narrow range for safe intake of this nutrient.

Various animal models have been used to show how alcohol affects vitamin A and the fetus, and how vitamin A may mitigate the effects of alcohol. In *Xenopus* (frog) embryos, it was found that late blastula and early gastrula development were periods of increased sensitivity to ethanol. Exposure to 1.5-2.5% ethanol solution led to a high incidence of developmental malformations similar to those of FASD, such as small eyes and microencephalopathy (Yelin et al., 2005). It was also confirmed that ethanol competes with vitamin A for alcohol and aldehyde-dehydrogenases (Yelin et al., 2005).

Marrs et al. (2010) used zebrafish embryos to show that adding retinoic acid at low concentration of 10^{-9} alongside 100 mM of ethanol reduced defects such as craniofacial cartilage formation and neural axis patterning seen in the ethanol only group. However, the retinoic acid plus ethanol treated group was more similar to the retinoic acid only group than the ethanol control group. This suggests that retinoic acid only rescued embryos to a similar point as retinoic acid toxicity.

In zebrafish, Sarmah et al. (2013) studied alcohol induced congenital heart defects such as defective myocardium, endocardium, and endocardial cushions. When embryos were supplemented with retinoic acid, there was a rescue of most of the defects except for endocardial cushions. In contrast, supplementation of folic acid rescued normal heart development in all aspects. More recently, retinoic acid supplementation was shown to rescue alcohol-induced retinal defects in zebrafish (Muralidharan et al., 2015).

Choline

Choline is an essential nutrient. This is because metabolic demands cannot be sufficiently met from the quantities produced (Zeisel, 2009). Choline, part of acetylcholine, plays a role in neurotransmission and cell signaling. It also provides structural integrity to cell plasma membranes and acts as a methyl donor (Ballard et al., 2012). For these reasons choline is in high demand during pregnancy and lactation (Zeisel, 2009). During pregnancy, choline is delivered to the fetus with many other nutrients via the placenta. Plasma and serum choline concentrations

are higher in pregnant women than non-pregnant women, and are even higher in the newborn infant compared to the mother (Ziesel, 2009).

Choline is available through various foods such as eggs and liver (Zeisel, 2011). In the body, choline is converted to lecithin, or phosphatidylcholine (PC) to become the main phospholipid in cell membranes (Zeisel, 2009). PC is then converted to phospholipase D for use in cell signaling (Ballard et al., 2012). Alcohol can interfere with the conversion of PC to phospholipase D, which can affect signaling pathways and ultimately protein expression (Ballard et al., 2012).

Many studies have used a rat model to study how choline mitigates the effects of alcohol. In an animal study, rats were exposed to 6.0 g/kg/day ethanol throughout gestation, with the treatment group receiving a prenatal choline supplement of 250 mg/kg/day. Both the treatment and control group (ethanol only) had BACs that were not significantly different. The treatment of choline saw improved brain weight and incisor emergence and some behavioral targets (Thomas et al., 2009).

In a study by Otero (2012), alcohol was given to rat pups during the neonatal period (postnatal days 2-10), with choline and saline provided subcutaneously from postnatal days 2-20. Postnatal days 2-10 are comparative to the third trimester in humans. Results showed that choline supplementation reduced hypermethylation in the hippocampus and pre frontal cortex (Otero, 2012). In control animals, choline supplementation resulted in increased DNA methylation in these areas of the brain. Another study in mice found that choline provided prior to ethanol exposure on postnatal days 1-5 resulted in better balance and coordination in males on dowel crossing tasks (Bearer et al., 2015). Choline treatment pre and post ethanol exposure improved these measures in both males and females.

A study by Thomas et al. (2010) looked at the effects of choline supplementation on behavioural alterations caused by prenatal alcohol exposure. Rat dams were fed 6 g/kg/day ethanol in a binge-like fashion from gestational days 5-20. Choline in the form of choline

chloride was given as 250 mg/kg/day to treatment groups. The ethanol group had delayed behaviours such as natural exploring and foraging (known as spontaneous alternation), and spatial working memory, which were improved through choline supplementation. Other studies have also found improvements in behavior of ethanol exposed rat pups through choline supplementation (Thomas et al., 2007; Thomas et al., 2004; Ryan et al., 2008, Monk et al., 2012).

Docosahexaenoic Acid

Docosahexaenoic acid (DHA, C22: 6n-3) is an omega-3 polyunsaturated fatty acid required for maintaining good health. DHA can be found in food sources such as cold-water fish (mackerel, salmon, trout etc.). It can also be created in our bodies through elongation and desaturation of another omega-3 polyunsaturated fatty acid (PUFA) called alpha-linolenic acid (ALA), found in flaxseed oil, canola oil, olive oil, soya oil, and nuts (Singh, 2005). In women, it has been shown that conversion rate from ALA to DHA is 9% (Burdge & Wootton, 2002).

DHA is critical for brain growth as it affects neuronal membrane structure, synaptogenesis, and myelination (Martinez, 1992; Georgieff, 2007). DHA is also important for preventing oxidative stress and minimizing apoptosis in the brain (Innis, 2007). DHA is the most plentiful omega-3 fatty acid found in the human brain, and is altered by the amounts eaten in the diet (Innis, 2007). The brain is the organ made up of the most fat with DHA contributing to 40% of the PUFAs (Singh, 2005). During the third trimester, the fetal brain accrues up to 14 grams per week of omega-3 fatty acids (Clandinin et al., 1980). This quick accumulation in the brain during late gestation and early infancy indicates that that time period may have consequences for long-term brain function if there is a deficiency in DHA (Innis, 2007).

Alcohol may limit dietary intake and therefore intake of DHA during pregnancy. Alcohol may also lower DHA levels in the body due to fatty acid breakdown (Beblo et al., 2005). For instance, in a study of pregnant inner city black women, it was found that plasma DHA levels were low in those who were habitual drinkers (drinking more than one drink per week) compared

to those not drinking (Stark et al., 2005). This study also found that DHA and arachidonic acid (AA, C20: 4n-6) were further decreased as alcohol consumption increased. However, this study is limited in its small sample of pregnant women drinking habitually (n=6) and their low intake of alcohol.

Animal studies have tested whether DHA can mitigate the effects of prenatal alcohol exposure. An animal study used rats to determine if DHA in the diets of rat dams consuming ethanol could reduce hyperactivity in their infants. Diets included 5% safflower oil; 3% safflower oil with 2% DHA; 5% safflower oil and 10% ethanol; and 3% safflower oil, 2% DHA and 10% ethanol. It was found that rat pups from the mothers fed ethanol with DHA had less hyperactivity than those in the ethanol group (Furuya et al., 2000). Another study in rats found that DHA provided at 10 g/kg postnatal in artificial rat milk improved rat social behavior in early to mid adolescence after prenatal alcohol exposure (Wellmann et al., 2015). This included isolation induced ultrasonic vocalization, play fighting, and somatosensory performance via gap crossing.

In a study using guinea pigs, females were given 6g/kg/day ethanol, ethanol with 0.5 g tuna oil per day (130mg DHA/day), or tuna oil alone both before and during pregnancy (Burdge et al., 1997). It was found that the ethanol only group had decreased amounts of PC and phosphatidylethanolamine (PE) in the brains of pups. In the ethanol + tuna oil, DHA levels were restored to above that of controls (66.7%) and ethanol exposed (284.6%) groups. Brain PC levels were also significantly improved in the ethanol + tuna oil group compared to control and ethanol only groups. Another study in rat pups found that supplementation with omega-3 fats during prenatal and post-natal ethanol exposure prevented neurodegeneration (Ol et al., 2015). These results indicate that DHA supplements may be needed help mitigate the effects of alcohol.

Folate

Folate plays an important role as a coenzyme in metabolic pathways and in DNA methylation, which controls gene expression (Ballard et al., 2012). During pregnancy, folate

requirements increase so that the fetus can use it for rapid growth and cell proliferation (Hutson et al. 2012). The placenta plays a role by concentrating folate into fetal circulation leading to fetal levels 2-4 times higher than maternal levels (Hutson et al., 2012).

Alcohol can create deficiencies through inhibiting intestinal absorption and renal reabsorption and cellular entry of folate (Ballard et al., 2012). Chronic and heavy alcohol consumption can also hinder folate transport (Ballard et al., 2012; Hutson et al., 2012) and inhibit folate metabolism through changes in coenzyme patterns (Lin et al., 1992). A deficiency in folate would affect metabolism, slowing it down, and leading to increased errors in DNA replication in the fetus (Ballard et al., 2012).

It has been shown in both animals and humans that ethanol can lead to lower serum folate levels. In rats fed a folate deficient diet, those with low (0.4g/kg) and high ethanol intakes (4.0 g/kg) during days 7-9 gestation (equivalent to 1st trimester in humans), had lower serum folate. It was also found that low quantities of alcohol in conjunction to folate deficiency led to higher rates of fetal death (Gutierrez et al., 2007). In a human study, mothers with heavy alcohol consumption were compared to controls consuming no alcohol for folate transfer to the fetus. This was done through the use of maternal and cord blood samples taken at delivery. They found that in mothers drinking alcohol, folate transport to the fetus was impaired (Hutson et al. 2012). Control groups showed results typical for pregnancy; higher folate levels in the fetus compared to the mother. This was not seen in mothers consuming alcohol (Hutson et al., 2012). Atypically, there was no difference in maternal folate levels between mother consuming alcohol and controls. This may be due to the type of alcohol consumed, as beer can provide a source of folate.

Few studies have looked at the supplementation of folic acid in mitigating the effects of alcohol. In an animal study on guinea pigs it was found that folic acid supplementation of 2mg/kg body weight per day did not mitigate the effects of ethanol at 4% body weight (Hewitt et al., 2011). It did however prevent the fetal liver folate from lowering at birth. With so few

studies, it is unclear whether folate supplementation above requirements would be useful for FASD prevention.

Zinc

Zinc is essential to human growth and development as it is imperative in transcription, translation and cellular differentiation (Coyle et al., 2013). Dietary zinc can be found in animal products such as seafood, eggs, and red meat (Massaro et al., 2006). Deficiency of zinc during pregnancy leads to poor outcomes. For instance, women in developing countries with low plasma zinc concentrations are 2.5 times more at risk for delivering an infant weighing less than 2000g, with women younger than 19 years being at even higher risk (Rwebembera et al., 2006). Both animal studies and epidemiological data have showed that zinc deficiencies can cause congenital malformations in the fetus (Shah & Sachdev, 2006). This is because the genetic code is regulated by "zinc-finger" transcription factors, and a deficiency can affect gene expression (Coyle et al, 2013).

It has been shown in animal studies that a combination of alcohol and a low zinc diet has more severe effects on the fetus then rat dams in the alcohol or low zinc groups alone (Keppen et al., 1985). High zinc groups (8.5 ug/ml—recommended for reproduction) and low zinc groups (0.3 ug zinc/ml) were given 0%, 15%, or 20% of calories from ethanol. Fetal malformations, such as open eyelids, as well as low fetal birth weight were more common in the low zinc/ethanol groups.

In a study by Carey et al. (2003), pregnant mice (gestational day 8) were given 2.9 g/kg ethanol (at time points 0 and 4 hours), or the same with a 250 μg/ml of zinc supplement, or zinc only. On gestation day 18 fetuses were checked for malformations. It was found that the ethanol only group experienced small litters, had malformations of the eye, and lower body weights and crown-rump lengths. Malformations were less common in the zinc + alcohol group. In study with similar methods, Summers et al. (2009) found that zinc supplementation was protective against physical malformations and post-natal mortality.

Antioxidants

Ethanol can directly induce oxidative stress in a variety of ways. The metabolism of ethanol creates ROS through the use of alcohol dehydrogenase (see Figure 1-1) and by inducing cytochrome P-450 2E1, especially in the brain (Montoliu et al., 1995). ROS can damage various cellular components such as proteins, lipids, and DNA (Cohen-Kerem & Koren, 2003; Brocardo et al., 2011). As a result of ethanol metabolism there is an increase in superoxide, hydrogen peroxide, and hydroxyl radical in the cell (Brocardo et al., 2011). Ethanol also has an indirect effect on oxidative stress by reducing intracellular antioxidant capacity through lowering levels of glutathione peroxides, superoxide dismutase, and glutathion (Cohen-Kerem & Koren, 2003; Brocardo et al., 2011).

Vitamins with antioxidant activity, such as vitamins C and E, have been shown via animal studies to help ameliorate the effects of alcohol in the fetus. For instance, in rat pups, ethanol was given from day 7-9 postnatally at 5.25 g/kg/day. Vitamin E supplements were concurrently given to half the pups at 2.0g/kg/day in milk six days after birth (Marino et al., 2004). It was found that the antioxidant treatment prevented hippocampus cell loss seen in the alcohol only group. It also reduced protein carbonyls in the brain (an indicator of oxidative protein damage) (Marino et al., 2004). In the embryos of frogs exposed to ethanol, ascorbic acid provided at 100 uM during the neural plate development period, prevented a reduction in brain size and growth retardation seen in those exposed only to ethanol (Peng et al., 2005). The vitamin C was seen to inhibit ROS production, making it a possibly effective protective against ethanol exposure. In an *in vitro* study, hippocampal neuronal cell cultures were exposed to varying levels of ethanol and supplemented by either vitamin E or β-carotene. It was found that vitamin E protected neuronal cells and resulted in increased survival compared to controls. This was also seen in the cultures supplemented with β-carotene (Mitchell et al., 1999).

Folic acid also has antioxidant capacities (Hutson et al., 2012). For instance, pregnant rats received a standard control diet (60 ug/day folic acid), ethanol plus a standard diet (60 ug/day

folic acid), or ethanol plus a folic acid supplement (152 ug/day). The folic acid supplement in the treatment group prevented an increase of thiobarbituric acid reactive substances (TBARS) in the pup livers formed as a product of lipid peroxidation seen with alcohol. It also prevented protein oxidation in the liver. In fact, this study found that the supplementation level lowered these markers of oxidative stress 15% below that of the pups of the control group (Cano et al., 2001).

Superoxide dismutase is an enzyme with antioxidant capacity used to break down the superoxide anion into oxygen and hydrogen peroxide (Chen et al., 2004). In a study on mouse embryos, exposure to ethanol on day 8 gestation revealed greater superoxide anion generation and greater lipid peroxidation, as well as cell death. When embryos exposed to ethanol were also provided 300 U/ml superoxide dismutase, these effects were reduced (Kotch, 1995). Chen et al., (2004) also tested a synthetic version of superoxide dismutase (EUK-134) with additional catalytic properties. The synthetic version allows the breakdown of the superoxide anion along with its by-product, hydrogen peroxide. Ethanol was given to pregnant mice at 2.9 g/kg/day alone or with EUK-134. It was found that cell death was reduced, as well as forelimb malformation (by 36%) in groups treated with EUK-134 (Chen et al., 2004).

Other nutrients, such as DHA, also scavenge for free radicals in the developing brain and protect against lipid and protein peroxidative damage (Innis, 2007). However, more studies on each nutrient's antioxidant effect are required.

Table 1-2: Summary of results: Nutrients in mitigating effects of prenatal ethanol exposure.

Nutrient	Amount	Animal	Result	Reference
Vitamin A	10^{-9} M	Zebrafish	Restored craniofacial cartilage formation and partially rescued brain development defects seen in embryos exposed to 100 mM of ethanol.	Marrs et al., 2010
Vitamin A & Folic Acid	1 nM Vitamin A 75μM Folic Acid	Zebrafish	Rescued congenital heart defects in the myocardium and endocardium cushions but not endocardial cushions. Folic acid rescued all cushion defects.	Sarmah et al., 2013
Vitamin A & Folic Acid	1 nM Vitamin A 75μM Folic Acid	Zebrafish	Rescued retinal cell differentiation defects caused by ethanol exposure. Retinoic acid also recused retinal cell differentiation defects.	Muralidharan et al., 2015
Choline	188 mg/kg/day	Rat	Attenuated alcohols effects on hyperactivity and errors related to reversal learning tasks seen with 6 g/kg/day ethanol exposure.	Thomas et al., 2004
	250 mg/kg/day	Rat	Pups of rat dams exposed to ethanol at 6g/kg/day given in a binge-like fashion had delayed spontaneous alternation behaviours and impaired spatial working memory. Choline improved these outcomes, but did not influence motor coordination tasks.	Thomas et al., 2010
	100mg/kg/day	Rat	Prevented hyperactivity seen in groups exposed to 5.25 g/kg/day ethanol.	Monk et al., 2012
	100 mg/kg/day	Rat	Ethanol at 5.25 g/kg/day affected spatial memory as tested via probe trial. Choline treatment mitigated these effects, but did not improve performance on Morris water maze spatial learning tasks.	Ryan et al., 2008

Choline	0.188 mg/d	Mice	Treatment with choline prior to ethanol exposure improved balance and coordination in male mice. Pre and post treatment saw improvements for both sexes.	Bearer et al., 2015
Folic Acid	2 mg/kg/day	Guinea Pig	Did not mitigate the decrease in fetal body weight, brain, and hippocampus weights when exposed to ethanol at 4g/kg/day.	Hewitt et al., 2011
	152 µg/day	Rat	Concurrent supplementation of folic acid with ethanol (6.6g/kg/day) prevented an increase in markers of oxidative stress in the offspring, including of thiobarbituric acid reactive substances (TBARS) and carbonyl groups found in proteins.	Cano et al., 2001
DHA	30 mg/100g	Rat	DHA and DHA+ betaine prevented brain neurodegeneration caused by ethanol induced oxidative stress.	Ol et al., 2015
	10g/kg	Rat	DHA supplementation in artificial rat milk improved rat social behavior and ultrasonic vocalization after prenatal alcohol exposure.	Wellmann et al., 2015
	Basel diet with 2% DHA	Rat	Rat pups from mothers fed DHA in addition to ethanol experienced reduced hyperactivity to those in the ethanol only group.	Furuya et al., 2000
	130 mg/day	Guinea Pig	In supplemented group, DHA levels were restored to above that of controls (66.7%) and ethanol exposed groups (284.6%).	Burdge et al., 1997.

