The Association Between Dietary Methyl Donor Intake During Pregnancy and Offspring Birth Weight

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Abstract

Maternal consumption of dietary methyl donors (DMDs) such as folate, methionine, choline, as well as co-factors including zinc, vitamins B_2, B_6 and B_{12} can lead to permanent alterations in the DNA and gene expression of the developing fetus. This study aimed to identify patterns of DMD intake during the second trimester of pregnancy and their associations with infant birth weight, small and large for gestational age (SGA and LGA, respectively). From food sources alone, most pregnant women were below the estimated average requirement for dietary folate equivalent (DFE) (69%) and below the adequate intake measure for choline (99%). Zinc seemed to be the most important nutrient for attaining adequate birth weight. DFE and vitamin B_{12} were positively associated with birth weight. DFE also reduced the risk for SGA whereas choline increased the risk for LGA. Therefore, DMD intake from food sources during pregnancy may be important to ensure optimal infant birth weight.

Résumé

La consommation maternelle de donneurs de méthyle alimentaire (DMDs) comme le folate, la méthionine, la choline et des cofacteurs comme le zinc, les vitamines B_2, B_6 et B_{12}, peuvent causer des altérations permanentes de l'ADN et de l'expression des gènes du fœtus. Cette étude vise à identifier des modèles de consommation des DMDs pendant le deuxième trimestre de la grossesse, ainsi que leurs associations avec le poids à la naissance (PN) et le risque d'avoir un poids faible ou élevé pour l'âge gestationnel (PF et PÉ, respectivement). La plupart des femmes étaient sous le besoin moyen estimatif pour l'équivalent de folate alimentaire (EFA) (69 %) et sous l'apport suffisant pour la choline (99 %). Le zinc est apparu comme le nutriment le plus important pour atteindre un PN suffisant. L'EFA et la vitamine B_{12} étaient positivement associés avec le PN. L'EFA est associé à un risque réduit de PF tandis que la choline à un risque augmenté de PÉ. Par conséquent, la consommation maternelle des DMDs qui viennent des sources alimentaires pendant la grossesse peut être importante pour assurer un PN optimal.

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3D	Découvrir, Développer, Devenir
AI	Adequate Intake
APrON	Alberta Pregnancy Outcomes and Nutrition Cohort
BMI	Body Mass Index
CEGEP	Collège d'enseignement général et professionnel
CH_3	Methyl
CHU	Centre Hospitalier Universitaire
CNF	Canadian Nutrient File
CpG	Cytosine-Guanosine
DHF	Dihydrofolate
DICE	Detection of Informative Combined Effects
DFE	Dietary Folate Equivalent
DNA MTase	DNA Methyltransferase
DMG	Dimethylglycine
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
FAD	Flavin Adenine Dinucleotide
IGF2	Insulin-like Growth Factor 2
IRNPQEO	Integrated Research Network in Perinatology of Québec and Eastern Ontario
IUGR	Intrauterine Growth Restriction
KMO	Kaiser-Meyer-Olkin
LGA	Large for Gestational Age
LSD	Fisher's Least Significant Difference
MS	Methionine Synthase
MTHFR	Methylenetetrahydrofolate Reductase
OR	Odds Ratio
PCA	Principal Components Analysis
PREFORM	Prenatal Folic Acid Exposure on DNA Methylation

RD	Registered Dietitian
RDA	Recommended Dietary Allowance
SAH	*S*-Adenosyl Homocysteine
SAM	*S*-Adenosyl Methionine
SD	Standard Deviation
SGA	Small for Gestational Age
SOGC	Society for Obstetricians and Gynaecologists of Canada
SPSS	Statistical Package for the Social Sciences
THF	Tetrahydrofolate

Thesis Outline

Chapter 1: Theoretical Foundations. An overview of the fundamental concepts, followed by the study rationale, hypothesis and research objectives are presented.

Chapter 2: Methodology. The methods used for data collection, dietary and outcomes assessments as well as statistical analyses are described.

Chapter 3: The Association Between Dietary Methyl Donor Intake and Birth Weight. The results from both objectives are presented and discussed in depth.

Chapter 4: Conclusion. A summary of the findings and suggestions for future research areas are proposed.

Chapter 1: Theoretical Foundations

In this chapter, the aim is to deliver an overview of the one-carbon metabolism cycle including the specific roles of all dietary methyl donors and cofactors involved. Thereafter, the determinants of offspring birth weight, including maternal nutrition, will be described. Finally, an explanation of nutritionally-induced epigenetic modifications (i.e. DNA methylation) that are capable of affecting birth weight will be presented. This overview will be followed by the study rationale, the hypothesis as well as the specific research objectives.

1.1 One-Carbon Metabolism Cycle

The maternal diet is of great influence and importance in determining offspring health. Maternal nutrition can lead to permanent alterations of methylation patterns in the DNA of the developing fetus, therefore altering gene expression.[1] These changes can be preserved over time and may lead to long-term consequences for health.[1] The one-carbon metabolism pathway (Figure 1) uses nutrients from the diet to provide methyl (CH_3) groups for the methylation of DNA. Methyl-group donors involved in the cycle, such as methionine, folate and choline, as well as cofactors including zinc, vitamins B_2, B_6 and B_{12} are derived entirely from dietary and supplemental intake.[2] This cycle consists of two methylation pathways, one folate-dependent and the other folate-independent, in which homocysteine is remethylated to form methionine, which then generates *S*-Adenosyl methionine (SAM): the universal methyl donor.[1,3]

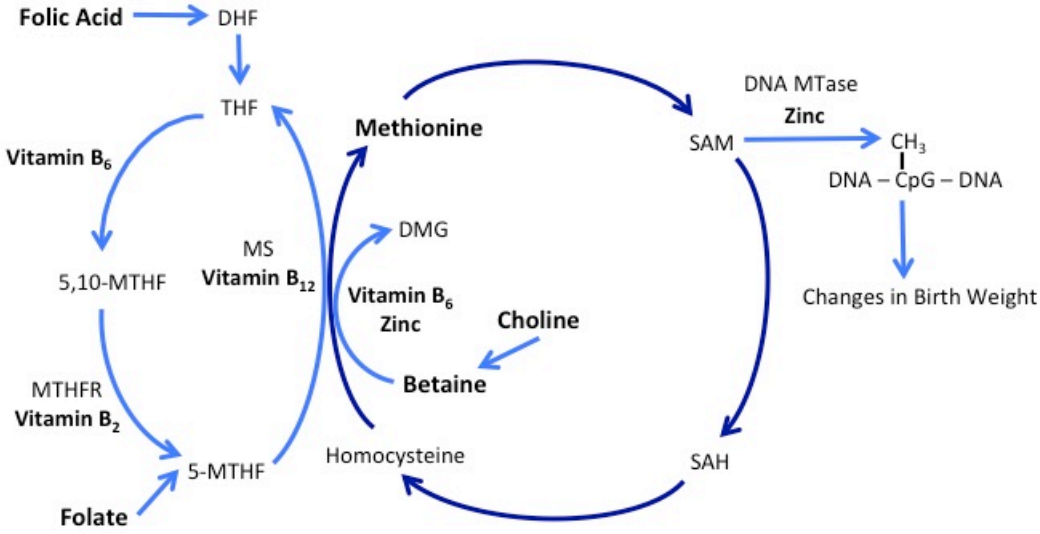

Figure 1. Involvement of Dietary Methyl Donors in One-Carbon Metabolism

This diagram outlines the interaction of methyl donors methionine, betaine, choline, folate, and folic acid as well as cofactors, vitamins B_2, B_6, B_{12} and zinc, in the methylation pathway as well as their putative relationship with DNA methylation and birth weight. **Abbreviations:** 5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; DHF, dihydrofolate; DMG, dimethylglycine; DNA MTase, DNA methyltransferase. MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; SAH, *S*-Adenosyl homocysteine; SAM, *S*-Adenosyl methionine; THF, tetrahydrofolate.

In the folate-dependent cycle, folic acid is reduced to dihydrofolate (DHF) and eventually to tetrahydrofolate (THF).[4] THF is then converted into 5,10-methylene-THF by serine hydroxymethyltransferase, which uses vitamin B_6 as a coenzyme.[5] Next, 5,10-methylene-THF is converted into 5-methyl-MTHF (5-MTHF) by the enzyme methylenetetrahydrofolate reductase (MTHFR) and cofactor flavin adenine dinucleotide (FAD) to which vitamin B_2 is a precursor.[6] Vitamin B_{12} acts as a cofactor for methionine synthase to convert 5-MTHF into methionine.[7] Betaine, which can be derived from choline or consumed directly from the diet, is employed as the enzyme, betaine homocysteine methyltransferase, where it catalyzes the folate-independent cycle.[1] Betaine donates a methyl group to homocysteine to form methionine. Methionine is converted into SAM, which supplies the methyl groups needed for DNA methylation.[2] Finally, DNA methyltransferase obtains a methyl group from SAM and uses zinc as a cofactor to methylate DNA.[8]