Zinc	62.5 μg	Mouse	Prevented ethanol-related growth deficits, as well as external abnormalities seen in mouse litters when the dam was given ethanol at 2.9 g/kg once or twice a day.	Carey et al., 2003
	200 mg/kg/day	Mouse	Protected fetuses from physical abnormalities such as eye malformation (microphthalmia, and anophthalmia) and limb abnormalities. It also led to higher zinc plasma concentrations than non- supplemented groups.	Summers et al., 2009
Superoxide Dismutase	20 mg/kg/day	Mouse	Ethanol given to mice dams at 5.8 g/kg/day caused apoptotic cell death in the apical ectodermal ridge of the forelimb buds in 67.3% of fetuses. Concurrent treatment with superoxide dismutase plus catalytic mimetic, EUK-134, reduced malformations to 35.9%.	Chen et al., 2004
Vitamin C	100 μM	Frog	Pretreatment and concurrent treatment of embryos reduced ethanol induced microencephaly and growth retardation. Also prevented a rise in hydrogen peroxide and malondialdehyde from ethanol.	Peng et al., 2005
Vitamin E	2.0 g/kg/day	Rat	Ethanol treated pups had impaired spatial navigation. They also had higher protein carbonyl formation in the hippocampus and fewer hippocampal CA1 pyramidal cells. Treatment with Vitamin E prevented the decrease in CA1 cells and the increase in protein carbonyl, but did not improve spatial navigation.	Marino et al., 2004

Chapter II. RESEARCH PLAN

Rationale

During pregnancy, optimal nutritional status is needed for proper fetal development. Macro and micronutrient needs increase throughout pregnancy due to physiological changes. Inadequate intake of these nutrients, as in the case of maternal malnutrition, is predictive of delivering a low birth weight baby and other negative birth outcomes (Mumbare et al., 2012; Brough et al., 2010; Imdad & Bhutta, 2012). Thus, providing adequate amounts of nutrients is key for a healthy pregnancy.

Alcohol can compromise maternal nutritional status in various ways, including displacing nutrients essential to fetal development and causing alcohol related gastrointestinal problems leading to secondary malnutrition (Zajac & Abel, 1992). The fetal brain is especially vulnerable to the teratogenic effects of prenatal alcohol consumption. Alcohol exposure during pregnancy has been reported to reduce fetal brain weight by 12% in animal models (Miller, 1996) and to induce oxidative stress and hypoxia (Bosco & Diaz, 2012). Together with compromised nutritional status, this may result in Fetal Alcohol Spectrum Disorder (FASD), a collective term for the cognitive and behavioural disabilities that may be seen in children exposed to alcohol prenatally. FASD can lead to disabilities in the child including difficulty with memory, learning life skills, and forming healthy relationships (Healthy Child Manitoba, 2013).

While there is no specified rate of FASD in Canada, an estimated 9 in 1,000 infants are born with FASD (PHAC, 2012). Studies in certain First Nations communities have estimated the rate to be much higher—between 7.2 to 101 cases per 1,000 births (Square, 1997; Williams et al., 1999). It is important to recognize that this does not likely represent the First Nations population, as active case ascertainment in at-risk communities may have led to these higher prevalence rates (Pacey, 2009). Owing to the physical defects and social disabilities of those with FASD, the cost of treatment, including direct and indirect costs, is significant. In 2013, the

estimated cost of FASD in Canada was approximately $1.8 billion dollars (Popova et al., 2015). The lifelong support required for those with FASD will likely continue to increase economic costs making it necessary to prevent or reduce the severity of FASD.

Public health campaigns have promoted the negative side effects of drinking during pregnancy. However, in Canada, 10.5% of post-partum women said they drank alcohol at some point while pregnant (PHAC, 2009). Of these women, 0.7% drank frequently during their pregnancy (PHAC, 2009). Theoretically, abstinence from alcohol during pregnancy is safest, but for women who have past trauma, who struggle with adequate housing, income, and safety, and whose lives are chaotic, this may not be a feasible option (M. Bryans, personal communication, April 27, 2016)

Prenatal alcohol exposure is associated with a variety of factors including maternal age, multi-parity, prenatal exposure to smoking and cocaine, and lower socioeconomic status (Sood et al., 2001). Healthy Child Manitoba (2012) reported that maternal alcohol consumption was related to those living in lower income areas, those that had not completed Grade 12, or were receiving income assistance. Due to a history of colonization, Indigenous women may have some of these demographics (Statistics Canada, 2011). Western patriarchy took Indigenous women from traditional positions of value and equality and led to a loss of identity and social and political disadvantage. This reduced access to the social determinants of health such as education and higher SES (Bourassa et al., 2004). With alcohol consumption comes the risk of various nutrient deficiencies, compromising the health of the fetus, and increasing the likelihood for FASD (Bingol et al., 1987).

Current research has focused on specific nutrient interventions (including DHA, choline, zinc, vitamin A, E, and selenium) to reduce FASD in animal models. For instance, prenatal ethanol exposure has been shown to decrease glutathione levels and increase lipid peroxidation, contributing to oxidative stress in rats (Patten et al., 2012). When these same rats were supplemented with omega-3 fatty acids (including DHA) there was an increase in glutathione

and a reduction in lipid peroxidation, thus undoing some of the negative oxidative stress from prenatal alcohol exposure. In studies on zinc supplementation, pregnant mice injected with 25% alcohol on the 8th day of gestation saw a lower incidence of birth defects when supplemented with dietary zinc (Summers et al., 2009). In the same study, post-natal mortality was also reduced in the zinc-supplemented group. Similarly, an animal study looking at the effects of choline supplementation on rat dams fed an alcohol containing diet showed protection to the neurons typically affected in FASD (Bekdash et al., 2013). The research of choline in animal models has also shown improvements in the severity of FAS, particularly in hyperactivity and learning deficits in rat models (Thomas et al., 2007; Thomas et al., 2010).

While these studies show the potential of supplementating various nutrients to reduce the effects of FASD, it is still unknown whether these nutrients are indeed required for mothers during pregnancy due to a lack of sufficient research data. Prior to nutrition provision during pregnancy, it is important to understand how much of these nutrients pregnant women at risk of drinking alcohol are consuming. Therefore, this study aimed to collect detailed nutrition intake data and maternal health information in such a population. This preliminary data on nutrient intake may allow future research to determine potential nutrition interventions in drinking pregnant women, which may ultimately decrease rates of FASD.

Hypothesis

The study hypothesis is that women at risk of drinking alcohol during pregnancy will not be consuming adequate amounts of nutrients important to fetal brain development. Further,

- Women who have alcohol exposure during their pregnancy will have lower intakes of these nutrients than those women who do not have alcohol exposure.
- Pregnant women at risk for prenatal alcohol exposure will not meet recommended intakes of Canada's Food Guide servings and Dietary Reference Intakes.

Objectives

The overall study objective is to identify the nutrient intake in pregnant women at risk of consuming alcohol. The specific objectives are:

- To identify the nutrient intake (vitamin A, choline, folate, DHA, zinc) of pregnant women with alcohol exposure during pregnancy in comparison to pregnant women without alcohol exposure.
- To identify macronutrient intake and food group consumption patterns among pregnant women using a 24-hour food recall.
- To correlate alcohol exposure with nutrient intake and other risk factors for prenatal drinking including age, parity, smoking and drug use.

Part III: EXPERIMENTAL DESIGN AND METHODS

Experimental design

Study design

The University of Manitoba Health Research Ethics Board (HS16448) approved study protocols and consent forms for this project. The principles set out by Tri-Council Policy on *Ethical Conduct for Research Involving Humans*, and specifically, *Research Involving the First Nations, Inuit and Métis Peoples of Canada* were used to guide this study (Tri-Council, 2014). This included community engagement, which seeks to build collaboration between the researcher and the communities involved (Tri-Council, 2014).

This method is inclusive and respects the values, knowledge and initiatives of the community being studied by involving them in the research process including identification of their needs (Ahmed & Palermo, 2010). The process of community engagement focuses on building relationships based on mutual respect and trust. Most importantly, it places the participants, especially those who are marginalized, including Indigenous women, those who have substance use issues, and those who live in poverty, as the "experts" in their own lives (Salmon, 2007). Due to the stigma and marginalization surrounding women who drink alcohol or use other substances during pregnancy, recruitment can be difficult making community engagement essential for the success of this study (Salmon, 2007).

Recruitment of participants took place through prenatal programs in the Downtown and Point Douglas areas of Winnipeg, Manitoba, Canada from December 2014 to July 2015. These programs serve a unique population. In Point Douglas, unemployment rates are 7.2% compared to 3.9% in the rest of Winnipeg, while average household incomes stand at $40,703 compared to $63,000 in all of Winnipeg (City of Winnipeg and Statistics Canada, 2006). This area also has

one of the highest populations identifying as Aboriginal, at 29%. The Downtown community is similar, with unemployment rates at 7.4%, average household incomes at $39,000, and 17.4% identifying as Aboriginal (City of Winnipeg and Statistics Canada, 2006).

The primary site of community engagement was the Mothering Project at Mount Carmel Clinic. The Mothering Project (Manito Ikwe Kagiikwe) specifically supports women with substance use issues who are pregnant or have young children. Engagement occurred through volunteering weekly at drop-ins, providing support for the community garden, cooking projects, and developing relationships with participants and staff. In this way, over the period of a year, trust was built between all parties, and I (K.D.) was seen as a reliable face in the "landscape" of the Mothering Project. When the Program Manager suggested collaboration for a research project, there was strong support. As a critical component of preparing this research, we (K.D., H.G.) met with the Women's Advisory Council, the group of women whose experiential background helps guide the program. During these meetings, feedback was received on the research tool, as well as recruitment and retention strategies. One of these strategies was to make it as easy as possible for women to participate by meeting them where they were at in their lives, without judgment. The Women's Advisory Council also suggested an appropriate honorarium for the women's time, which included a gift card to a local grocery store. Program management was supportive of these strategies.

The Program Manger reviewed the research tool to ensure it was appropriate for the population and would not stimulate any past trauma. Alcohol and drug use can be connected to past histories and painful or traumatic events (Salmon, 2007). A trauma informed approach seeks to have awareness of the impacts of trauma, and practices in a way that builds genuine and compassionate relationships (Klinic, 2013). It also aims to prevent re-traumatization. Additional

training was provided to the researchers on how to deliver the questionnaire using a trauma informed approach.

Although posters were used to recruit from other prenatal clinics in the area, this was not found to be highly effective. As with the Mothering Project, regular attendance at prenatal program such as Health Start for Mom and Me, and Pregnancy and Family Support Services (PFSS) resulted in increased participation in the study. Important to this was providing a safe, comfortable and convenient place for women to participate. This meant that the researchers completed interviews in participants' homes, public spaces, or the cultural room at the Mothering Project. Snacks and water were provided. Women were told the purpose of the study using an info-graph to provide a visual representation and aid comprehension. This visual helped inform participants why they were being asked sensitive questions related to alcohol and drug use. As part of the trauma informed practice, participants were told that they did not have to answer any questions they felt uncomfortable with, and that they could stop participating in the study at any time. During the interview, if participants appeared to hesitate when answering the question, the interviewer would again provide the option of moving on in the questionnaire. A visual aid for the consent form was also used to help participants understand each component, and if participants agreed, the consent form was signed.

Participants

Power calculations were not conducted for this study, as it was a pilot. A target was set for interviewing 60 pregnant women. Inclusion criteria were pregnant women aged 14-50 living in and accessing prenatal services in Point Douglas or Downtown Winnipeg. Participants also had to be able to read and communicate in English. Pregnant women under the age of 18 were included in this study because Manitoba has one of the highest rates of teen pregnancy in Canada, especially in Indigenous populations, with rates of 23% in First Nations compared to

7.5% in non-First Nations (Hallett, et al. 2000). The exclusion criteria were women who were not pregnant, those outside of the target age range, those unable to communicate in English, and living in areas outside of Point Douglas and Downtown. The rationale for participants accessing services in these areas is to increase the chance that participants will be similar in terms of background, education, and SES. In total, interviews were conducted with 62 pregnant women, however, 6 did not meet the inclusion criteria as they lived outside of the Point Douglas and Downtown areas. Therefore, 56 women were included in the results for this study.

Research Tool

An iPad version of the *Nutrition for Two* questionnaire developed in our lab was used for interviewing pregnant women at risk for alcohol consumption during pregnancy. Participants had the option of entering the data themselves, with the researcher providing guidance along the way, or having the researcher input the data as the participant provided information. This simplified the documentation process, allowing participants to engage in a new way and providing more time for conversation and questions with the interviewer.

The questionnaire contains information on participant demographics including identification as Aboriginal or non-Aboriginal, education levels, employment, and number of people living per household. During development of the research tool feedback from stakeholders, including an FASD community coordinator, noted that asking for income levels may make participants feel uncomfortable. Therefore, direct questions on income were not included in the questionnaire, but mothers could indicate whether they were on social assistance or not. Information on food patterns was collected in regards to meals eaten per day, eating out, cooking habits, and what factors were important to mothers when choosing the foods they ate.

Food intake data was collected using a 24-hour food recall, which was administered by a Dietitian and trained nutritionists. Participants were asked to describe each food and beverage

they had taken from the time they woke up till the time they went to bed the day before. Interviewers asked clarifying questions on the time of day food was eaten, portion sizes, and any ingredients added, such as condiments.

The semi-quantitative food frequency questionnaire (FFQ) was created using the top food sources (as per the Canadian Nutrient File) for the nutrients important to fetal brain development, which have the potential to mitigate the effects of FASD, as known from an extensive literature review (Young et al., 2014). Foods that were commonly consumed (based on the CCHS), and traditional foods were also included for a total of 104 food items. Participants were asked if they ate that food during their pregnancy. If they had, they could specify how often ("Rarely", 1-3 times per month, "Sometimes" 1-2 times a week, "Often" 3-5 times a week, "Everyday" 1 time a day, and "All the time", 2 times or more a day). Participants were also asked when they ate that food item and what amount they would typically consume (small, medium, or large portions). Food models were used to represent medium portion sizes, which was the equivalent to one Canada's Food Guide serving. Researchers asked for further clarification when participants seemed unsure.

Data was collected on anthropometrics when possible. As part of the methods to make participants feel comfortable, safe, and non-medicalized, women were asked for a weight and height from their most recent prenatal visit. For many participants, weight can be a sensitive subject and women were given the opportunity to refuse to answer. Women were asked to rate their health before and during pregnancy. Information on medication and supplement use was collected, as well as alcohol, smoking, and drug use. Questions on alcohol consumption included when the last time they had a drink was, how much they typically consumed when drinking, and how often they drank. The same questions were asked regarding smoking. Women were asked if

they had used drugs at any point during their pregnancy, and which drugs were taken. The final section provided information on pregnancy including the effects of morning sickness on food intake.

The primary tool for analysis of the 24 hour recall and food frequency questionnaire was the Canadian Nutrient File (CNF) 2007b (Health Canada, 2007c). For 24-hour food recalls, a research assistant (also a nutritionist) input each food item and amount, along with the corresponding code from the CNF. Excel sheet comments were included when the serving sizes in the 24-hour food recall differed from those in the CNF. For example, one sugar packet in the food recall was converted into 4 grams for the CNF. When the brand of products was specified, the nutrient label of that product was preferentially used. If the item was not available on the CNF or on restaurant and company websites, the most similar item in the CNF was taken. This database was used to find the kilocalories, grams of protein, carbohydrates, fat and sugar for each food item. It was also recorded which food group each item was categorized under, and the number of servings eaten. The daily total from each category was tallied and a dietitian reviewed the data set for accuracy.

A reference sheet was created to identify the amount of each nutrient (zinc, vitamin A, folate, choline, and DHA) in a medium serving size for each item on the FFQ. A medium portion was based on the CFG serving size. For each item, the nutrient values from various forms of that food were found and then averaged. For example, under the food item 'tomatoes', the nutrient values for both raw and canned tomatoes were included, then averaged.

Nutrient intake was calculated per day. To determine this, the frequency of consumption was translated into an amount per day. For instance, items eaten "Everyday" were described as items eaten 1 time a day. In this case, the entire nutrient value of that item was then used to contribute to their overall intake. Items eaten "Often" are described as those eaten 3-5 times per week. If the average intake was 4 days out of 7 days, then the nutrient value was multiplied by 4 and then divided by 7 to get a daily amount (or multiplied by 0.57). The nutrients from items

eaten “All the time” were multiplied by a factor of 2, as the definition was items eaten 2 times or more a day. Other frequencies were treated similarly.

To account for portion size, medium portions were used as a reference for each item due to their correspondence to a single CFG serving. Similar methods in previous studies used medium portions as the 50^{th} percentile, with small and large portions as 25^{th} and 75^{th} percentiles (Cardoso & Stocco, 2000; Forster et al., 2014). The nutrient intake from each food item consumed was tallied into a total daily amount. Nutrient adequacy was calculated using the EAR based on pregnant women (19-30 years). If the EAR was not available, AI was used.

Statistical analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute Inc., Toronto, ON). Data was tested for normal distribution using Kolmogorov-Smirnov. To determine the difference in intake between trimesters, one-way ANOVA and Duncan’s Multiple Range Test were used. To compare differences between non-alcohol exposed and alcohol exposed groups, T-tests were used for data that was normally distributed and non-parametric tests for those not normally distributed. For categorical data, Cochran-Mantel-Haenszel was used to compare between groups. Correlations between alcohol exposure and participant variables were done using Spearman correlation. The level of significance was set at $p \leq 0.05$. Data were presented as mean ± standard deviation for normally distributed data, median (interquartile range) for data that was not normally distributed, or percentage (%) of participants.

Chapter IV. RESULTS

Basic demographic data

The demographic information of the study group is outlined in Table 4-1. The mean age of participants was 29.4 ± 5.8 years. Body Mass Index (BMI) data was available and calculated for 46 participants. Average usual BMI (pre-pregnancy) was 24.4 ± 5.5, while current BMI was 27.9 ± 6.6 at the time of data collection. Of the 56 pregnant women who participated, 71.4% (n=40) identified as Aboriginal. For those identifying as Aboriginal, 50% (n=28) were First Nations and 21.4% (n=12) were Metis. The majority of participants reported their highest level of education as high school (80.4%, n=45). The majority (69.6%, n=39) of participants reported receiving social assistance. Only 8.9% (n=5) of women were in their 1st trimester, while 46.4% (n=26) were in their 2nd, and 41.1% (n=23) were in their 3rd. Mean gravida was 4.1 ± 2.7 and parity was 2.2 ± 2.2.

Alcohol and substance use

Just under half of participants had alcohol exposure during pregnancy (46.6%, n=26). Of the women who drank, 92.3% (n=24) reported that their last drink was over a month ago. Only one mother reported that her last drink was "last week" and one mother reported that it was "sometime this week". Over half of mothers had smoked during their pregnancy (58.9%, n=33). Of these, 76.5% reported smoking every day, with the average of all smokers having 4.2 cigarettes per day. Only 26.8% (n=15) of mothers reported exposure to drugs during their pregnancy. Of theses mothers 46.7% (n=7) had used marijuana, 26.7% (n=4) had used crack cocaine, and 26.7% (n=4) had used multiple substances including marijuana, cocaine, and crystal meth.

Table 4-1 Maternal demographic characteristics

Characteristics	**(N= 56)**
Age (years)	29.4 ± 5.8
Usual BMI*	24.4 ± 5.5
Current BMI*	27.9 ± 6.6
Ethnicity	
First Nations	28 (50.0)
Metis	12 (21.4)
Non-Aboriginal	16 (28.6)
Education	
Jr. High	24 (42.9)
High School	21 (37.5)
Certificate	3 (5.3)
University	8 (14.3)
Receiving Social Assistance	
Yes	39 (69.6)
No	17 (30.4)
Pregnancy	
Gravida	4.1 ± 2.7
Parity	2.2 ± 2.2
Trimester	
First	5 (8.9)
Second	26 (46.4)
Third	23 (41.1)
Alcohol Exposure**	
Yes	26 (46.4)
No	30 (53.6)
Smoking	
Yes	33 (58.9)
No	21 (37.5)
Drug-Exposure	
Yes	15 (26.8)
No	41 (73.2)

Data are mean ± SD or N (%). *Based on data from n=46

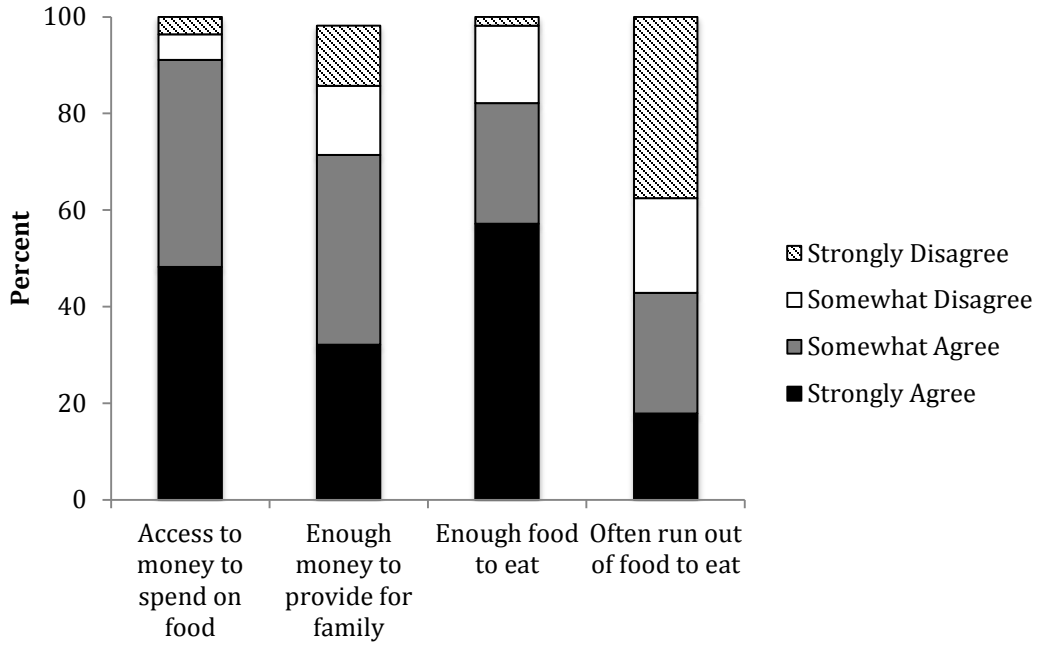
** Presented as alcohol exposure at least once during pregnancy

Self-reported access to food

Participants were asked a number of questions related to money and the money they had available to purchase food, which may be an indirect indication of food security. Participants could respond with "strongly agree", "somewhat agree", "somewhat disagree", and "strongly disagree". When asked if they have access to money to spend on food, 91.1% (n=51) of participants agreed (48.2% strongly agreed, 42.9% somewhat agreed). Fewer participants (71.4%, n=40) agreed when asked if they have enough money to provide for themselves and their families (32.1% strongly agreed, 39.3% somewhat agreed). Participants were also asked if they usually had enough food to eat, with the majority of participants agreeing that they did (82.1%, n=46). However, when asked if they often run out of food to eat, almost half of participants (42.9%, n= 24) agreed. Results can be seen in Figure 4-1a.

Participants were also asked about access, availability, and affordability of food. Participants could again respond with "strongly agree", "somewhat agree", "somewhat disagree", and "strongly disagree". When asked if they have access to all of the foods they want to eat, 71.4% (n=40) agreed (44.6% strongly agreed, 26.8% somewhat agreed). Of the food they want to eat, 62.5% (n=35) of participants agreed that they could afford to buy them (30.4% strongly agreed, 32.1% somewhat agreed), while 67.9% (n=38) agreed that the food they want to eat are available from a store near their home (37.5% strongly agreed, 30.4% somewhat agreed). Finally, when asked if they feel the food they eat is adequate to keep them healthy, 87.5% (n=49) of participants agreed (48.2% strongly agreed, 39.3% somewhat agreed). Results can be seen in Figure 4-1b.

4-1 a)



4-1 b)

Figure 4-1 Self-reported access to food. a) Participant reponses on access to money and food. b) Participant responses to questions on affordability, availability, and adequacy of food. Data in each category represents a percentage of total participants (n=56).

Self-rated health during pregnancy

When rating their health during pregnancy, 21.4% (n=12) of participants reported that their health was "excellent", 32.1% (n=18) "very good", 32.1% (n=18) "good", 8.9% (n=5) "fair", and 5.4% (n=3) "poor" (Table 4-2). Compared to before pregnancy, 35.7% of women (n=20) gave an improved rating to their health during pregnancy, while 17.9% (n=10) gave a poorer rating. Appendix A breaks down how women rated their health before pregnancy, based on their self-rated health during pregnancy.

Only 12.5% (n=7) of women reported a chronic illness. One participant was diagnosed with gestational diabetes, other illness reported include hyperthyroidism, HIV, multiple sclerosis, and asthma. Reports on prescription medications include thyroid hormone replacements (n=2), antiretrovirals (n=1), inhalers (n=2), anti-emetics (n=6), and anti-anxiety drugs (n=1). Almost all participants (89.3%, n=50) reported taking a supplement regularly (at least once a week) during their pregnancy. Of those that took a supplement, 75% (n=42) were taking a prenatal multivitamin with iron, and 1 participant was taking only an iron supplement. Seven participants were taking multiple supplements, which can be found in Table 4-3.

Table 4-2 Self-rated health during pregnancy

Category	**(N= 56)**
Self-rated health during pregnancy	
Excellent	12 (21.4%)
Very Good	18 (32.1%)
Good	18 (32.1%)
Fair	5 (8.9%)
Poor	3 (5.4%)
Chronic Illness During Pregnancy	
Yes	6 (10.7%)
No	50 (89.3%)
Prescription Medications*	
Yes	13 (24.1%)
No	41 (75.9%)
Supplement Use	
Prenatal with iron	42 (75.0)
Iron	1 (1.8)
Multiple Supplements	7 (12.5)
None	6 (10.7%)

Data are N (%).
*Based on data from n=54

Table 4-3 Multiple supplement use during pregnancy

Participant	**Prenatal**	**Multivit**	**Fe**	**Folic Acid**	**Vit D**	**Ca**	**B12**	**Mg**
1	X			X	X			
2		X	X					
3	X				X	X		
4							X	
5	X		X					
6	X		X					
7			X	X		X		X

X = reported use
Multivit = multivitamin that is not a prenatal formula; Fe = iron; Vit D = vitamin D; Ca = calcium, Mg = magnesium

Comparison of food and nutrient intake at each trimester

Food group consumption, based on the 24-hour food recall, was compared to Canada's Food Guide. Median (IQR) vegetable and fruit consumption was 3.0 (3.5) servings, grain products 5.0 (3.8) servings, milk and alternatives 1.3 (2.5) servings, and meat and alternatives 2.0 (2.0) servings. Few participants met CFG recommendations for vegetables and fruit (12.5%), while less than half met recommendations for grain products and milk and alternatives (42.9% for both groups). The food group in which most (69.6%) of participants met recommendations was meat and alternatives (Fig. 4-2). Vegetable and fruit intake did not significantly differ between trimesters. At $p = 0.10$, vegetable and fruit intake was significantly higher in the 3rd trimester than the 1st.

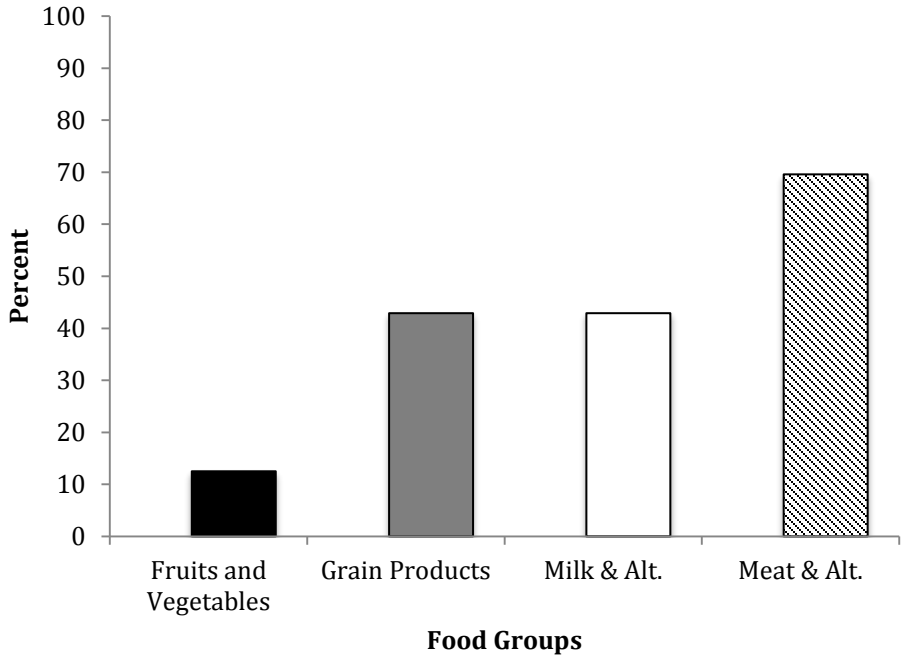


Figure 4-2 Food consumption meeting recommended servings for each food group based on Canada's Food Guide. Data derived from 24-hour food recall and presented as a percentage of all participants (n=56).

In regards to macronutrients, participants had a median (IQR) intake of 2047.2 (620.0) kcal, 254.5 (106.3) grams of carbohydrates, 76.5 (26.3) grams of protein, and 77.7 (43.5) grams of fat, based on the 24-hour food recall. Protein was also calculated in g/kg/day with participants having a median intake of 1.09 (0.45) g/kg/day (Table 4-4). Data was analyzed using one-way ANOVA to determine if trimester impacted nutrient intake. No significant results were found for macronutrients.

Acceptable Macronutrient Distribution Ranges (AMDR) for those 19 years and older are 45-65% for total carbohydrates, 10-35% total protein, and 20-35% total fat, with a limit of no more than 25% of total energy. Compared to the AMDR, this cohort's macronutrient intakes were within range, with carbohydrates as 49.7% of caloric intake, protein as 14.9%, fat as 34.1% and sugar as 19.8%. When looking at macronutrient intake on an individual level, 41.1% (n=23) of participant had fat intakes that exceeded the AMDR, while 28.6% (n=16) had intakes of sugar that exceeded the AMDR. Few participants exceeded the AMDR for protein and carbohydrates (1.8%, n=1 and 3.6%, n=2 respectively), however, 8.9% (n=5) of participants were below the AMDR for protein, and 19.6% (n=11) below for carbohydrates.

The median (IQR) intake of nutrients per day was 48.4 (102.8) mg for zinc, 1503.6 (843.1) μg of vitamin A, 471.2 (285.8) μg of folate, 448.6 (242.6) mg of choline, and 97.0 (81.7) mg of DHA. Compared to DRIs, almost all participants meet the EAR for zinc and vitamin A (96.4%). Less than half met the EAR for folate (44.6%), AI for choline (48.2%), and recommended intake for DHA (16.1%) (Fig. 4-3). Intake from supplements was not counted towards dietary intake. When analyzed by trimester, no significant differences were found for micronutrient intake. At $p = 0.07$, DHA intake was significantly higher in the 3rd trimester compared to the 1st (Table 4-5).

Table 4-4 Macronutrient intake by trimester

	DRI	All (n=56)	1st Trimester (n=5)	2nd Trimester (n=26)	3rd Trimester (n=23)*	P value
Macronutrient						
Energy (Kcal)	--	2047.2 (620.0)	1647.0 (769.4)	2078.2 (501.9)	2055.0 (775.0)	0.51
CHO (g)	175	254.5 (106.3)	199.7 (190.4)	251.0 (105.6)	268.1 (51.1)	0.23
Protein(g)	71	76.5 (26.3)	66.1 (30.9)	75.6 (29.4)	77.3 (24.0)	0.84
Fat (g)	--	77.7 (0.45)	65.5 (23.4)	79.3 (44.2)	78.1 (39.1)	0.74
Sugar (g)	--	101.5 (43.5)	59.8 (158.9)	92.4 (92.6)	107.2 (47.8)	0.96
Met CFG						
Veg/Fruit		7 (12.5)	0 (0)	3 (11.5)	4 (17.4)	0.10
Grains		24 (42.9)	1 (20.0)	11 (42.3)	11 (47.8)	0.27
Milk & Alt		24 (42.9)	0 (0)	10 (38.5)	13 (56.5)	0.50
Meat & Alt		39 (69.6)	3 (60.0)	15 (57.7)	19 (82.6)	0.81

Values are median (IQR) for macronutrients and N (%) for CFG. The significant differences between groups were tested by one way ANOVA. No significant effect of trimesters was identified.
CFG= Canada's Food Guide
*Trimester data available for n=54

Table 4-5 Micronutrient intake by trimester

	DRI	All (n=56)	1st Trimester (n=5)	2nd Trimester (n=26)	3rd Trimester (n=23)*	P value
Micronutrient						
Zinc (mg)	11	48.4 (102.8)	23.8 (17.1)	39.7 (85.3)	119.3 (101.9)	0.36
Vitamin A (µg)	770	1503.6 (843.1)	1595.9 (560.5)	1277.5 (820.7)	1534.7 (1161.6)	0.33
Folate (µg)	600	471.2 (285.8)	486.7 (402.5)	446.0 (243.7)	476.5 (251.3)	0.67
Choline (mg)	450	448.6 (242.6)	458.0 (354.7)	404.8 (284.8)	449.3 (161.3)	0.45
DHA (mg)	200	97.0 (81.7)	58.1 (76.2)	95.2 (75.4)	97.1 (148.3)	0.07
Met DRI						
Zinc		54 (96.4)	4 (80.0)	25 (96.2)	23 (100)	
Vitamin A		54 (96.4)	4 (80.0)	22 (84.6)	22 (95.7)	
Folate		25 (44.6)	2 (40.0)	7 (26.9)	8 (34.8)	
Choline		27 (48.2)	3 (60.0)	12 (46.2)	14 (60.9)	
DHA		9 (16.1)	0 (0)	2 (7.7)	7 (30.4)	

Values are median (IQR) for micronutrients and N (%) for DRIs. The significant differences between groups were tested by one way ANOVA. No significant effect of trimesters was identified.
CFG= Canada's Food Guide
*Trimester data available for n=54

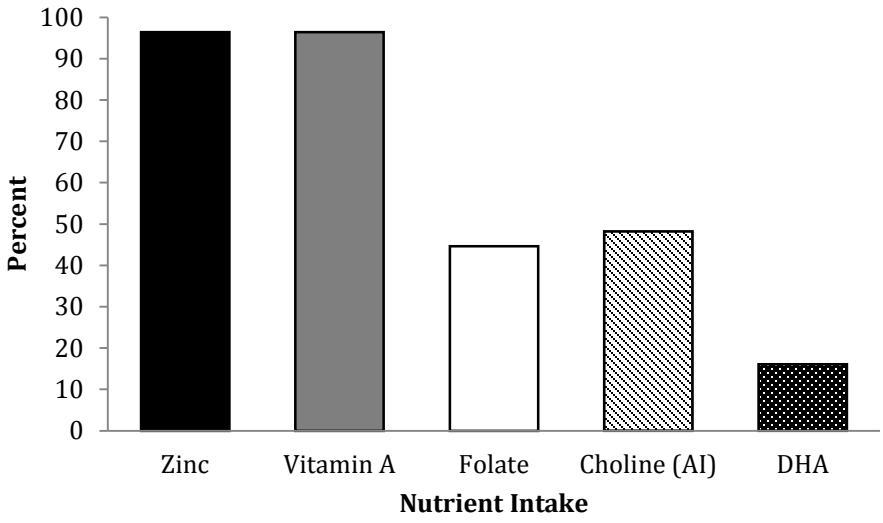


Figure 4-3 Nutrient intake meeting Dietary Reference Intakes (DRIs). Data derived from semi-quantitative Food Frequency Questionnaire and presented as a percentage of all participants (n=56).

Comparison of non-alcohol expsosed and alcohol exposed participants

Demographic data on non-alcohol exposed and acohol exposed participants are shown in Table 4-6. There were no significant differences between these groups, except for smoking behaviours during pregnancy (p= 0.016).