Without sufficient methyl donors, homocysteine, produced from the breakdown of methionine, increases and cannot be remethylated to form methionine.[9] Therefore, homocysteine levels are influenced by intake of dietary methyl donors and cofactors involved in the one-carbon metabolism process.[4,10] Elevated homocysteine levels have been associated with fetal growth restriction and shorter gestation, emphasizing the influence of the one-carbon metabolism cycle on fetal growth and development as well as the potential modification of maternal nutrition as a strategy for its improvement.[7,10–12]

1.2 Dietary Reference Intakes (DRIs)

Dietary Reference Intakes (DRIs) are reference values for energy, macro and micronutrients developed by the Institute of Medicine's Food and Nutrition Board to

promote sufficient nutrient intake for healthy North American populations. Depending on available scientific evidence, each nutrient has an Estimated Average Requirement (EAR) value and either a Recommended Dietary Allowance (RDA) or an Adequate Intake (AI) value. The EAR is the median daily intake of a nutrient that is expected to be sufficient for half of the individuals in that sex or life-stage group.[13] The RDA is the mean daily intake that is expected to meet the requirement for nearly all individuals (97.5%) in the specified sex or life-stage group (obtained from the EAR + 2 standard deviations [SD]).[13] The AI is derived when there is insufficient data to determine an EAR and thus an RDA. It is the recommended mean daily intake of a nutrient that is expected to meet or exceed requirements for a specific sex or life-stage group and is based on estimates of intake from healthy people who seem to be maintaining sufficient nutritional intake.[13] A Tolerable Upper Intake Level (UL) has also been established for most nutrients and is the highest mean daily intake of a nutrient that is unlikely to pose any adverse health risk for most people.[13]

1.3 Dietary Methyl Donor Intake and Pregnancy Outcomes

1.3.1 Folic Acid

Folic Acid During Pregnancy

Folate, vitamin B_9, has been proven to be a key micronutrient required for the rapid proliferation of cells, the development of the uterus and placenta as well as for the increased maternal blood volume during pregnancy.[14] Folate is vital for the growth of the spine, brain and skull of the fetus, particularly throughout the first four weeks of

pregnancy.[15] Moreover, several studies have demonstrated the importance of folic acid in preventing neural tube defects (NTDs).[16–19]

The risk of NTDs is reduced if women take a multivitamin containing folic acid three months prior to conception until the closure of the neural tube (approximately 21 to 28 days after conception).[20] This reduced risk was observed in women who consumed 200 – 300 μg of folate per day as well as a supplement containing folic acid ranging from 360 – 800 μg.[21–23] Moreover, a case-control study by Daly *et al.*[18] (1995) found women with red blood cell folate concentrations greater than 906 nmol/L had the lowest NTD risk. Additionally, the risk of NTDs was shown to be fourfold lower when blood folate concentration was 1,200 nmol/L when compared to 500 nmol/L.[24] Crider *et al*.[24] (2014) suggested blood cell folate concentrations of approximately 1,000 – 1,300 nmol/L to be effective for preventing folate-dependent NTDs. Although the amount of daily folate intake needed to achieve a blood folate concentration in the proposed range is unknown,[24] some studies have found a daily folate intake of 400 μg for two to six months to be sufficient to reach a red blood cell folate concentration of at least 906 nmol/L.[25,26]

As a result, since 1998, white flour, pasta and cornmeal have been fortified with folic acid in Canada.[27] The objective of this fortification was to increase the average folic acid intake of women of childbearing age without exceeding the 1,000 μg UL for the rest of the population.[28] Since food fortification has been implemented, the overall prevalence of NTDs in Canada has decreased from 1.5 to 0.86 cases per 1,000 births: a 46% reduction.[28] Folate, the natural form of folic acid, is found in dark green leafy vegetables, fruits, nuts, beans and peas whereas folic acid, the synthetic form, is found in the aforementioned fortified grain products and dietary supplements.[29] The synthetic form

has a greater bioavailability than does dietary folate and is rapidly absorbed across the intestine.[30] In fact, folic acid is equivalent to 1.7 times that of natural folate if consumed with food and 2.0 times natural folate if consumed on an empty stomach.[30] Therefore, dietary folate equivalent (DFE), the weighted sum of the natural and synthetic forms, is used as a measure of folate and folic acid intake.