Table 4-6 Demographics of non-alcohol exposed and alcohol exposed participants

	Non-Alcohol Exposed (N= 30)	Alcohol Exposed (N=26)	p-value *
Age (years)	30.5 ± 5.6	28.2 ± 5.9	0.78
Usual BMI*	24.5 ± 5.8	24.3 ± 5.1	1.00
Current BMI*	27.6 ± 6.7	28.3 ± 6.6	0.70
Ethnicity			0.83
First Nations	13 (23.2)	15 (26.8)	
Metis	6 (10.7)	6 (10.7)	
Non-Aboriginal	11 (19.6)	5 (8.9)	
Education			0.08
Jr. High	10 (33.3)	14 (53.8)	
High School	12 (40)	9 (34.6)	
Certificate	2 (6.7)	1 (3.8)	
University	6 (20)	2 (7.7)	
Receiving Social Assistance			
Yes	19 (63.3)	20 (76.9)	0.274
No	11 (36.7)	6 (23.1)	
Smoking			
Yes	13 (46.4)	20 (76.9)	0.016
No	15 (53.6)	6 (23.1)	
Drug-Exposure			
Yes	6 (20)	9 (34.6)	0.222
No	24 (80)	17 (65.4)	

Values are mean ± SD or N (%).

** Presented as alcohol exposure at least once during pregnancy

The significant differences between groups were tested by Cochran-Mantel-Haenszel Test. No significant effect of alcohol was identified, except on smoking.

Nutrient intake in non-alcohol and alcohol exposed groups

A sub-analysis was undertaken to determine if nutrient intake differed between women who had no alcohol exposure and women who had alcohol exposure during their pregnancy. There were no significant differences in intake found between participants alcohol exposed pregnancies and those without (Fig. 4-4). When comparing macronutrient intake as a percentage of total energy, non-alcohol exposed participants did not differ from alcohol exposed participants (Fig 4-5). All nutrient intake data between groups is displayed in Table 4-7.

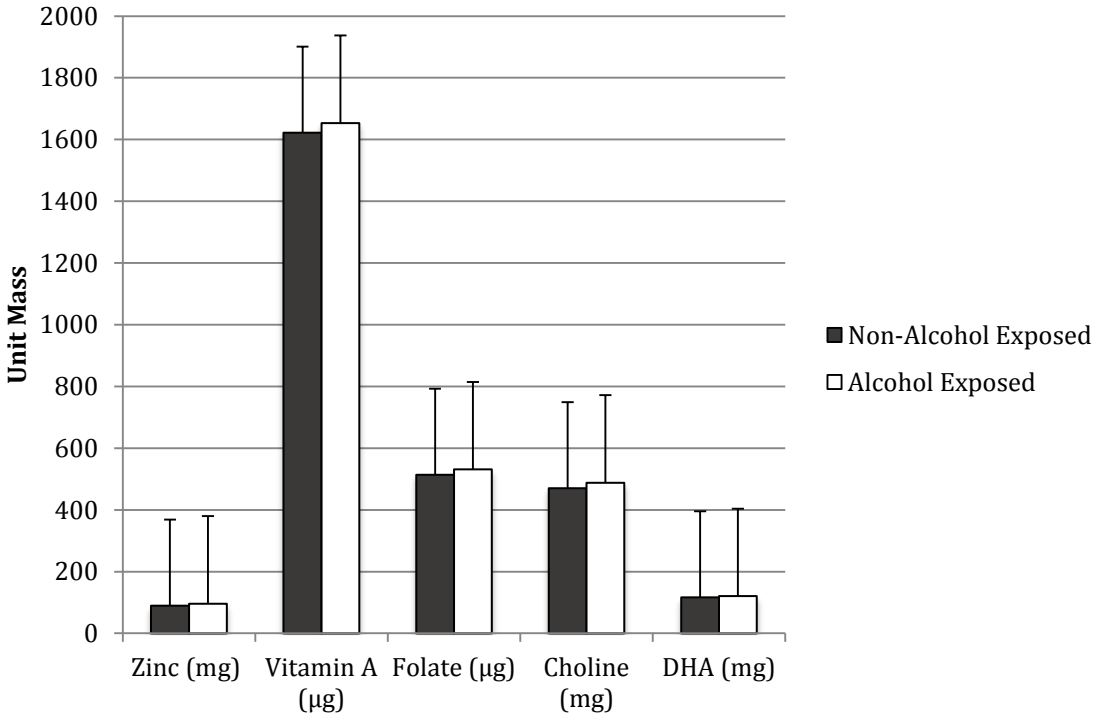
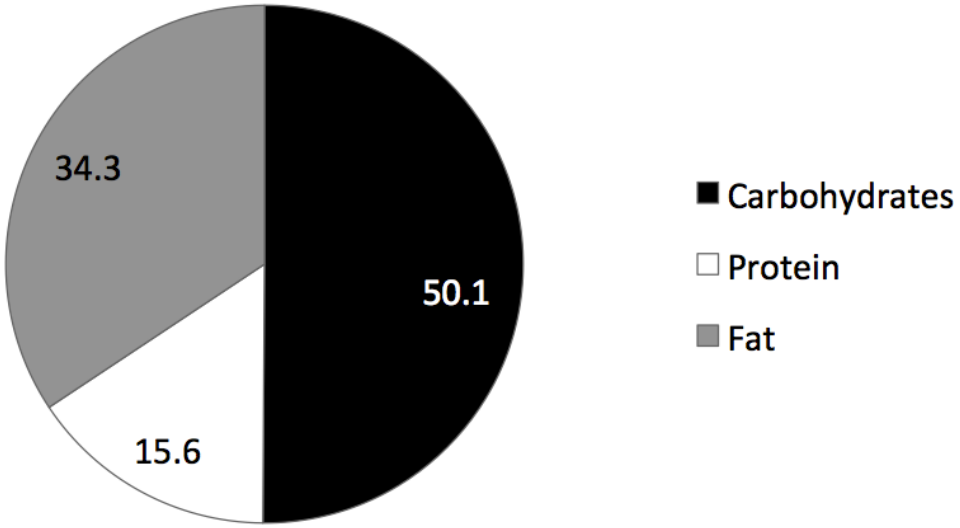


Figure 4-4 Micronutrient intake in non-alcohol and alcohol exposed groups Data derived from Food Frequency Questionnaire (n=56). Values are mean ± SD

a)



b)

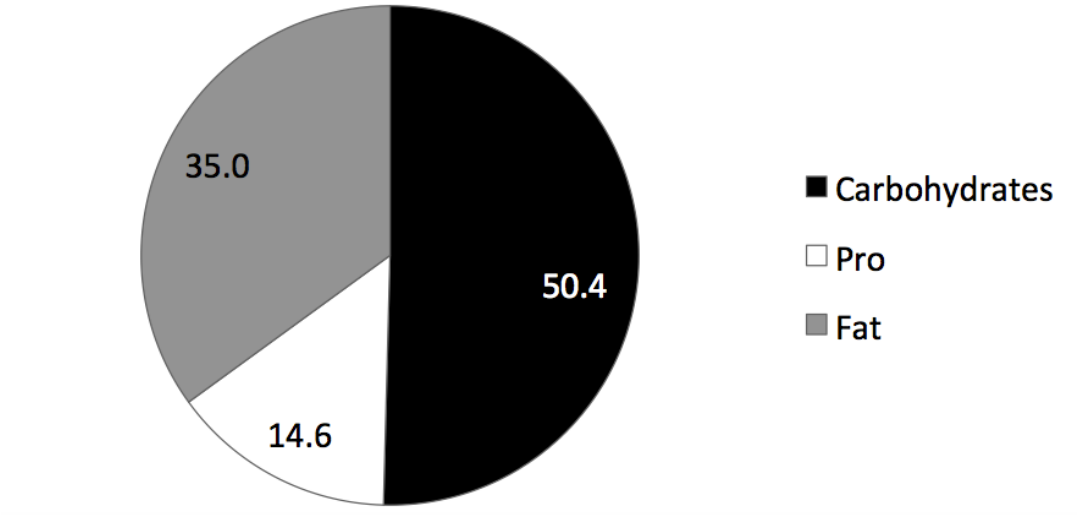


Figure 4-5 Fat, protein, and carbohydrates as a percentage of total energy intake in a) non-alcohol and b) alcohol exposed participants. Data derived from 24 hour food recall (n=56).

Table 4-7 Macro and micronutrient intake in non-alcohol and alcohol exposed groups

Nutrient	DRI	Non-Alcohol		Alcohol Exposed		P-value
Energy and Macronutrients						
Energy (Kcal)	--	2036.7	(550.0)	2080.6	(858.2)	0.92
Carbohydrates (g)	135	255.2	(89.2)	250.7	(113.8)	0.90
Protein (g)	71	79.3	(23.8)	72.6	(31.3)	0.91
Fat (g)	--	77.7	(44.1)	77.3	(42.3)	0.74
Sugar (g)		98.9	(78.5)	105.3	(79.2)	0.66
Micronutrients						
Zinc (mg)	11	39.7	(104.1)	53.1	(100.8)	0.73
Vitamin A (μg)	770	1504.3	(793.1)	1503.6	(1017.9)	0.90
Folate (μg)	600	481.6	(220.6)	458.5	(363.9)	0.92
Choline (mg)	450	457.2	(246.9)	446.5	(239.5)	0.78
DHA (mg)	200	98.0	(92.8)	89.0	(72.0)	0.81

Values are median (IQR). The significant differences between groups were tested by t-test when normally distributed and non-parametric tests when not normally distributed. No significant effect of alcohol was identified.

Correlations of nutrient intake to alcohol exposure

Alcohol exposure was tested to see if a correlation existed between it and nutrient intake (Table 4-8). Using Spearman's correlations, no significant results were found. Correlations were also tested with other demographic factors of age, parity, smoking, and drug use (Table 4-8). Smoking showed a significant positive correlation to alcohol exposure during pregnancy at p=0.02.

Table 4-8 Correlations with prenatal alcohol exposure

Nutrient	R value	P-value
Micronutrients		
Zinc	-0.076	0.73
Vitamin A	0.034	0.82
Folate	0.009	0.92
Choline	0.013	0.91
DHA	0.044	0.81
Demographics		
Age	0.212	0.10
Parity	0.080	0.68
Smoking	0.263	0.02
Drugs	-0.142	0.23

The correlation was tested by Spearman's correlation. No correlation exists in alcohol with micronutrients and demographic factors.

Chapter V: DISCUSSION

This pilot study is the first to examine the intake of nutrients important to fetal brain development in women at risk of consuming alcohol during pregnancy. Based on Canada's Food Guide, the majority (69.6%) of participants met recommendations for meat and alternatives, while only 42.9% met recommended intakes of grain products and milk and alternatives. The least amount of participants met recommendations for vegetables and fruit (12.5%). In this study population, macronutrient intake for the cohort was within the AMDR, but on an individual level, intakes of fat and sugar exceeded the AMDR in 41.1% and 28.6% of participants respectively. The majority of participants had dietary intakes that met the DRI for zinc and vitamin A, while only 44.6% met recommendations for folate, 48.2% for choline, and 16.1% for DHA. Alcohol exposure during pregnancy did not affect intake, as there was no statistically significant difference between participants who had consumed alcohol during their pregnancy and those who had not. No correlation was found between alcohol exposure and the nutrient intake of zinc, vitamin A, folate, choline and DHA. However, a positive correlation was found between alcohol exposure and smoking during pregnancy.

Study population

Demographic data shows that the majority (71.4%) of participants identify as Indigenous. This represents a much higher proportion than the Point Douglas or Downtown community areas (29% and 17.4% respectively) and is likely due to the type of prenatal programs where recruitment occurred, which target women who are more "at risk, or in need" (PFSS, 2016). Indigenous women are more likely to fall into this category due to a history of colonization and socioeconomic marginalization, which can cause them to not access prenatal care (Di Lallo, 2014). Because SES was not measured in this study, there is no direct income comparison to the

Point Douglas and Downtown area. However, almost 70% of participants were on social assistance. The maximum total income from Employment and Income Assistance is $28,980 per year (Provincial and Federal Benefits for General Assistance Recipients, based on household of 2 adults and 3 children), which is well below the average household income of $63,000 in Winnipeg (Government of Manitoba, 2016).

Alcohol and Substance Use

This study found that 46.4% of participants reported alcohol exposure at least once during pregnancy, which is higher than the rates typically seen in the community. Other reports have found prenatal alcohol consumption of 23.8% in Point Douglas, and 18.2% in Downtown (Heaman, et al., 2012). The high level of prenatal alcohol exposure in this study may be due to the specific populations where recruitment was done, including a program that supports women with substance use issues. Another potential reason for this difference is due to the community engagement, which allowed for women to provide information in a safe, non-judgmental environment. Knowing prenatal alcohol exposure data is important due to the potential harm to the fetus including hypoxia and the formation of reactive oxygen species. This may also contribute to outcomes such as FASD. Variables such as maternal drinking patterns, genetic susceptibility, and maternal alcohol metabolism play a role in severity of these effects (Maier & West, 2001).

Smoking rates during pregnancy were 58.9%, similar to rates found in Aboriginal women in Manitoba (61.2%) (Heaman & Chalmers, 2005). Data from the Downtown and Point Douglas community areas have shown lower rates of prenatal smoking, at 28.2% and 39.8% respectively (Heaman, et al., 2012). Reported prenatal drug use of 26.8% was also higher than that of Manitoba (3.6%) as well as the Point Douglas (13.5%) and Downtown (8.9%) communities

(Heaman, et al., 2012). It has been shown that alcohol, smoking, and drug use during pregnancy is more likely in women who are younger, are lone parents, who live in lower income areas, have not completed Grade 12, or do not have adequate prenatal care (Healthy Child Manitoba, 2012). The higher rates seen in this study may be due to the population having many of these demographic characteristics.

Access to food

The United Nations defines food security as when "all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (United Nations Food and Agriculture Organization, 1996). Although this study did not directly measure food security, we found indirect indicators that there is difficulty with access and affordability of food. Although most participants agreed that they had access to money to spend on food (91.1.%), fewer agreed that they had enough food to eat (82.1%), and 42.9% of participants agreed that they often ran out of food. Most participants agreed that food was able to be accessed (71.4%), however, less participants said they could afford the foods they want to eat (62.5%) and or that they were available from a store near their home (67.9%).

In 2011, 12.4% of Manitoban households experienced food insecurity (Tarasuk et al., 2013). One of the most important factors in food security is poverty (Malabar & Grant, 2010). Food insecurity in Canada affects 65% of households that rely on social assistance. Other factors that have been associated with increased food insecurity include being Aboriginal, renting rather than owning one's home, and being a female lone parent (Tarasuk et al., 2013). An assessment of the Downtown community showed that 62.5% of people used grocery money to pay for shelter, utilities, and medicine (Food Matters Manitoba, 2013). With almost 70% of this study's

participants reporting being on social assistance, and 71.4% identifying as Aboriginal, it is likely that a more in depth interview would find higher rates of food insecurity in this population.

Geographic and economic access to food has been identified as key issues in the Downtown area (Food Matters Manitoba, 2013). The Downtown and Point Douglas community areas have been identified as food deserts. Food deserts are "areas where vulnerable populations have poor geographic access to nutritious food" (Health Canada, 2013). Corner stores, with very little nutritious food, or highly priced nutritious options, are the most abundant and centrally located establishments at which to purchase food. Larger grocery stores sit on the periphery of the community area; however, few community members have access to transportation to reach these stores (Malabar & Grant, 2010). For community members using public transit, safety and the challenges of travelling with small children can be barriers to accessing full-service grocery stores (Food Matters Manitoba, 2013). This is especially important, as proximity to supermarkets has been associated with higher quality diets in pregnant women (Laraia et al., 2004).

Indigenous Peoples have also undergone a nutrition transition, which has replaced traditional diets, and the physical activities associated with these diets, with market-based foods that are often high in sugar and fat (Earle, 2011). Traditional foods have also been shown to play a role in food security in Indigenous peoples, where limited access to traditional foods creates reliance on less healthy options. Food insecurity has been impacted by colonization through forcing Indigenous peoples from their lands and away from traditional practices, creating a reliance on food assistance programs (Gurney et al., 2015). Beyond the nutritional value of traditional foods, the tie to culture and identity provide a holistic health benefit to their consumption (Earle, 2011). With the high proportion of Indigenous women in this study,

incorporating culture and traditional foods can be an important component in improving nutrient intake, overall health, and greater food security during pregnancy (Elliott et al., 2012).

Self-rated health during pregnancy and supplement use

This study asked participants to rate their health both before and during pregnancy. Self-rated health has been shown to be associated with objective health status including health-related factors, prevalence of diseases and laboratory parameters (Wu et al., 2013). It was found that 21.4% of women rated their health as "excellent" during pregnancy, which is higher than the 10.9% found in a US study of pregnant women with lower socioeconomic backgrounds (Christian et al., 2013).

Rates of prenatal vitamin intake of 89.3% is similar to other Canadian pregnant women (66.7-82.9%) (Grigoriadis et al., 2010; Roy et al., 2012). Few women in this study were taking single vitamins in addition to a prenatal supplement. Similar to a cohort of pregnant women in Quebec, folic acid and calcium are more commonly taken as single supplements. However, Greigoriadis et al. (2010) found that 11.4% of women were taking omega-3 fatty acids, whereas none the participants in this study were.

Knowledge of supplementation use by pregnant women alongside dietary intake data is important to determine if nutrient intake via supplements are helping meet, or even exceed requirements during pregnancy. Single nutrient data is also necessary, as some prenatal supplements do not contain DHA or choline, which we have shown to be lacking in the diet of our study population. Folic acid, on the other hand, is found in prenatal vitamins, and may help women reach recommended intakes when not achieving them from diet alone.