Because folic acid is essential for the growth of the fetus during the first few weeks of pregnancy[15] and because many pregnancies are unplanned, Health Canada recommends that women of reproductive age take a daily multivitamin containing 400 μg of folic acid and continue supplementation throughout pregnancy.[31] The Society of Obstetricians and Gynaecologists of Canada (SOGC) recommends women take a supplement ranging from 400 μg to 1,000 μg periconceptionally, throughout pregnancy and postpartum if they are at low risk for NTDs.[32] This recommendation increases to 5,000 μg of folic acid per day if they are at high risk with conditions such as epilepsy, insulin-dependent diabetes mellitus, obesity with Body Mass Index (BMI) > 35 kg/m^2, of a certain ethnic group (e.g. Sikh) or have a family history of neural-tube defects.[32] For women of reproductive age, the RDA for dietary folate equivalent is 400 μg per day.[13] This recommendation increases to 600 μg of DFE per day during pregnancy.[13]

Folic Acid and Birth Weight

Folic acid is a crucial part of placental development.[33] In fact, high homocysteine levels, those of which folic acid serves to decrease,[34,35] provoke oxidative and cytotoxic stress on endothelial and placental vascular functions[36–38] and may increase apoptosis of trophoblasts, the cells that eventually become the placenta.[39] Therefore, periconceptional

folic acid supplementation may be important in preventing placental developmental disorders that result in preterm birth.[40–44]

Several studies have shown beneficial effects of daily (1,000 μg or less) folic acid supplementation on growth restriction and preterm birth.[45–47] A study conducted on Spanish pregnant women observed a 60% decrease in infants born small for gestational age (SGA) by weight for women who consumed greater than 245 μg of dietary folate per day compared to women who consumed less than 211 μg per day, independent of supplementation.[48] Similarly, an American study yielded comparable results in which folate intakes below 240 μg/day were associated with low birth weight and preterm delivery.[46] Additionally, a systematic review and meta-analysis conducted by Fekete *et al*.[14] (2012) found a significant dose-response relationship between maternal folate intake (250 – 5,000 μg/day of folic acid) and offspring birth weight. Likewise, a systematic review with meta-analysis conducted on a multiethnic population found folic acid to be successful in reducing the risk for small for gestational age infants below the 5th percentile, but only if supplementation was started before conception.[49]

Serum and cord-blood concentrations of folate have proven to be significant predictors of fetal growth and birth weight. A study by Weber *et al*.[50] (2013) found that higher cord-blood concentrations of 5-MTHF, the biologically active form of folic acid (Figure 1),[51] in the periconceptional period and early pregnancy significantly reduced the risk of low birth weight, small for gestational age and preterm newborns. Furthermore, low maternal serum folate and high homocysteine concentrations are associated with reduced fetal growth in midpregnancy.[52] Thus, folic acid supplementation during pregnancy may be important for delivering an infant at term and of normal birth weight.

Several studies that examined folic acid supplementation in early-to-mid pregnancy on fetal growth and gestational age provided inconsistent results[48,53–55] and some studies found that folate status in the second and third trimesters was not associated with birth weight or gestational age.[56,57] Moreover, the relationship between maternal folate consumption and birth weight is suggestive of a threshold effect, thus excessive consumption of folic acid would have no benefit in promoting optimal birth weight.[58] Finally, the results of folic acid supplementation for preventing low birth weight and preterm births are inconclusive.[59]

Excessive Folic Acid Intake

Despite the well-defined success of folate in preventing NTDs,[16–19] concerns have been raised regarding high folate status, with the greatest increase observed in pregnant women.[60,61] The Prenatal Health Project, which is a study consisting of pregnant women from London, Ontario, showed that the majority of women consuming dietary folate while taking a folic acid supplement consumed an estimated mean daily intake of 2,148 μg/day; however, DFE intake of these women from food alone averaged 473 μg/day, well below the 600 μg RDA.[62] Likewise, the Prenatal Folic Acid Exposure on DNA Methylation (PREFORM) study conducted on pregnant women living in Toronto, Ontario found that 41% of participants had dietary folate intakes below the EAR.[63] However, supplemental folic acid consumption alone reached the UL with a median intake of 1,000 μg.[63]