Food and nutrient intake

It is recommended by Canada's Food Guide that women ages 19-50 have 7-8 servings of vegetables and fruits, 6-7 servings of grain products, 2 servings of milk and alternatives, and 2 servings of meat and alternatives, with pregnant women having an additional 2-3 servings from any of these groups (Health Canada, 2011). Compared to another study on pregnant women in Ontario, more women in this study were meeting recommended servings of grains (42.9% compared to 8.6%), but fewer were getting adequate amounts of vegetables and fruit (12.5% compared to 25%) (Fowler et al., 2012). This study also found lower intakes of vegetables and fruits compared to Canadian women ages 19-30 years, who had mean intakes of 5.01 ± 0.18 servings compared to the median (IQR) 3.0 (3.5) in this study (Jessri, Nishi & L'Abbé, 2015).

Fruits and vegetables are nutrient dense and are rich in folate, vitamin A and C, potassium, dietary fibre and phytochemicals (Liu, 2013). Therefore, low intakes of these nutrients, especially in pregnant women at risk for drinking, can contribute to nutrient intakes that do not meet recommendations. Some of the barriers affecting access to vegetables and fruit in low-income populations include the cost, transportation, lack of quality (especially at local businesses), lack of variety, the changing food environment, and the shift from cooking to convenience food (Haynes-Maslow et al., 2013).

Macronutrient intake in participants is also similar to that of pregnant women in Winnipeg. Hui et al. (2014), found that in women < 20 weeks pregnant in Winnipeg and with BMI's > 25, consumed similar total calories (2089 ± 517) and carbohydrates (280.1) prior to a lifestyle intervention targeting gestational weight. However, in our study, pregnant women had lower intakes of protein (91.2 ± 26.1) and higher intakes of fat (68.0 ± 23.3). This may be accounted for in the difference in demographics, as Hui et al. (2014)'s cohort had a family

annual income of $54,404 (± 33, 689) and only had 4% (n=1) identify as First Nations. One study found that a higher intake of energy from added sugar may displace important nutrients including protein, iron, vitamin A, C, B-6, B-12 and potassium (Bhargava & Amialchuk, 2007).

Results on micronutrient intake show that the majority of participants are meeting DRIs for zinc and vitamin A, however, only 48.2% are meeting the DRI for choline, 44.6% for folate and only 16.1% for DHA. While no other study has looked at intake of all of these nutrients, some have looked at them individually in pregnant women.

Zinc intake in this study was 48.4 (102.8) mg, much higher than the 11 mg recommended by the DRIs. Studies from Canada and other countries have also shown similar intakes of zinc (Behboudi-Gandevani et al., 2013; Blumfield et al., 2013). Zinc is important for fetal development, as it is needed for transcription, translation, and cellular differentiation (Coyle et al., 2013). Most importantly, zinc supplementation in animal models given alcohol has been shown to be protective against physical malformations. Dietary sources of zinc include animal products such as seafood, eggs, and red meat (Massaro et al., 2006). Zinc intake in this population is likely high due to the adequate servings of meat and alternatives consumed by most study participants. Whether this level of zinc intake is protective in a humans fetus against malformations caused by alcohol consumption, is still unknown.

Vitamin A intake in this study was found to be above the EAR at 1503.6 (843.1) µg. This is similar to a meta-analysis by Blumfield et al. (2013) where pregnant women in the US and Canada had 3.5 times greater intake of vitamin A than the EAR. Preformed vitamin A (retinol and retinyl ester) are highest in liver and fish oils, but can also be found in milk and eggs. Pro-vitamin A (carotenoids) is found in leafy green vegetables, fruits, orange and yellow vegetables (Office of Dietary Supplements, 2016a). The high intake of vitamin A in some participants may be due to milk and egg consumption.

During pregnancy, a mother's vitamin A stores are usually sufficient for a developing fetus (Manhan et al., 2012, p358). In cases of heavy drinking however, alcohol can be preferentially oxidized over retinol, leading to potential retinoic acid deficiencies in various tissues (Duester, 1991). Among this cohort of women with alcohol exposure, only n=1 woman reported recent consumption of a large quantity of alcohol, which is why vitamin A intake was likely not affected.

Choline intake at 448.6 (242.6) mg/day was somewhat higher than seen in other pregnancy cohorts from Canada (383 ± 98.6 mg/day) (Wu et al., 2012) and the US (328 ± 63 mg) (Boeke, et al., 2013). However, in this study, almost 50% of participants were not meeting the AI. The demand for choline is greater during pregnancy due to its role as a methyl donor, and as part of acetylcholine (involved in neurotransmission and cell signaling) (Ballard et al., 2012). Top food sources of choline include eggs, liver, beef, and chicken (Zeisel, 2011). Although this study found that, based on a 24-hour food recall, intakes of meat and alternatives met recommendations, food choices within this group may have impacted choline intake. For instance, the lower choline content of peanut butter would result in poorer intakes than eating an egg, while choices such as poultry would not contribute as much dietary choline as organ meats.

Choline supplements have been shown in animal models to mitigate the effects of alcohol (Thomas et al., 2009; 2010; Otero, 2012). With lower dietary intake, and the lack of choline as part of prenatal supplements, this nutrient may be an excellent choice for a potential harm reduction method through a nutrition intervention.

Folate during pregnancy is important for the rapid growth and cell proliferation in the fetus (Hutson et al. 2012) and prevents neural tube defects. This is due to its role in DNA methylation, which controls gene expression (Ballard et al., 2012). Median (IQR) intake of folate in this study was 471.2 (285.8) μg and only 44.6% met the EAR recommendations. Others

studies have found that similar proportions of pregnant women do not meet the EAR for folate (Masih et al., 2015; Sherwood et al., 2006), While another study found slightly higher intakes (972 ± 392 μg and 1,268 ± 381 μg) during the first and second trimester respectively (Boeke et al., 2013). However, the majority of women recruited in that study were Caucasian, with post-secondary education, and household incomes >$70,000 per year.

Low folate intake in this study is likely due to the low intake of vegetables and fruits, as sources include dark green leafy vegetables, fruits, nuts, beans, and grains (Office of Dietary Supplements, 2016b). As many pregnant women do not meet the DRI for folate through diet alone, a folic acid supplement is recommended during pregnancy. Typical doses of folic acid in prenatal supplements are 1000 μg, which may actually place women's intake above the UL for folate (1000 μg) (Masih et al., 2015).

As seen in other studies, maternal intake of DHA is low at 97.0 (81.7) mg. A study of dietary intake of DHA in pregnant Canadian women, found mean intakes of 82 ± 33 mg/day and only 10% of participants meeting recommendations (Denomme et al., 2005). In another Canadian study on pregnant women with higher education and income levels, it was found that DHA intake from food was 237 ± 164 mg/day with 46.3% of participants meeting recommendations (Arsenault Bishop, 2015). The best sources for DHA are fatty fish including salmon, herring, sardines, trout and tuna (Singh, 2005). Low intake of these products is likely the cause for lower intakes of DHA.

DHA is involved in neuronal membrane structures, synaptogenesis, and myelination (Martinez, 1992; Georgieff, 2007) and can minimize apoptosis in the brain (Innis, 2007) making it essential for the growing fetus. The incredible accumulation of DHA in the fetal brain during the 3rd trimester, or 14 grams per week also makes it important during pregnancy (Clandinin et al., 1980). As well, DHA has been shown to mitigate the effects of alcohol in animal models. As this study and others have found that DHA intake from food sources is low, supplementation

may be an important way for women to meet recommended intakes. In a recent APrON study in Alberta, pregnant and lactating women taking a DHA supplement during pregnancy were more likely to meet recommended intakes (Jia et al., 2015). Although some prenatal supplements include DHA, many of the women in this study receive their prenatal vitamins from the prenatal programs that they attend, making them reliant on the type distributed. Low incomes may also prevent women from purchasing an additional DHA supplement.

Overall, it is important to understand the intake of these nutrients due to their role in mitigating the effects of alcohol during pregnancy. It has been shown in the literature that these nutrients important to fetal brain development can potentially reduce the risk of FASD (Bekdash et al., 2013; Patten et al., 2012; Summers et al., 2009; Young et al., 2014). With the high rates of alcohol exposure reported in this cohort (46.4%), low intakes of nutrients such as choline, folate and DHA may provide an opportunity for a nutrition intervention. This may be in the form of supplements or whole foods, as the intakes of food groups such as vegetables and fruits, and grain products, and choices of meat and alternatives may be contributing to these low nutrient intakes.

Comparison of non-alcohol expsosed and alcohol exposed participants

No other known studies have looked at the difference in nutrient intake between pregnant mothers without alcohol exposure and those with alcohol exposure. This study found no significant difference in macro or micro nutrient intakes between non-alcohol exposed and alcohol exposed participants. This may be due to the timing and quantity of alcohol consumed by participants. Primary malnutrition through the decreased intake of macro and micronutrients requires heavy alcohol intake (Lieber, 2003). Heavy alcohol intake can be defined as 8 or more drink per week for women (Center for Disease Ctonrol and Prevention, 2014) or >30% of daily calories from alcohol (Lieber, 2003). With only 1 participant reporting recent alcohol intake as

heavy drinking, this sample will likely not have enough power to show significance. In this population as well, issues of food security and food knowledge and skills may affect nutrient intake.

Correlations to Alcohol Exposure

This study found no correlations between alcohol exposure during pregnancy and nutrient intake. This is likely due to the small sample size, and few women who would be categorized as chronic drinkers. There was a positive correlation however, between alcohol exposure and smoking during pregnancy. This is consistent with the literature (Lange et al., 2015; Meschke, et al., 2013). Although age was not significantly correlated with prenatal alcohol exposure ($p = 0.10$), a larger sample size may show significant results, as greater maternal age has been shown to be a significant risk factor (Meschke et al., 2013).

Summary

This is the first pilot study to show that pregnant women who are at risk of consuming alcohol are not getting enough nutrients important for fetal brain development, including folate, choline, and DHA. Although there was no significant difference in women who had consumed alcohol, further studies with women who are heavy drinkers may provide more definitive results. This cohort is also different from Canadian pregnant women in that fewer are meeting recommended intakes of vegetables and fruits, and have higher intakes of fat. Tied to this may be geographic and economic access to food, which affects the many participants living on social assistance, and those trying to purchase food in food deserts. Nutrition interventions through the supplementation of nutrients such as folate, DHA, and choline may be important for pregnant women at risk for consuming alcohol during pregnancy.

Conclusion

The intake of nutrients important to fetal brain development during pregnancy is valuable information for creating strategies to enhance the health of mothers consuming alcohol during pregnancy, and in turn their infants. By determining low intakes of nutrients, such as choline and DHA, opportunities for intervention can be identified. Nutrition interventions may have the potential to mitigate the effects of alcohol and reduce the risk of FASD, through an overall improvement of the mother's nutritional status and therefore health, or by providing nutrients that are protective for the fetal brain. For those in health-care, including physicians and dietitians, this information may help inform best practices and policies for pregnant women at risk for consuming alcohol. The growing research in this area can impacts the individual, their families, and the community at large. Through investment in preventative research, Canada may help reduce the social and economic impact of FASD.

Strengths and Limitations

This study's strength lies in its community engagement. Trust based relationships are needed when doing research with populations that are marginalized. This is especially true when collecting sensitive information on alcohol, smoking, and drug use during pregnancy due to the stigma that is often attached. The research team worked hard to build relationships within the community and with participants, and to practice using kindness and a non-judgmental, trauma informed approach in order to minimize the impact. The relationship building also allowed the community to be involved in the study's development and meet their particular needs. The value in this pilot study was also to be able to test the research tool, and determine the logistics for delivering this project on a larger scale.

Various limitations are present in this pilot study. One limitation was sample size. Although appropriate for a pilot study, the sample size limited the ability to further divide the cohort into sub-groups for analysis. For instance, further analysis of differences in intake related

to frequency and amount of alcohol exposure were not possible, as only 1 participant reported regular chronic drinking. Future studies may need a much larger sample size, or to recruit based on desired categories, such as level of alcohol intake.

Another limitation is the reliance on self-reported data for food intake. The FFQ and 24-hour food recall rely on memory and accuracy in estimating portion size, and are subject to social desirability bias. It has been shown that self-reported dietary data tends to underestimate true consumption (Subar et al., 2015). Although memory is required for these tools, training on how to deliver them can help improve accuracy. For the 24-hour food recall, the interviewer can use questions to help prompt memories including, "Did you have anything to drink with your meal?" Food models can also help participants recall the amount of food they ate.

The collection of alcohol data had limitations. The format of the questionnaire meant that not all participants were able to adequately describe their alcohol consumption within the options provided. For instance, when asked how much they usually drank in an occasion, mothers that had stopped drinking when they realized they were pregnant, would say zero. However, when asked about their drinking during the 1st trimester, women would describe episodes of binge drinking and how much alcohol they had during that time. The lack of specificity in this questionnaire makes it difficult to compare alcohol intakes within this study and with other populations.

Currently, face and content validity have been completed on the questionnaire. The questionnaire was based on validated surveys including the Canadian Community Health Survey (Giesbrecht, 2015). Key stakeholders including nutrition experts and the Women's Advisory Committee at the Mothering Project also reviewed the questionnaire extensively, including for readability. A pre-test was also completed with 10 pregnant First Nations women from the target community, which provided written and verbal feedback. These methods of validation were similar to those undertaken in another study (Czuber-Dochan et al., 2014; Giesbrecht, 2015). However, further measures should be taken to ensure overall validity and reliability.

Finally, alcohol is often used in conjunction with other substances during pregnancy, including smoking. This can make it difficult to separate the effects of alcohol and other substances on nutrient intake. For instance, smoking can decrease appetite and women who smoke during pregnancy have been found to have significantly lower intakes of micronutrients such as zinc, retinol and carotenoids, and folate (Haste et al., 1990). Marijuana users have been shown to have higher intakes of energy, lower intakes of fruits, and lower serum carotenoid levels (Smit & Crespo, 2001). Use of other drugs including cocaine and crystal meth can also have impacts.

Recommendations for Future Research

It is first recommended that obtaining a larger sample size would provide more robust data and allow for greater sub analysis, especially in participants with alcohol exposure during pregnancy. It would also be important to follow mothers throughout their pregnancy, from the 1st trimester, through till the birth of the baby. Determining changes in intake is valuable, as some nutrients are especially important during certain trimesters (i.e. folate during the first trimester, DHA during the 3rd trimester). This would also allow for more data connecting infant outcomes to nutritional status.

To gain a better understanding of the nutritional status of mothers and levels of nutrient sufficiency and deficiency, it is recommended that future studies collect blood and urine samples. While it is important to know what nutrients pregnant mothers are consuming, it is also essential to see how intake is affecting nutrient status in the body. Biological data can be difficult to collect in populations where participants may be more vulnerable and transient, and where experiences with research and sample collection may have negative associations. Building strong relationships within communities and with participants, and showing the value of this data is key to collecting it.

With adequate data on the nutrient intakes of pregnant women at risk of consuming alcohol, future studies may allow for a nutrition intervention. This may be done in the form of a supplement or specific foods high in nutrients such as choline or DHA provided to pregnant women consuming alcohol, with a control group of those not consuming alcohol. This type of data could help set best practice guidelines for health professionals working with pregnant women. There is also value in understanding this cohort's lived experience. These women who are consuming alcohol may experience food insecurity, poverty, racism and other substance use. A qualitative approach may be used in the future to better understand these connections from the women's perspective and allow them to express it in their own voice. This may also help understand the intersection between colonization, gender inequality, poverty and FASD.

References

Abel, E. L. (1995). An update on incidence of FAS: FAS is not an equal opportunity birth defect. *Neurotoxicology and Teratology, 17*(4), 437-443.

Abel, E. L., & Hannigan, J. H. (1995). Maternal risk factors in fetal alcohol syndrome: Provocative and permissive influences. *Neurotoxicology and Teratology, 17*(4), 445-462.

Abu-Saad, K., & Fraser, D. (2010). Maternal nutrition and birth outcomes. *Epidemiologic Reviews, 32*(1), 5-25.

Ahmed, S. M. & Palermo, A. G. (2010). Community engagement in research: Frameworks for education and peer review. *American Journal of Public Health, 100*(8), 1380-1387.

Arsenault Bishop, N. B. A. (2015). *Apport en ADH et en AEP chez un groupe de femmes enceintes de la région de Moncton*. (Unpublished masters dissertation). University of Moncton, Moncton.

Bailey, B. N., Delaney-Black, V., Covington, C. Y., Ager, J., Janisse, J., Hannigan, J. H., & Sokol, R. J. (2004). Prenatal exposure to binge drinking and cognitive and behavioral outcomes at age 7 years. *American Journal of Obstetrics and Gynecology, 191*(3), 1037-1043.

Bakdash, A., Burger, P., Goecke, T. W., Fasching, P. A., Reulbach, U., Bleich, S., . . . Kornhuber, J. (2010). Quantification of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in meconium from newborns for detection of alcohol abuse in a maternal health evaluation study. *Analytical and Bioanalytical Chemistry, 396*(7), 2469-2477.

Bakker, R., Pluimgraaff, L. E., Steegers, E. A. P., Raat, H., Tiemeier, H., Hofman, A., & Jaddoe, V. W. V. (2010). Associations of light and moderate maternal alcohol consumption with fetal growth characteristics in different periods of pregnancy: The generation R study. *International Journal of Epidemiology, 39*(3), 777-789.

Ballard, M. S., Sun, M., & Ko, J. (2012). Vitamin A, folate, and choline as a possible preventive intervention to fetal alcohol syndrome. *Medical Hypotheses,78*(4), 489-493.

Bangalore, L. (2007). In Chudler E. H. (Ed.), *Brain development*. New York, NY: Chelsea House.

Barr, H. M., & Streissguth, A. P. (2001). Identifying maternal self-reported alcohol use associated with fetal alcohol spectrum disorders. *Alcoholism: Clinical and Experimental Research, 25*(2), 283-287.

Bearer, C. F., Jacobson, J. L., Jacobson, S. W., Barr, D., Croxford, J., Molteno, C. D., . . . Cwik, A. S. (2003). Validation of a new biomarker of fetal exposure to alcohol. *Journal of Pediatrics, 143*(4), 463-469.

Bearer, C. F., Stoler, J. M., Cook, J. D., & Carpenter, S. J. (2004). Biomarkers of alcohol use in pregnancy. *Alcohol Research and Health, 28*(1), 38-43.

Bearer, C. F., Wellmann, K. A., Tang, N., He, M. & Monney, S. M. (2015). Choline ameliorates deficits in balance caused by acute neonatal ethanol exposure. *The Cerebellum, 14*(4), 413-420.