In the Alberta Pregnancy Outcomes and Nutrition (APrON) cohort, which was conducted on a group of Canadian women with high socioeconomic status, the majority of women were taking a multivitamin or mineral supplement during pregnancy and had a

median daily intake of 1,000 μg of folic acid, exceeding the RDA by more than 200%.[64] Also, over half of the women from this study had high folate status (> 1,360 nmol/L) during pregnancy[65] and more than 20% of participants consumed folic acid levels above the UL in each trimester of pregnancy.[64] Therefore, the authors concluded folic acid supplementation during pregnancy to be inappropriate for healthy women with low nutrient deficiency risk.[65] Additionally, in a study of American pregnant women, median intake of folic acid was 1,129 μg and intake of other methyl donors such as vitamins B_2, B_6, B_{12} and zinc were 1.3 to 5.5 times above the recommended amounts.[66] Although it appears that pregnant women are consuming less than the recommended amount of dietary folate, when incorporating a supplement into the diet, median daily intake rises substantially and often equates with the UL. Finally, despite the proposed UL, there is no consensus on a safe upper limit of blood folate concentration.[67]

Red blood cell folate concentrations greater than 1,300 nmol/L have little to no benefit in preventing neural tube defects and high levels of consumption may actually be detrimental to health.[24] Folic acid supplementation in the first trimester is associated with a greater prevalence of respiratory illness and wheeze[68] whereas supplementation in late pregnancy is linked to an increased risk of asthma in early childhood.[69] Maternal folate consumption greater than 500 μg/day in the third trimester is associated with eczema in children[70] and supplemental folic acid intake greater than 1,000 μg/day is associated with reduced birth height.[48] Finally, the long-term effects of high folate levels have yet to be elucidated.[58]

There is concern over high amounts of folic acid consumption as they may overpower the liver metabolic capacity, but the potential effects are unclear.[71] For

instance, a high dose (5,000 μg) of folic acid intake for 12 weeks nearly doubles the median plasma concentration of unmetabolized folic acid.[72] However, even with sustained supplementation, a significant decline in unmetabolized folic acid concentration is observed, suggesting that a compensatory mechanism exists.[72] In fact, unmetabolized concentrations of folic acid are not significantly different after 30 weeks of supplementation when compared to baseline.[72] This regulatory mechanism may be caused by a reduction of folic acid to its active form, 5-MTHF, that plays a significant role in one-carbon metabolism (Figure 1).[73] Furthermore, there seems to be no difference in plasma concentration between women of childbearing age exposed to 1,100 μg and 5,000 μg of folic acid ten hours post-ingestion[74] and no difference in the relative distribution of folate forms in neither pregnant nor nonpregnant women.[75] Additionally, for the majority of pregnancies, folic acid is not believed to accumulate in the fetus, unlike 5-MTHF and THF.[71,76]

In conclusion, the risk of high folic acid intake during pregnancy is uncertain. Folate is a crucial nutrient, especially during pregnancy, and its fortification in several countries has proven to be a remarkable remedy for folate-dependent NTDs. However, NTDs are uncommon in Canada (< 1%) and 40% of Canadians have high folate status.[77] Accordingly, concerns over the effects of high folate consumption as well as other dietary methyl donor intakes are beginning to rise.

1.3.2 Vitamin B_2 (Riboflavin)

Vitamin B_2 serves as a precursor to FAD, a cofactor for the enzyme, methylenetetrahydrofolate reductase (MTHFR) that converts 5,10-methylene-THF into 5-MTHF.[6] Thus, like folate, vitamin B_2 plays a key role in the recycling of homocysteine in

the one-carbon metabolism cycle (Figure 1). The EAR and RDA for vitamin B_2 intake during pregnancy are 1.2 mg/day and 1.4 mg/day, respectively.[13] Amongst the limited research on vitamin B_2 intake during pregnancy, one study conducted on rodents concluded vitamin B_2 and choline intake to be important for the prevention of congenital heart defects.[78] Furthermore, riboflavin may be effective at reducing hypertensive disorders, such as preeclampsia, especially in women with the less efficient MTHFR polymorphism.[79] Maternal and fetal concentrations of vitamin B_2 have been found to be positively correlated with both birth length[80] and weight in which an additional 1 mg of riboflavin led to an increase of 0.72 cm in birth length and 149 g in birth weight.[81] Another study found that changes in riboflavin intake from 28 weeks to 36 weeks were predictive of infant birth weight and that women who decreased their intake of vitamin B_2 by 0.41 mg/day, on average, in late pregnancy gave birth to offspring who were 87 g lighter, on average.[82] However, the effects of differing riboflavin concentrations during pregnancy have not been adequately addressed.

1.3.3 Vitamin B_6 (Pyroxidine)

Vitamin B_6 acts as a coenzyme to serine hydroxymethyltransferase to convert THF into 5,10-methylene-THF,[5] the precursor to the molecule that supplies the methyl group necessary for the recycling of homocysteine into methionine (Figure 1).[67] The EAR and RDA intakes for vitamin B_6 during pregnancy are 1.6 mg/day and 1.9 mg/day, respectively.[13] Vitamin B_6 supplementation is prescribed to treat nausea and vomiting during pregnancy, often at doses much higher than recommended (up to 40 mg/day).[83] In addition, fetal concentration of plasma pyridoxal 5-phosphate, the metabolically active form of vitamin B_6, was found to be significantly higher during the second and third

trimesters of pregnancy when compared to the mother, implying fetal requisition of the vitamin.[84] However, the risk of deficiency in the infant has been shown to increase as maternal vitamin status declines.[80]

A meta-analysis examined three intervention studies and found that vitamin B_6 supplementation led to an increase of 217 g in mean birth weight.[84] In fact, the case-control study conducted by Shrim *et al.*[83] (2006) found that vitamin B_6 intakes greater than 50 mg/day were not associated with major malformations at birth or any other adverse fetal outcome. In addition, they found that women who consumed a mean dose of 132.3 mg/day of vitamin B_6 gave birth to significantly bigger babies that weighed, on average, 221 g more than the control group.[83] However, when adjusting for maternal weight as a confounder, vitamin B_6 dose did not appear to affect birth weight.[83] Finally, one study found that a mean decrease (0.23 μg/day) in vitamin B_6 intake between 28 and 36 weeks led to a mean decrease in infant birth weight of 59 g.[82] Therefore, vitamin B_6 intake during pregnancy may be important to ensure an infant of optimal birth weight.

Vitamin B_6 deficiency in pregnancy has been linked to preterm birth.[55] A case-control study conducted by Ronnenberg *et al.*[55] (2002) on Chinese women found that less than 3% of participants reported using nutritional supplements during pregnancy and more than 20% of women were deficient for vitamin B_6. In fact, women with adequate plasma pyridoxal 5-phosphate concentration (≥ 30 nmol/L) had a 50% less risk of giving birth to a preterm infant than women who were vitamin B_6 deficient (< 30 nmol/L); however, this result was not statistically significant ($p = .09$).[55] Overall, no significant associations between low birth weight or small for gestational age were found with a deficiency in vitamin B_6.[55]

Vitamin B_6 supplementation may be important for achieving adequate intake during pregnancy. The PREFORM study found that 50% of participants had vitamin B_6 intakes below the EAR (< 1.6 mg/day); however, when examining supplemental intake, women consumed a median amount of 1.9 mg/day, thus meeting the RDA from supplements alone.[63] Additionally, the APrON study, in which the majority of women were consuming a multivitamin or nutritional supplement, found vitamin B_6 deficiency to be extremely rare (< 1%).[65] Finally, additional studies are needed to confirm the role of vitamin B_6 intake during pregnancy on infant birth weight.[84]

1.3.4 Vitamin B_{12} (Cobalamin)

Vitamin B_{12} is involved in the final step of the one-carbon metabolism cycle where it acts as a cofactor for methionine synthase (Figure 1).[7] Without vitamin B_{12}, folate is confined as 5-MTHF, thereby inhibiting methionine regeneration and increasing homocysteine concentration.[7] Folic acid and vitamin B_{12} may be involved in preventing central nervous system disorders including dementia and Alzheimer's disease in the elderly and there is concern that high folate intake will mask a vitamin B_{12} deficiency and subsequently affect the nervous system with conditions such as depression, psychosis and violent hallucinations.[85–87] This is because folic acid may temporarily improve the anaemia caused by a vitamin B_{12} deficiency, but will eventually lead to a severe haematological and neurological relapse.[88] However, the role of vitamin B_{12} in pregnancy including its interaction with other dietary methyl donors is unclear.

Vitamin B_{12} is acquired from animal and microbial foods; therefore, deficiency is common in women who do not regularly consume animal products.[7] The EAR and RDA

for vitamin B_{12} during pregnancy are 2.2 μg/day and 2.6 μg/day, respectively.[13] Vegetarian diets are often low in vitamin B_{12} and methionine and are common in India.[7,89] A unique longitudinal study called the Pune Maternal Nutrition study was conducted in rural regions near Pune, India and attempted to understand the relationship between maternal nutritional status and birth and adulthood outcomes. The study found low vitamin B_{12} concentrations in the second trimester and high maternal folate concentrations in the third trimester were associated with high insulin resistance in offspring at 6-years-old.[90] Additionally, higher maternal folate levels were predictive of increased adiposity in children, in regards to both body fat percentage and fat mass.[90] Thus, varying amounts of these nutrients as well as their interactions with one another may be important in predicting disease risk.