Beblo, S., Stark, K. D., Murthy, M., Janisse, J., Rockett, H., Whitty, J. E., . . . Salem Jr., N. (2005). Effects of alcohol intake during pregnancy on docosahexaenoic acid and arachidonic acid in umbilical cord vessels of black women. *Pediatrics, 115*(2), e194-e203.

Behboudi-Gandevani, S., Kafary, K., Moghaddam-Benaem, L., Lamyian, M., Goshtasebi, A., & Alian-Moghaddam, N. (2013). The relationship between maternal serum iron and zinc levels and their nutritional intakes in early pregnancy with gestation diabetes. *Biological Trace Elements Research, 154*(1), 7-13. DOI: 10.1007/s12011-013-9703-y

Berti, P. R., Soueida, R., & Kuhnlein, H. V. (2008). Dietary assessment of indigenous Canadian arctic women with a focus on pregnancy and lactation. *International Journal of Circumpolar Health, 67*(4), 349-362.

Bhargava, A. & Amialchuk, A. (2007). Added sugars displaced the use of vital nutrients in the national food stamp program survey. *Journal of Nutrition, 137*(2), 453-460.

Bingol, N., Schuster, C., Fuchs, M., Iosub, S., Turner, G., Stone, R. K., & Gromisch, D. S. (1987). The influence of socioeconomic factors on the occurrence of fetal alcohol syndrome. *Advances in Alcohol and Substance Abuse, 6*(4), 105-118.

Black, R. E. (2001). Micronutrients in pregnancy. *British Journal of Nutrition, 85*(SUPPL. 2), S193-S197.

Blumfield, M. L., Hure, A. J., MacDonald-Wicks, L., Smith, R., & Collins, C. E. (2013). A systematic review and meta-analysis of micronutrient intakes during pregnancy in developed countries. *Nutrition Reviews, 71*(2), 118-132. doi 10.1111/nure.12003

Boeke, C. E., Gillman, M. W., Hughes, M. D., Rifas-Shiman, S. L., Villamor, E. & Oken, E. (2013). Choline intake during pregnancy and child cognition at age 7 years. *American Journal of Epidemiology, 177*(12), 1338-1347.

Bonthius, D. J., Goodlett, C. R., & West, J. R. (1988). Blood alcohol concentration and severity of microencephaly in neonatal rats depend on the pattern of alcohol administration. *Alcohol, 5*(3), 209-214.

Bosco, C., & Diaz, E. (2012). Placental hypoxia and foetal development versus alcohol exposure in pregnancy. *Alcohol and Alcoholism, 47*(2), 109-117.

Bourassa, C., McKay-McNabb, K. & Hampton, M. (2004). Racism, sexism, an colonialism: The impact on the health of Aboriginal women in Canada. *Canadian Woman Studies, 24*(1), 23-30.

Brocardo, P. S., Gil-Mohapel, J., & Christie, B. R. (2011). The role of oxidative stress in fetal alcohol spectrum disorders. *Brain Research Reviews, 67*(1-2), 209-225.

Brough, L., Rees, G. A., Crawford, M. A., Morton, R. H., & Dorman, E. K. (2010). Effect of multiple-micronutrient supplementation on maternal nutrient status, infant birth weight and gestational age at birth in a low-income, multi-ethnic population. *British Journal of Nutrition, 104*(3), 437-445.

Burd, L., Blair, J., & Dropps, K. (2012). Prenatal alcohol exposure, blood alcohol concentrations and alcohol elimination rates for the mother, fetus and newborn. *Journal of Perinatology, 32*(9), 652-659.

Burdge, G. C. & Wootton, S. A. (2002). Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *British Journal of Nutrition, 88*(4), 411-420.

Burdge, G. C., Wright, S. M., Warner, J. O., & Postle, A. D. (1997). Fetal brain and liver phospholipid fatty acid composition in a guinea pig model of fetal alcohol syndrome: Effect of maternal supplementation with tuna oil. *Journal of Nutritional Biochemistry, 8*(8), 438-444.

Cameron, C. M., Davey, T. M., Kendall, E., Wilson, A., & McClure, R. J. (2013). Changes in alcohol consumption in pregnant Australian women between 2007 and 2011. *Medical Journal of Australia, 199*(5), 355-357.

Cano, M. J., Ayala, A., Murillo, M. L., & Carreras, O. (2001). Protective effect of folic acid against oxidative stress produced in 21-day postpartum rats by maternal-ethanol chronic consumption during pregnancy and lactation period. *Free Radical Research, 34*(1), 1-8.

Cardoso, M. A. & Stocco, P. R. (2000). Development of a quantitative questionnaire of food intake in Japanese immigrants and their descendants residents in Sao Paulo, Brazil. *Cadernos de Saúde Pública, 16*(1), 107-114.

Carey, L. C., Coyle, P., Philcox, J. C., & Rofe, A. M. (2003). Zinc supplementation at the time of ethanol exposure ameliorates teratogenicity in mice. *Alcoholism: Clinical and Experimental Research, 27*(1), 107-110.

Center for Disease Control and Prevention (2014). Alcohol and public health. Retrieved from http://www.cdc.gov/alcohol/faqs.htm#excessivealcohol.

Chen, S., Dehart, D. B., & Sulik, K. K. (2004). Protection from ethanol-induced limb malformations by the superoxide dismutase/catalase mimetic, EUK-134. *FASEB Journal, 18*(11), 1234-1236.

Chesnutt, A. N. (2004). Physiology of normal pregnancy. *Critical Care Clinics, 20*(4), 609-615.

Christian, L. M., Iams, J., Porter, K. & Leblebicioglu, B. (2014). Self-rated health among pregnant women: Associations with objective health indicators, psychological functioning, and serum inflammatory markers. *Annals of Behavioural Medicine, 46*(3). doi: 10.1007/s1260-013-9521-7

Chudley, A. E., Conry, J., Cook, J. L., Loock, C., Rosales, T., & LeBlanc, N. (2005). Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. *Canadian Medical Association Journal,172*(5 SUPPL.), S1-S21.

Church, M. W., Abel, E. L., Dintcheff, B. A., & Matyjasik, C. (1990). Maternal age and blood alcohol concentration in the pregnant long-evans rat. *Journal of Pharmacology and Experimental Therapeutics, 253*(1), 192-199.

City of Winnipeg and Statistics Canada. 2006. 2006 Census Data: Point Douglas Community Area, Winnipeg.

Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R., & Chance, G. W. (1980). Intrauterine fatty acid accretion rates in human brain: Implications for fatty acid requirements. *Early Human Development, 4*(2), 121-129.

Clarke, M. E., & Gibbard, W. B. (2003). Overview of fetal alcohol spectrum disorders for mental health professionals. *The Canadian Child and Adolescent Psychiatry Review, 12*(3), 57-63.

Clarren, S. K., Randels, S. P., Sanderson, M., & Fineman, R. M. (2001). Screening for fetal alcohol syndrome in primary schools: A feasibility study. *Teratology, 63*(1), 3-10.

Cogswell, M. E., Weisberg, P., & Spong, C. (2003). Cigarette smoking, alcohol use and adverse pregnancy outcomes: Implications for micronutrient supplementation. *Journal of Nutrition, 133*(5), 1722S-1731S.

Cohen-Kerem, R., & Koren, G. (2003). Antioxidants and fetal protection against ethanol teratogenicity: I. review of the experimental data and implications to humans. *Neurotoxicology and Teratology, 25*(1), 1-9.

Cook, J. L., Green, C. R., Lilley, C. M., Anderson, S. M., Baldwin, M. R., Chudley, A. E., ….Rosales, T. (2015). Fetal alcohol spectrum disorder: A guideline for diagnosis across the lifespan. *CMAJ,* doi:10.1503/cmaj.141593

Coyle, P., Summers-Pearce, B., Cowley, C. J., & Rofe, A. M. (2013). Dietary zinc supplementation and prenatal ethanol exposure. In R. R. Watson, V. R. Preedy & S. Zibadi (Eds.), *Alcohol, nutrition, and health consequences*. New York: Springer.

Czuber-Dochan, W. C., Norton, P., Bassett, S., Berliner, F., Bredin, M., Darvell, A…Terry, H. (2014). Development and psychometric testing of inflammatory bowel disease fatigue (IBD-F) patient self-assessment scale. *Journal of Crohns and Colitis*, 8, 1398-1406.

Dell, C. A., & Roberts, G. (2005). *Alcohol use and pregnancy: An important Canadian public health and social issue.* Public Health Agency of Canada. Retrieved from http://www.addictionresearchchair.ca/wp-content/uploads/Alcohol-Use-and-Pregnancy-An-Important-Canadian-Health-and-Social-Issue.pdf

Deltour, L., Ang, H. L., & Duester, G. (1996). Ethanol inhibition of retinoic acid synthesis as a potential mechanism for fetal alcohol syndrome. *FASEB Journal, 10*(9), 1050-1057.

Denomme, J. Stark, K. D. & Holub, B. J. (2005). Directly quantitated dietary (n-3) fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations. *Journal of Nutrition, 135*(2), 206-211.

Di Lallo, S. (2014). Prenatal care through the eyes of Canadian Aboriginal women. *Nursing for Women's Health, 18*(1), 38-46.

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Human Development, 3*(1), 79-83.

Duester, G. (1991). A hypothetical mechanism for fetal alcohol syndrome involving ethanol inhibition of retinoic acid synthesis at the alcohol dehydrogenase step. *Alcoholism: Clinical and Experimental Research, 15*(3), 568-572.

Earle, L. (2011). *Traditional Aboriginal diets and health.* National Collaborating Centre for Aboriginal Health. Retrieved from http://www.nccah-ccnsa.ca/docs/social%20determinates/1828_NCCAH_mini_diets_health_final.pdf

Elliott, B., Jayatilaka, D., Brown, C., Varley, L. & Corbett, K. K. (2012). "We are not being heard": Aboriginal perspectives on traditional foods access and food security. *Journal of Environmental and Public Health*, 130945. doi: 1155/2012/130956

Ethen, M. K., Ramadhani, T. A., Scheuerie, A. E., Canfield, M. A., Wyszynski, D. F., Druschel, C. M., Romitti, P. A., & National Birth Defects Prevention Study. (2009). Alcohol consumption by women before and during pregnancy. *Maternal and Child Health Journal, 13*(2), 274-285.

Feldman, H. S., Jones, K. L., Lindsay, S., Slymen, D., Klonoff-Cohen, H., Kao, K., . . . Chambers, C. (2012). Prenatal alcohol exposure patterns and alcohol-related birth defects

and growth deficiencies: A prospective study. *Alcoholism: Clinical and Experimental Research, 36*(4), 670-676.

First Nations and Inuit Health Committee, Canadian Paediatric Society, & First Nations, Inuit and Métis Health Committee. (2002). Fetal alcohol syndrome. *Paediatric Child Health, 7*(3), 161-174.

Fowler, J. K., Evers, S. E. & Campbell, M. K. (2012). Inadequate dietary intakes among pregnant women. *Canadian Journal of Dietetic Practice Research, 73*(2): 72-77.

Food Matters Manitoba (2013). *Downtown Winnipeg Community Food Assessment*. The Winnipeg Foundation. Retrieved from http://www.foodmattersmanitoba.ca/wp-content/uploads/2014/06/CFA-Report_Downtown_Final.pdf

Forster, H., Fallaize, R., Gllagher, C., O'Donovan, C. B., Woolhead, C., Walsh, M. C., … Gibney, E. R. (2014). Online dietary intake estimation: The Food4Me food frequency questionnaire. *Journal of Medical Internet Research, 16*(6), e150.

Furuya, H., Aikawa, H., Yoshida, T., & Okazaki, I. (2000). The use of docosahexaenoic acid supplementation to ameliorate the hyperactivity of rat pups induced by in utero ethanol exposure. *Environmental Health and Preventive Medicine, 5*(3), 103-110.

Gareri, J. Lynn, H., Handley, M., Rao, C. & Koren, G. (2008). Prevalence of fetal ethanol exposure in a regional population-based sample by meconium analysis of fatty acid ethyl esters. *Therapeutic Drug Monitoring, 2, 239-245.*

Georgieff, M. K. (2007). Nutrition and the developing brain: Nutrient priorities and measurement. *American Journal of Clinical Nutrition, 85*(2), 614S-620S.

Giesbrecht, H. E. (2015). *Laying the groundwork for prenatal dietary assessment research among First Nations women: Implications for fetal alcohol spectrum disorder.* (Unpublished masters dissertation). University of Manitoba, Winnipeg.

Gladstone, J., Levy, M., Nulman, I., & Koren, G. (1997). Characteristics of pregnant women who engage in binge alcohol consumption. *Canadian Medical Association Journal, 156*(6), 789-794.

Goh, Y., Hutson, J. R., Lum, L., Roukema, H., Gareri, J., Lynn, H., Koren, G. (2010). Rates of fetal alcohol exposure among newborns in a high-risk obstetric unit. *Alcohol, 44*(7-8), 629-634.

Golan, H., & Huleihel, M. (2006). The effect of prenatal hypoxia on brain development: Short- and long-term consequences demonstrated in rodent models. *Developmental Science, 9*(4), 338-349.

Government of Manitoba. (2016). *Employment and income assistance for the general assistance category, 2015-2016.* Retrieved from http://www.gov.mb.ca/jec/eia/pubs/eia_general.pdf

Grigoriadis, S., Barrett, J., Pittini, R., Herer, E., Zaltz, A., Bazinet, R. P., . . . Levitt, A. (2010). Omega-3 supplements in pregnancy: Are we too late to identify the possible benefits? *Journal of Obstetrics and Gynaecology Canada, 32*(3), 209-216.

Gurney, R. M., Caniglia, B. S., Mix, T. L. & Baum, K. A. (2015). Native American food security and traditional foods: A review of the literature. *Sociology Compass, 9*(8), 681-693.

Gutierrez, C. M., Ribeiro, C. N. D. M., De Lima, G. A., Yanaguita, M. Y., & Peres, L. C. (2007). An experimental study on the effects of ethanol and folic acid deficiency, alone or in combination, on pregnant swiss mice. *Pathology, 39*(5), 495-503.

Habbick, B. F., Nanson, J. L., Snyder, R. E., Casey, R. E., & Schulman, A. L. (1996). Foetal alcohol syndrome in Saskatchewan: Unchanged incidence in a 20-year period. *Canadian Journal of Public Health, 87*(3), 204-207.

Hallett, B. Nemeth, M, Stevens, H., & Stewart, D. (2000). *Aboriginal people in Manitoba 2000*. Government of Canada. Retrieved from http://www.gov.mb.ca/ana/apm2000/pubs/apm2000.pdf

Han, A., Rotermann, M., Fuller-Thomson, E., & Ray, J. G. (2009). Pre-conceptional folic acid supplement use according to maternal country of birth. *Journal of Obstetrics and Gynaecology Canada, 31*(3), 222-226.

Haste, F. M., Brooke, O. G., Anderson, H. R., Bland, J. M., Shaw, A., Griffin, J. & Peacock, J. L. (1990). Nutrient intakes during pregnancy: Observations on the influence of smoking and social class. *American Journal of Clinical Nutrition, 51*(1), 29-36.

Haynes-Maslow, L., Parsons, S. E., Wheeler, S. B. & Leone, L. A. (2013). A qualitative study of perceived barriers to fruit and vegetable consumption among low-income populations, North Carolina, 2011. *Preventing Chronic Disease. 10,* E34. doi: 10.5888/pcd10.120206

Health Canada (2007a). Background on the food guide: The evidence base. Retrieved from http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/context/evid-fond-eng.php.

Health Canada (2007b). Food guide basics: How much food you need every day. Retrieved from http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/quantit-eng.php.

Health Canada. (2007c). Canadian nutrient file 2007b version. Health Canada; Ottawa, Canada: 2007.

Health Canada (2011a). Advice for different ages and stages: Pregnancy and breastfeeding. Retrieved from http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/choose-choix/advice-conseil/women-femmes-eng.php.

Health Canada. (2012).Healthy pregnancy. Retrieved from http://www.hc-sc.gc.ca.proxy1.lib.umanitoba.ca/hl-vs/preg-gros/index-eng.php

Health Canada. (2013). Measuring the food environment in Canada. Government of Canada. Retrieved from http://publications.gc.ca/collections/collection_2013/sc-hc/H164-155-2012-eng.pdf

Healthy Child Manitoba. (2012). *2012 report on Manitoba's Children and Youth.* Government of Manitoba. Retrieved from https://www.gov.mb.ca/healthychild/publications/hcm_2012report.pdf

Healthy Child Manitoba. (2013). FASD. Retrieved from http://www.gov.mb.ca.proxy1.lib.umanitoba.ca/healthy child/fasd/

Heaman, M. I. & Chalmers, K. (2005). Prevalence and correlates of smoking during pregnancy: a comparison of aboriginal and non-aboriginal women in Manitoba. *Birth, 32*(4), 299-305.

Heaman, M., Kingston, D., Delewa, M. E., Brownell, M., Derksen, S. Bogdanovic, B., McGowan, K., & Bailly, A. (2012). *Perinatal services and outcomes in Manitoba*. Manitoba Centre for Health Policy. Retrieved from http://mchp-appserv.cpe.umanitoba.ca/reference/perinatal_report_WEB.pdf

Hewitt, A. J., Knuff, A. L., Jefkins, M. J., Collier, C. P., Reynolds, J. N., & Brien, J. F. (2011). Chronic ethanol exposure and folic acid supplementation: Fetal growth and folate status in the maternal and fetal guinea pig. *Reproductive Toxicology, 31*(4), 500-506.

Horton, D. K., Adetona, O., Aguilar-Villalobos, M., Cassidy, B. E., Pfeiffer, C. M., Schleicher, R. L., . . . Naeher, L. P. (2013). Changes in the concentrations of biochemical indicators of diet and nutritional status of pregnant women across pregnancy trimesters in Trujillo, Peru, 2004-2005. *Nutrition Journal*,*12*(1).

Hui, A. L., Back, L, Ludwig, S., Gardiner, P., Sevenhuysen, G., Dean, H. J… Shen, G. X. (2014). Effects of lifestyle intervention on dietary intake, physical activity level, and gestational weight gain in pregnant women with different pre-pregnancy body mass index in a randomized control trail. *BMC Pregnancy and Childbirth, 14*(331). doi:10.1186/1471-2393-14-331

Hutson, J. R., Stade, B., Lehotay, D. C., Collier, C. P., & Kapur, B. M. (2012). Folic acid transport to the human fetus is decreased in pregnancies with chronic alcohol exposure. *PLoS One, 7*(5) doi:10.1371/journal.pone.0038057

Idänpään -Heikkilä, J., Jouppila, P., Åkerblom, H. K., Isoaho, R., Kauppila, E., & Koivisto, M. (1972). Elimination and metabolic effects of ethanol in mother, fetus, and newborn infant. *American Journal of Obstetrics and Gynecology*, *112*(3), 387-393.

Imdad, A., & Bhutta, Z. A. (2012). Maternal nutrition and birth outcomes: Effect of balanced protein-energy supplementation. *Paediatric and Perinatal Epidemiology, 26*(SUPPL. 1), 178-190.

Innis, S. M. (2007). Dietary (n-3) fatty acids and brain development. *Journal of Nutrition, 137*(4), 855-859.

Insel, P., Ross, D., McMahon, K., & Bernstein, M. (2013). Life cycle: Maternal and infant nutrition. *Discovering nutrition* (4th ed., pp. 499-538). Burlington, M.A.: Jones and Bartlett Learning.

Institute of Medicine. (1997). *Food and nutrition board; dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride.* Washington, DC: National Academic Press.

Institute of Medicine. (2001). *Food and nutrition board: Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, moybdenum, mickel, silcon, vanadium, and zinc*. Washington, DC: National Academic Press.

Institute of Medicine. (2002). *Food and nutrition board; dietary reference intakes for energy and the macronutrients, carbohydrate, fibre, fat, and fatty acids*. Washington, DC.: National Academic Press.

Institute of Medicine (2014). Dietary reference intakes (DRIs). Retrieved from http://iom.edu/Activities/Nutrition/SummaryDRIs/~/media/Files/Activity%20Files/Nutrition/DRIs/New%20Material/5DRI%20Values%20SummaryTables%2014.pdf

Jacobson, J. L., Jacobson, S. W., & Sokol, R. J. (1996). Increased vulnerability to alcohol-related birth defects in the offspring of mothers over 30. *Alcoholism: Clinical and Experimental Research, 20*(2), 359-363.

Jessri, M., Nishi, S. K. & L'Abbé, M. R. (2015). Assessing the nutritional quality of diets of Canadian adults using the 2014 health Canada surveillance tool tier system. *Nutrients, 7*(12), 10447-10468. doi:10.3390/nu7125543

Jia, X., Pakseresht, M., Wattar, N, Wildgrube, J., Sontag, S. Andrews, M… Field, C. J. (2015). Women who take n-3 long-chain polyunsaturated fatty acid supplements during pregnancy and lactation meet the recommended intake. *Applied Physiology, Nutrition and Metabolism, 40*(5), 474-481.

Jones, K. L. (2011). The effects of alcohol on fetal development. *Birth Defects Research Part C: Embryo Today: Reviews, 93*(1), 3-11.

Jones, K. L., Smith, D. W., Ulleland, C. N., & Streissguth, P. (1973). Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet, 1*(7815), 1267-1271.

Joya, X., Friguls, B., Ortigosa, S., Papaseit, E., Martínez, S. E., Manich, A., . . . Pichini, S. (2012). Determination of maternal-fetal biomarkers of prenatal exposure to ethanol: A review. *Journal of Pharmaceutical and Biomedical Analysis, 69*, 209-222.

Keen, C. L., Uriu-Adams, J. Y., Skalny, A., Grabeklis, A., Grabeklis, S., Green, K., . . . Chambers, C. D. (2010). The plausibility of maternal nutritional status being a contributing factor to the risk for fetal alcohol spectrum disorders: The potential influence of zinc status as an example. *Biofactors, 36*(2), 125-135.

Keppen, L. D., Pysher, T., & Rennert, O. M. (1985). Zinc deficiency acts as a co-teratogen with alcohol in fetal alcohol syndrome. *Pediatric Research, 19*(9), 944-947.

King, J. C. (2000). Physiology of pregnancy and nutrient metabolism. *The American Journal of Clinical Nutrition, 75*(5), 1218s-1225s.

Klinic. (2013). *Trauma-informed.* The Trauma Toolkit, 2nd ed. Retrieved from http://trauma-informed.ca/wp-content/uploads/2013/10/Trauma-informed_Toolkit.pdf

Kotch, L. E., Chen, S., & Sulik, K. K. (1995). Ethanol-induced teratogenesis: Free radical damage as a possible mechanism. *Teratology, 52*(3), 128-136.

Kot-Leibovich, H., & Fainsod, A. (2009). Ethanol induces embryonic malformations by competing for retinaldehyde dehydrogenase activity during vertebrate gastrulation. *Disease Models and Mechanisms, 2*(5-6), 295-305.

Laraia, B. A., Siega-Riz, A. M., Kaufman, J. S. & Jones, S. J. (2004). Proximity of supermarkets is positively associated with diet quality index for pregnancy. *Preventative Medicine, 39*(5), 869-875.

Lange, S. Probst, C., Quere, M., Rehm, J. & Popova, S. (2015). Alcohol use, smoking and their co-occurrence during pregnancy among Canadian women, 2003 to 2011/12. *Addictive Behaviors, 50*, 102-109. doi:1.1016/j.addbeh.2015.06.018

Lee, S. H., Shin, S. J., Won, S., Kim, E., & Oh, D. (2010). Alcohol use during pregnancy and related risk factors in Korea. *Psychiatry Investigation,7*(2), 86-92.

Leo, M. A., & Lieber, C. S. (1999). Alcohol, vitamin A, and ß-carotene: Adverse interactions, including hepatotoxicity and carcinogenicity. *American Journal of Clinical Nutrition, 69*(6), 1071-1085.

Lieber, C. S. (2003). Relationships between nutrition, alcohol use, and liver disease. *Alcohol Research and Health, 27*(3), 220-231.

Lin, G. W., McMartin, K. E., & Collins, T. D. (1992). Effect of ethanol consumption during pregnancy on folate coenzyme distribution in fetal, maternal, and placental tissues. *The Journal of Nutritional Biochemistry, 3*(4), 182-187.

Lindsley, T. A., Comstock, L. L., & Rising, L. J. (2002). Morphologic and neurotoxic effects of ethanol vary with timing of exposure in vitro. *Alcohol, 28*(3), 197-203.

Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. *Advanced Nutrition 4*(3), 384S-392S.

Livy, D. J., Miller, E. K., Maier, S. E., & West, J. R. (2003). Fetal alcohol exposure and temporal vulnerability: Effects of binge-like alcohol exposure on the developing rat hippocampus. *Neurotoxicology and Teratology, 25*(4), 447-458.

Mahan, L. K., & Escott-Stump, S. (2008). Nutrition during pregnancy and lactation. In Y. Alexopoulos, K. Hebberd & H. Bays (Eds.), *Krause's Food & Nutrition Therapy* (12th ed., pp. 160-199). Philadelphia, PA: Saunders.

Mahan, L. K., Escott-Stump, S., & Raymond, J. L. (2012). Nutrition in pregnancy and lactation. In Y. Alexopoulos, & D. M. Frazier (Eds.), *Krause's Food and The Nutrition Care Process* (13th ed., pp. 340-374). St. Louis, Missouri: Elsevier.

Maier, S. E., & West, J. R. (2001). Drinking patterns and alcohol-related birth defects. *Alcohol Research and Health, 25*(3), 168-174.

Malabar, M. & Grant, K. (2010). North end food assessment report Winnipeg. *Food Matters Manitoba and Public Health Agency of Canada.* Retrieved from http://foodmattersmanitoba.ca/sites/default/files/Winnipeg%20North%20End%20FINAL%20ASSESSMENT%20REPORT%20-%20April%2030,%202010.pdf

Marchetta, C. M., Denny, C. H., Floyd, R. L., Cheal, N. E., Sniezek, J. E., & McKnight-Eily, L. R. (2012). Alcohol use and binge drinking among women of childbearing age — United States, 2006–2010. *Morbidity and Mortality Weekly Report, 61*(28), 534-538.

Marino, M. D., Aksenov, M. Y., & Kelly, S. J. (2004). Vitamin E protects against alcohol-induced cell loss and oxidative stress in the neonatal rat hippocampus. *International Journal of Developmental Neuroscience, 22*(5-6), 363-377.

Marrs, J. A., Clendenon, S. G., Ratcliffe, D. R., Fielding, S. M., Liu, Q., & Bosron, W. F. (2010). Zebrafish fetal alcohol syndrome model: Effects of ethanol are rescued by retinoic acid supplement. *Alcohol, 44*(7-8), 707-715.

Martinez, M. (1992). Tissue levels of polyunsaturated fatty acids during early human development. *Journal of Pediatrics, 120*(4 II SUPPL.), S 129-S 138.

Masih, S. P., Plumptre, L., Ly, A., Berger, H., Lausmann, A. Y., Croxfod, R., Kim, Y. & O'Connor, D. L. (2015). Pregnant Canadian women achieve recommended intakes of one-carbon nutrients through prenatal supplementation but the supplement composition, including choline, requires reconsideration. *Journal of Nutrition, 145*(8), 1824-1834.

Massaro, A. N., Rothbaum, R., & Aly, H. (2006). Fetal brain development: The role of maternal nutrition, exposures and behaviors. *Journal of Pediatric Neurology, 4*(1), 1-9.

May, P. A., Blankenship, J., Marais, A., Gossage, J. P., Kalberg, W. O., Joubert, B., . . . Seedat, S. (2013a). Maternal alcohol consumption producing fetal alcohol spectrum disorders (FASD): Quantity, frequency, and timing of drinking. *Drug and Alcohol Dependence, 133*(2), 502-512.

May, P. A., Blankenship, J., Marais, A., Gossage, J. P., Kalberg, W. O., Barnard, R., . . . Seedat, S. (2013b). Approaching the prevalence of the full spectrum of fetal alcohol spectrum disorders in a South African population-based study. *Alcoholism: Clinical and Experimental Research, 37*(5), 818-830.

May, P. A., Fiorentino, D., Coriale, G., Kalberg, W. O., Hoyme, H. E., Aragón, A. S., . . . Ceccanti, M. (2011). Prevalence of children with severe fetal alcohol spectrum disorders in communities near Rome, Italy: New estimated rates are higher than previous estimates. *International Journal of Environmental Research and Public Health, 8*(6), 2331-2351.

May, P. A., & Gossage, J. P. (2011). Maternal risk factors for fetal alcohol spectrum disorders: Not as simple as it might seem. *Alcohol Research and Health,34*(1), 15-26.

May, P. A., Gossage, J. P., Brooke, L. E., Snell, C. L., Marais, A., Hendricks, L. S., . . . Viljoen, D. L. (2005). Maternal risk factors for fetal alcohol syndrome in the western cape province of South Africa: A population-based study. *American Journal of Public Health, 95*(7), 1190-1199.

May, P. A., Gossage, J. P., Kalberg, W. O., Robinson, L. K., Buckley, D., Manning, M., & Hoyme, H. E. (2009). Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Developmental Disabilities Research Reviews, 15*(3), 176-192.

May, P. A., Gossage, J. P., Marais, A., Hendricks, L. S., Snell, C. L., Tabachnick, B. G., . . . Viljoen, D. L. (2008). Maternal risk factors for fetal alcohol syndrome and partial fetal alcohol syndrome in South Africa: A third study. *Alcoholism: Clinical and Experimental Research, 32*(5), 738-753.

Meschke, L. L., Holl, J. & Messelt, S. (2013). Older not wiser: Risk of prenatal alcohol use by maternal age. *Maternal and Child Health Journal, 17*, 147-155.

Miller, M. W. (1996). Effect of early exposure to ethanol on the protein and DNA contents of specific brain regions in the rat. *Brain Research, 734*(1-2), 286-294.

Mitchell, J. J., Paiva, M., & Heaton, M. B. (1999). The antioxidants vitamin e and ß-carotene protect against ethanol- induced neurotoxicity in embryonic rat hippocampal cultures. *Alcohol, 17*(2), 163-168.

Monk, B. R., Leslie, F. M., & Thomas, J. D. (2012). The effects of perinatal choline supplementation on hippocampal cholinergic development in rats exposed to alcohol during the brain growth spurt. *Hippocampus, 22*(8), 1750-1757.

Montoliu, C., Sancho-Tello, M., Azorin, I., Burgal, M., Valles, S., Renau-Piqueras, J., & Guerri, C. (1995). Ethanol increases cytochrome P4502E1 and induces oxidative stress in astrocytes. *Journal of Neurochemistry, 65*(6), 2561-2570.

Muralidharan, P., Sarmah, S. & Marrs, J. A. (2015). Zebrafish retinal defects induced by ethanol exposure are rescued by retinoic acid and folic acid supplement. *Alcohol, 49*(2), 149-163.

Muckle, G., Laflamme, D., Gagnon, J., Boucher, O., Jacobson, J. L., & Jacobson, S. W. (2011). Alcohol, smoking, and drug use among Inuit women of childbearing age during pregnancy and the risk to children. *Alcoholism: Clinical and Experimental Research, 35*(6), 1081-1091.

Muhajarine, N., D'Arcy, C., & Edouard, L. (1997). Prevalence and predictors of health risk behaviours during early pregnancy: Saskatoon pregnancy and health study. *Canadian Journal of Public Health, 88*(6), 375-379.

Mumbare, S. S., Maindarkar, G., Darade, R., Yengl, S., Tolani, M. K., & Patole, K. (2012). Maternal risk factors associated with term low birth weight neonates: A matched-pair case control study. *Indian Pediatrics, 49*(1), 25-28.

National Academy of Science. (2016). *Dietary reference intakes tables and application: Background information. Retrieved from* *http://www.nationalacademies.org/hmd/Activities/Nutrition/SummaryDRIs/DRI-Tables.aspx*

Nicolás, J. M., Fernández-Solà, J., Fatjó, F., Casamitjana, R., Bataller, R., Sacanella, E., . . . Estruch, R. (2001). Increased circulating leptin levels in chronic alcoholism. *Alcoholism: Clinical and Experimental Research, 25*(1), 83-88.

Ohara, N., Tsujino, T., & Maruo, T. (2004). The role of thyroid hormone in trophoblast function, early pregnancy maintenance, and fetal neurodevelopment. *Journal of Obstetrics and Gynaecology Canada, 26*(11), 982-990.

Office of Dietary Supplements a. (2016a). *Vitamin A: Fact sheet for health professionals.* National Institute of Health. Retrieved from https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/#h3

Office of Dietary Supplements b. (2016b). *Folate: Fact sheet for health professionals.* National Institute of Health. Retrieved from https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/#h3

Ol, K. K., Kanbak, G., Ilhan, A. O. & Burukoglu, D. (2015). The investigation of the prenatal and postnatal alcohol exposure-induced neurodegeneration in rat brain: protection by betaine and/or omega-3. *Child's Nervous System.* doi:10.1007/s00381-015-2990-1

O'Rahilly, R., & Müller, F. (2008). Significant features in the early prenatal development of the human brain. *Annals of Anatomy, 190*(2), 105-118.

Otero, N. K. H., Thomas, J. D., Saski, C. A., Xia, X., & Kelly, S. J. (2012). Choline supplementation and DNA methylation in the hippocampus and prefrontal cortex of rats exposed to alcohol during development. *Alcoholism: Clinical and Experimental Research, 36*(10), 1701-1709.

Pacey, M. (2009). *Fetal alcohol syndrome & fetal alcohol spectrum disorder among aboriginal peoples: A review of prevalence*. Prince George, B.C.: National Collaborating Centre for Aboriginal Health. Retrieved from http://www.nccah-ccnsa.ca/Publications/Lists/Publications/Attachments/34/2010_01_27_FASDAboriginalReviewPrevalence_EN_Updated_Web.pdf

Patten, A. R., Brocardo, P. S., & Christie, B. R. (2013). Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure. *Journal of Nutritional Biochemistry, 24*(5), 760-769.

Peng, Y., Kwok, K. H. H., Yang, P., Ng, S. S. M., Liu, J., Wong, O. G., . . . Lin, M. C. M. (2005). Ascorbic acid inhibits ROS production, NF-κB activation and prevents ethanol-induced growth retardation and microencephaly. *Neuropharmacology, 48*(3), 426-434.

Peterson, K. (2004). Biomarkers for alcohol use and abuse: A summary. *Alcohol Research and Health, 28*(1), 30-37.

Petkovic, G., & Barišic, I. (2010). FAS prevalence in a sample of urban schoolchildren in Croatia. *Reproductive Toxicology, 29*(2), 237-241.

Pichini, S., Morini, L., Marchei, E., Palmi, I., Rotolo, M. C., Vagnarelli, F., . . . Zuccaro, P. (2009). Ethylglucuronide and ethylsulfate in meconium to assess gestational ethanol exposure: Preliminary results in two Mediterranean cohorts. *Canadian Journal of Clinical Pharmacology, 16*(2), e370-e375.

Pierce, D. R., & West, J. R. (1986). Blood alcohol concentration: A critical factor for producing fetal alcohol effects. *Alcohol, 3*(4), 269-272.

Popova, S., Lange, S., Burd, L. & Rehm, J. (2015). The burden and economic impact of fetal alcohol spectrum disorder in Canada. *Centre for Addiction and Mental Health.* Retrieved from http://www.camh.ca/en/research/news_and_publications/reports_and_books/Documents/Burden%20and%20Eco%20Costs%20FASD%20Feb%202015.pdf

Popova, S., Lange, S., Shield, K., Mihic, A., Chudley, A. E., Mukherjee, R. A. S…Rehm, J. (2016). Comorbidity of fetal alcohol spectrum disorder: A systematic review and meta-analysis. *Lancet, 387*(10022), 978-987.