Maternal vitamin B_{12} deficiency is associated with preterm birth,[55] low birth weight and intrauterine growth restriction.[91] In a cohort of Chinese women, the risk of preterm birth was 60% lower among those who had adequate vitamin B_{12} levels ($\geq$ 258 pmol/L) when compared to women with lower concentrations.[55] Moreover, one of the studies conducted by Dhobale *et al.*[41] (2012) in Pune, India found high vitamin B_{12} levels to be significantly associated with preterm birth. Because homocysteine concentrations were also found to be high, the authors speculated a possible defect in the one-carbon metabolism cycle whereby homocysteine was not being remethylated into methionine.[41] Additionally, maternal vitamin B_{12} status during pregnancy is predictive of vitamin B_{12} and homocysteine status in the 2-year-old child.[92] However, other studies conducted primarily on Southeast Asians found no association between vitamin B_{12} concentration

and intrauterine growth restriction,[52,93] gestational age, preterm birth, small for gestational age, birth length or weight.[94]

In Canada, vitamin B_{12} deficiency appears to be uncommon as many women are achieving adequate intake of this nutrient. For instance, the APrON study found vitamin B_{12} deficiency to be extremely rare (< 1%). Results from the PREFORM study found that vitamin B_{12} intake from supplements alone was sufficient to meet the RDA (2.6 μg/day).[63] In a well-nourished woman, maternal B_{12} stores are greater than 1,000 μg whereas the total needs of the fetus are predicted to be only 50 μg.[95] Unlike most dietary methyl donors, no UL exists for vitamin B_{12} for the general population or for pregnant women as no adverse effects from high intake have been recorded.[84] Finally, determining optimal vitamin B_{12} intake during pregnancy and its relation to other dietary methyl donors is essential.

1.3.5 Choline and Betaine

Approximately 60% of methyl groups come from choline, 20% from methionine and 10 – 20% from folate, demonstrating choline's fundamental role as a methyl donor.[2] Thus, choline and methionine, which are both derived solely from food, provide 80% of methyl groups. Betaine, which can be derived from choline or consumed directly from the diet, is employed as the enzyme, betaine homocysteine methyltransferase, where it catalyzes the folate-independent cycle (Figure 1).[1] Choline levels are believed to increase during pregnancy to accommodate for the heightened demands of the fetus.[96]

Similar to folic acid, choline is involved in neural tube closure[97] and neurogenesis and the depletion of either nutrient results in neural cell death.[98] The AI for choline during pregnancy is 450 mg/day.[13] An American study showed NTD risk to be lowest

when women consumed high intakes of choline (> 498.46 mg/day), betaine (> 258.02 mg/day) and methionine (> 2,491.85 mg/day) three months before conception.[97] Additionally, in women who consumed low amounts of folate (≤ 246.39), those who had low choline consumption (≤ 290.41) were four times more likely to give birth to an infant with a NTD than women who consumed high amounts of choline (> 498.46).[97] The authors speculated that deficiencies in methyl donors, not solely folic acid, may play a role in NTD risk.[97] Nevertheless, choline and folate are connected via the one-carbon metabolism pathway. When humans are deficient in choline, they use more 5-MTHF to remethylate homocysteine thereby increasing dietary folate demands.[2] Likewise, when humans are deprived of folate, they derive methyl groups from betaine, thereby increasing the demand for choline.[2] Although there is an increased demand for choline during pregnancy, the PREFORM study found that 87% of women had intakes of choline below the AI.[63] Likewise, a study conducted on pregnant women from Alberta found that only 23% of women met the AI for choline during pregnancy.[99] However, women who consumed at least one egg or 500 mL of milk per day were up to eight times more likely to attain an adequate amount of choline than women who consumed less eggs and milk.[99]

When compared to maternal blood, choline concentration is 10-fold higher in amniotic fluid, suggesting fetal sequestration of the nutrient.[96] High choline and betaine concentrations may not be optimal for pregnancy as increased umbilical choline and betaine concentrations in the third trimester were found to be associated with an increased risk of low birth weight in a group of Dutch women.[100] Because birth weight was positively correlated with dimethylglyine (DMG), the substrate generated after betaine donates its methyl group to homocysteine, the authors speculated that a change in

choline metabolism or placental dysfunction could have been probable causes.[100] Finally, the role of maternal choline and betaine consumption during pregnancy on offspring birth weight has yet to be determined.