Pregnancy and Family Support Services [PFSS]. (2016). *Mission & philosophy*. Retrieved from http://www.pfsswinnipeg.com/about-pfss-winnipeg/mission-and-philosophy/

Public Health Agency of Canada [PHAC]. (2009). *What mothers say: The Canadian maternity experiences survey.* Retrieved from file:///Users/karleedyck/Documents/-sites-webphac-htdocs-archives-survey-eng%20(1).pdf

Public Health Agency of Canada [PHAC]. (2012). *Fetal alcohol spectrum disorder (FASD): A framework for action*. Retrieved from http://www.phac-aspc.gc.ca/publicat/fasd-fw-etcaf-ca/index-eng.php

Ramakrishnan, U., Grant, F., Goldenberg, T., Zongrone, A., & Martorell, R. (2012). Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: A systematic review. *Paediatric and Perinatal Epidemiology, 26*(SUPPL. 1), 285-301.

Randall, C. L., Ekblad, U., & Anton, R. F. (1990). Perspectives on the pathophysiology of fetal alcohol syndrome. *Alcoholism, Clinical and Experimental Research, 14*(6), 807-812.

Ritchie, L. D., & King, J. C. (2008). Nutrient recommendations and dietary guidelines for pregnant women. In C. J. Lammi-Keefe, S. C. Couch & E. H. Philipson (Eds.), *Handbook of Nutrition and Pregnancy* (pp. 3-26). Totowa, NJ: Humana Press. doi:10.1007/978-1-59745-112-3

Robinson, G. C., Conry, J. L., & Conry, R. F. (1987). Clinical profile and prevalence of fetal alcohol syndrome in an isolated community in British Columbia. *Canadian Medical Association Journal, 137*(3), 203-207.

Roozen, S., Peter, G. Y., Kok, G., Townend, D., Nijhuis, J. & Curfs, L. (2016). Worldwide prevalence of fetal alcohol spectrum disorders: A systematic literature review including meta-analysis. *Alcoholism: Clinical and Experimental Research, 40*(1), 18-32.

Roy, A., Evers, S. E., & Campbell, M. K. (2012). Dietary supplement use and iron, zinc and folate intake in pregnant women in London, Ontario. *Chronic Diseases and Injuries in Canada, 32*(2), 76-83.

Rwebembera, A. A., Munubhi, E. K. D., Manji, K. P., Mpembeni, R., & Philip, J. (2006). Relationship between infant birth weight </=2000 g and maternal zinc levels at Muhimbili national hospital, Dar es Salaam, Tanzania. *Journal of Tropical Pediatrics, 52*(2), 118-125.

Ryan, S. H., Williams, J. K., & Thomas, J. D. (2008). Choline supplementation attenuates learning deficits associated with neonatal alcohol exposure in the rat: Effects of varying the timing of choline administration. *Brain Research, 1237*, 91-100.

Salmon, A. (2007). Walking the talk: How participatory interview methods can democratize research. *Qualitative Health Research,* 17, 982-993.

Sampson, P. D., Streissguth, A. P., Bookstein, F. L., Little, R. E., Clarren, S. K., Dehaene, P., . . . Graham Jr., J. M. (1997). Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. *Teratology, 56*(5), 317-326.

Sanchis, R., Sancho-Tello, M., Chirivella, M., & Guerri, C. (1987). The role of maternal alcohol damage on ethanol teratogenicity in the rat. *Teratology,36*(2), 199-208.

Santolaria, F. & González-Reimers, E. (2012). Alcohol and nutrition: An overview. In R. R. Watson, V. R. Preedy & S. Zibadi (Eds.), *Alcohol, nutrition, and health consequences* (3-14). New York: Humana Press.

Sarkola, T., Eriksson, C. J. P., Niemelä, O., Sillanaukee, P., & Halmesmäki, E. (2000). Mean cell volume and gamma-glutamyl transferase are superior to carbohydrate-deficient transferrin and hemoglobin-acetaldehyde adducts in the follow-up of pregnant women with alcohol abuse. *Acta Obstetricia Et Gynecologica Scandinavica, 79*(5), 359-366.

Sarmah, S., & Marrs, J. A. (2013). Complex cardiac defects after ethanol exposure during discrete cardiogenic events in zebrafish: Prevention with folic acid. *Developmental Dynamics, 242*(10), 1184-1201.

Schenker, S., Becker, H. C., Randall, C. L., Phillips, D. K., Baskin, G. S., & Henderson, G. I. (1990). Fetal alcohol syndrome: Current status of pathogenesis. *Alcoholism: Clinical and Experimental Research, 14*(5), 635-647.

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., & Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in Neurobiology, 106-107*, 1-16.

Shah, D., & Sachdev, H. P. S. (2006). Zinc deficiency in pregnancy and fetal outcome. *Nutrition Reviews, 64*(1), 15-30.

Shankar, K., Hidestrand, M., Liu, X., Xiao, R., Skinner, C. M., Simmen, F. A., . . . Ronis, M. J. J. (2006). Physiologic and genomic analyses of nutrition-ethanol interactions during gestation: Implications for fetal ethanol toxicity. *Experimental Biology and Medicine, 231*(8), 1379-1397.

Sherwood, K. L., Houghton, L. A., Tarasuk, V., & O'Connor, D. L. (2006). One-third of pregnant and lactating women may not be meeting their folate requirements from diet alone based on mandated levels of folic acid fortification. *Journal of Nutrition, 136*, 2820-2826.

Singh, M. (2005). Essential fatty acids, DHA human brain. *Indian Journal of Pediatrics, 72*(3), 239-242.

Skagerström, J., Alehagen, S., Häggström-Nordin, E., Årestedt, K., & Nilsen, P. (2013). Prevalence of alcohol use before and during pregnancy and predictors of drinking during pregnancy: A cross sectional study in Sweden. *BMC Public Health, 13*(1)

Skagerström, J., Chang, G., & Nilsen, P. (2011). Predictors of drinking during pregnancy: A systematic review. *Journal of Women's Health, 20*(6), 901-913.

Smit, E., & Crespo, C. J. (2001). Dietary intake and nutritional status of US adult marijuana users: Results from the third national health and nutrition examination survey. *Public Health Nutrition, 4*(3), 781-786.

Sood, B., Delaney-Black, V., Covington, C., Nordstrom-Klee, B., Ager, J., Templin, T., . . . Sokol, R. J. (2001). Prenatal alcohol exposure and childhood behavior at age 6 to 7 years: I. dose-response effect. *Pediatrics, 108*(2), E34.

Square, D. (1997). Fetal alcohol syndrome epidemic on Manitoba reserve. *Canadian Medical Association Journal, 157*(1), 59-60.

Stade, B., Ali, A., & Bennett, D. E. A. (2009). The burden of prenatal exposure to alcohol: Revised measurement of cost, 2007. *Canadian Journal of Clinical Pharmacology, 16*, e91-e102.

Stark, K. D., Beblo, S., Murthy, M., Whitty, J. E., Buda-Abela, M., Janisse, J., . . . Salem Jr., N. (2005). Alcohol consumption in pregnant, black women is associated with decreased plasma and erythrocyte docosahexaenoic acid. *Alcoholism: Clinical and Experimental Research, 29*(1), 130-140.

Statistics Canada. (2011). First nations, Métis and Inuit women. *Women in Canada: A gender based statistical report*. Retrieved from http://www.statcan.gc.ca.proxy1.lib.umanitoba.ca/pub/89-503-x/2010001/article/11442-eng.htm#a14

Stiles, J., & Jernigan, T., L. (2010). The basics of brain development. *Neuropsychol Rev, 20*(4), 327-348.

Storck, S. (2013). Fetal development. Retrieved from http://www.nlm.nih.gov.proxy1.lib.umanitoba.ca/medlineplus/ency/article/002398.htm

Subar, A. F., Freedman, L. S., Tooze, J. A., Kirkpatrick, S. I., Boushey, C., Neuhouser, M. L., …Thompson, F. E. (2015). Addressing current criticism regarding the value of self-report dietary data. *Journal of Nutrition, 145*(12).

Summers, B. L., Rofe, A. M., & Coyle, P. (2009). Dietary zinc supplementation throughout pregnancy protects against fetal dysmorphology and improves postnatal survival after prenatal ethanol exposure in mice. *Alcoholism: Clinical and Experimental Research, 33*(4), 591-600.

Sunderland, M. A. (2001). Initial formation of the nervous system: Gastrulation and neurulation. In D. Purves, G. J. Augustine, D. Fitzpatrick & et al. (Eds.), *Neuroscience* (2nd Edition ed.,) Sinauer Associates.

Tarasuk, V., Mitchell, A. & Dachner, N. (2013). Research to identify policy options to reduce food insecurity (PROOF). *Household food insecurity in Canada 2011*. Retrieved from http://nutritionalsciences.lamp.utoronto.ca/

Taylor, C. L. (2008). Framework for DRI development: Components "known" and components "to be explored". Institute of Medicine and Health Canada. Retrieved from http://www.nationalacademies.org/hmd/~/media/Files/Activity%20Files/Nutrition/DRIs/New%20Material/11Bckgrd%20PaperFramework%20for%20DRI%20Devel.pdf

Thanh, N. X., & Jonsson, E. (2010). Drinking alcohol during pregnancy: Evidence from Canadian community health survey 2007/2008. *Journal of Population Therapeutics and Clinical Pharmacology*, *17*(2), e302-e307.

Thomas, J. D., Abou, E. J., & Dominguez, H. D. (2009). Prenatal choline supplementation mitigates the adverse effects of prenatal alcohol exposure on development in rats. *Neurotoxicology and Teratology, 31*(5), 303-311.

Thomas, J. D., Biane, J. S., O'Bryan, K. A., O'Neill, T. M., & Dominguez, H. D. (2007). Choline supplementation following third-trimester-equivalent alcohol exposure attenuates behavioral alterations in rats. *Behavioral Neuroscience, 121*(1), 120-130.

Thomas, J. D., Garrison, M., & O'Neill, T. M. (2004). Perinatal choline supplementation attenuates behavioral alterations associated with neonatal alcohol exposure in rats. *Neurotoxicology and Teratology, 26*(1), 35-45.

Thomas, J. D., Idrus, N. M., Monk, B. R., & Dominguez, H. D. (2010). Prenatal choline supplementation mitigates behavioral alterations associated with prenatal alcohol exposure in rats. *Birth Defects Research. Part A, Clinical and Molecular Teratology, 88*(10), 827-837.

Tri-Council. (2014). *Tri-council policy statement: Ethical conduct for research involving humans.* Government of Canada. Retrieved from http://www.pre.ethics.gc.ca/pdf/eng/tcps2-2014/TCPS_2_FINAL_Web.pdf

United Nations Food and Agriculture Organization (1996). *Rome declaration on world food security, world food summit plan of action,* paragraph 1, Rome: November 1996. Retrieved from http://www.fao.org/docrep/003/w3613e/w3613e00.htm

Walker, M. J., Al-Sahab, B., Islam, F., & Tamin, H. (2011). The epidemiology of alcohol utilization during pregnancy: An analysis of the Canadian maternity experiences survey (MES)**.** *BMC Pregnancy and Childbirth, 11*(52) doi:10.1186/1471-2393-11-52

Wellmann, K. A., George, F., Brnouti, F. & Mooney, S. (2015). Docosahexaenoic acid partially ameliorates deficits in social behavior and ultrasonic vocalizations caused by prenatal ethanol exposure. *Behavioural Brain Research, 286*, 201-211.

Whitfield, J. B., Heath, A. C., Madden, P. A. F., Pergadia, M. L., Montgomery, G. W., & Martin, N. G. (2013). Metabolic and biochemical effects of low-to-moderate alcohol consumption. *Alcoholism: Clinical and Experimental Research, 37*(4), 575-586.

Williams, R. J., Odaibo, F. S., & McGee, J. M. (1999). Incidence of fetal alcohol syndrome in northeastern Manitoba. *Canadian Journal of Public Health,90*(3), 192-194.

World Health Organization. (2008). *World-wide prevalence of anemia 1993-2005*. WHO Press. Retrieved from http://www.who.int/vmnis/publications/anaemia_prevalence/en/

World, M. J., Ryle, P. R., & Thomson, A. D. (1985). Alcoholic malnutrition and the small intestine. *Alcohol and Alcoholism, 20*(2), 89-124.

Wu, B. T. F., Dyer, R. A., King, J., Richardson, K. J. & Innis, S. M. (2012). Early second trimester maternal plasma choline and betaine are related to measures of early cognitive development in term infants. *PLoS ONE, 7*(8): e43448. doi:10.1271/journal.pone.0043448

Wu, D., & Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol Research and Health, 27*(4), 277-284.

Wu, S., Wang, R., Zhao, Y., Ma, X., Wu, M., Yan, X. & He, J. (2013). The relationship between self-rated health and objective health status: A population-based study. *BMC Public Health, 13*:320. doi:10.1186/1471-2458-13-320

Yelin, R., Schyr, R. B., Kot, H., Zins, S., Frumkin, A., Pillemer, G., & Fainsod, A. (2005). Ethanol exposure affects gene expression in the embryonic organizer and reduces retinoic acid levels. *Developmental Biology, 279*(1), 193-204.

Yeomanset, M. R., Caton, S., & Hetherington, M. M. (2003). Alcohol and food intake. *Current Opinion in Clinical Nutrition and Metabolic Care, 6*(6), 639-644.

Young, J. K., Giesbrecht, H. E., Eskin, M. N., Aliani, M. & Suh, M. (2014). Nutrition implications for fetal alcohol spectrum disorder. *Advances in Nutrition, 5*(6), 675-692.

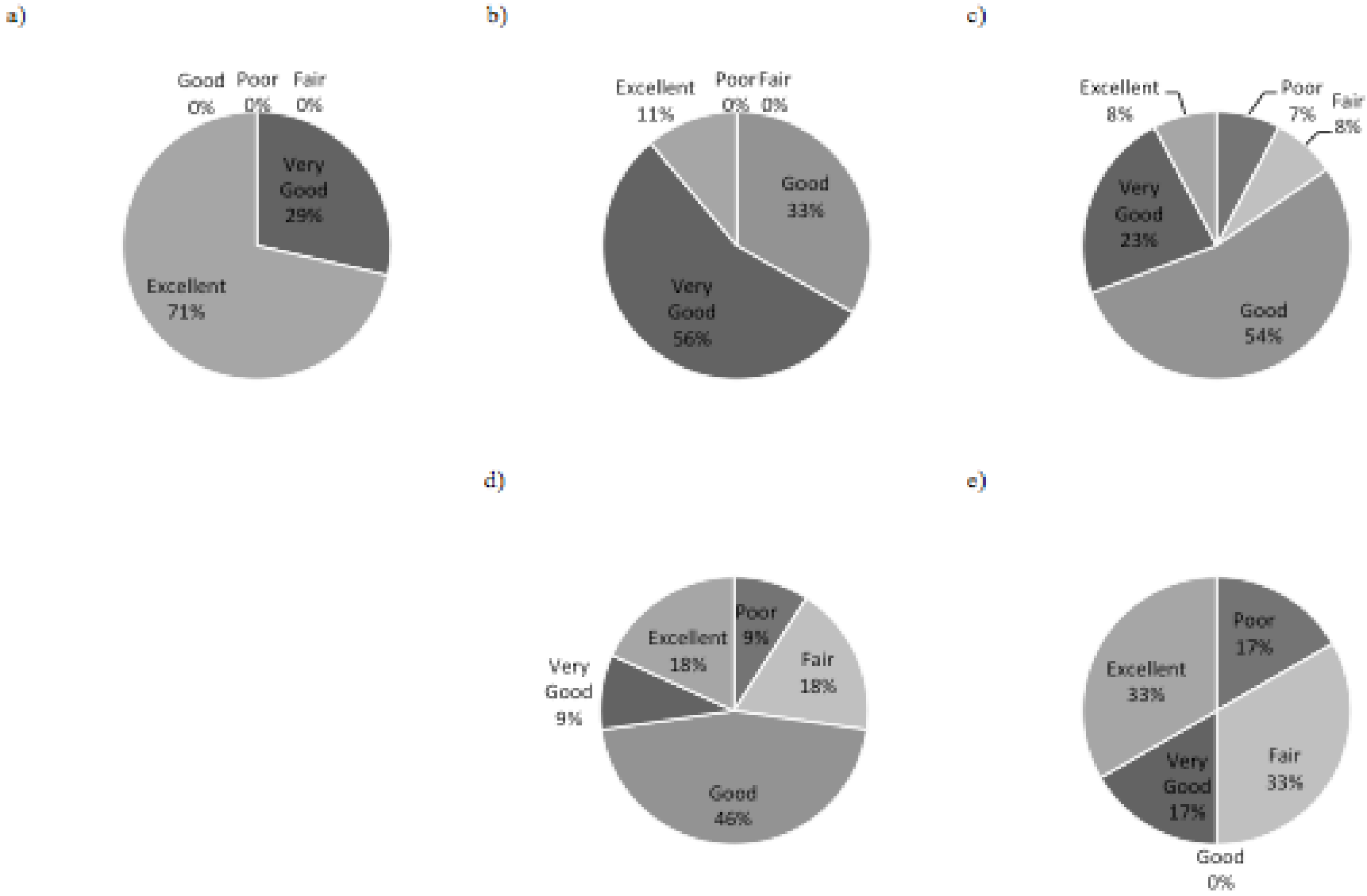
Zajac, C. S., & Abel, E. L. (1992). Animal models of prenatal alcohol exposure. *International Journal of Epidemiology, 21*(Suppl 1), S24-S32.

Zakhari, S. (2006). Overview: How is alcohol metabolized by the body? *Alcohol Research & Health, 29*(4), 245-254.

Zeisel, S. H. (2011). What choline metabolism can tell us about the underlying mechanisms of fetal alcohol spectrum disorders. *Molecular Neurobiology,44*(3), 185-191.

Zeisel, S. H., & Da Costa, K. (2009). Choline: An essential nutrient for public health. *Nutrition Reviews, 67*(11), 615-623.

Appendix A



Appendix A: Self-rated health prior to pregnancy, based on current health rating. a) Self-rated "excellent" during pregnancy, b) self-rated "very good" during pregnancy, c) self-rated "good" during pregnancy d) self-rated "fair" during pregnancy e), self-rated "poor" during pregnancy.