1.3.6 Methionine

Methionine is an essential amino acid and is one of the main sources of methyl groups in the diet.[2] In the one-carbon metabolism cycle, methionine is converted into SAM, which supplies the methyl groups needed for DNA methylation (Figure 1).[2] The amino acid cysteine, which can be derived from methionine, is also important for growth and the synthesis of proteins.[101] Thus, the EAR and RDA for methionine during pregnancy are reported in conjunction with cysteine and equate to 20 mg/kg/day and 25 mg/kg/day, respectively.[13]

Methionine concentration measured from amniotic fluid in the second trimester of pregnancy was found to be significantly positively associated with both birth length ($p < .02$) and weight ($p < .001$).[102] The authors observed a significant increase in birth weight with each increasing methionine quartile (< 23.6 μmol/L to > 33.5 μmol/L).[102] Unexpectedly, homocysteine concentration was positively correlated with methionine, but inversely related to both folate and vitamin B_{12}.[102] Additionally, maternal betaine supplementation has been shown to increase serum levels of methionine in piglets as well as increase insulin-like growth factor-2 (IGF2) expression.[103]

Methionine intake during pregnancy may be important for preventing NTDs. One study found women who consumed greater than the lowest quartile for methionine (> 1,341.86 mg/day) were up to 40% less likely to have an infant born with a NTD, irrespective of folate intake.[104] Similarly, an American study conducted on primarily

Caucasian women found methionine intake greater than the lowest quartile (1,580 mg/day) was associated with a 30% reduction in NTD risk.[105] Women who were in the highest quartile for both dietary folate (> 457.36 μg/day) and methionine (> 2,830 mg/day) were roughly 70% less likely to give birth to a child with a NTD compared to those who were in the lowest quartile for both (< 235.79 μg/day and < 1,580 mg/day, respectively).[105] Additionally, being in the highest quartile for methionine (> 2,830 mg/day) and the second highest quartile for vitamin B_{12} (6.59 – 12.55 μg/day) led to a 50% reduction in NTD risk compared to being in the lowest quartile for both (< 1,580 mg/day and < 4.19 μg/day, respectively).[105]

Earlier studies have shown that an imbalance of methionine intake among other amino acids leads to growth retardation in rats.[106–108] Among those, one study found that rats fed a diet supplemented with 0.3% methionine gave birth to offspring that were 10% lighter compared to offspring of dams whose diets were supplemented with 0.1% methionine.[107] The authors also observed significant changes in organ size and noted that offspring birth weight correlated with body size in adulthood.[107] Additionally, consuming high levels of methionine has been shown to alter one-carbon metabolism in mammals.[109] A reduction in folate, vitamin B_{12} and methionine in the maternal diet at the time of conception led to epigenetic modifications associated with greater insulin resistance, adiposity, high blood pressure and altered immune systems in adult sheep offspring.[110] However, it is uncertain whether high levels of methionine are associated with an increase or decrease in methylation patterns or offspring birth weight in humans.[109]

1.3.7 Zinc

DNA methyltransferase obtains a methyl group from SAM and uses zinc as a cofactor to methylate DNA (Figure 1).[8] Therefore, maternal consumption of zinc during pregnancy may influence DNA methylation patterns in the offspring. The EAR and RDA for zinc during pregnancy are 9.5 mg/day and 11 mg/day, respectively.[13] Several studies have reported dietary zinc intakes below the RDA in women of reproductive age and during pregnancy. The majority of women who participated in the Prenatal Health Project were taking a supplement containing zinc (70%) and meeting the RDA for zinc during pregnancy (82%).[62] In fact, women who were not taking a supplement containing zinc had an average intake of 11 mg/day.[62] In contrast, women who were consuming zinc via food and supplement intake averaged 18 mg/day, thus exceeding the RDA.[62] Because a significant proportion of the cohort was not meeting the RDA for zinc (18%) or folate (16%), the authors concluded that daily micronutrient supplement use is crucial to combat the increased nutrient demand during pregnancy;[62] however, the effects of zinc supplementation during pregnancy are unclear.

Because plasma zinc concentrations have been shown to decline throughout pregnancy and plateau near the 22nd week of gestation,[111] some studies have reported zinc supplementation to be beneficial in preventing adverse pregnancy outcomes, while others have found no such effect.[111–113] For instance, maternal leucocyte zinc concentration in the third trimester has been shown to be positively associated with birth weight.[114] In fact, maternal zinc concentration below 120 nmol/10^9 leucocytes strongly predicted fetal growth restriction.[114] Median leucocyte zinc concentration was significantly lower (112 nmol/10^9 leucocytes) in women who gave birth to infants weighing equal to or below the

10^{th} percentile for birth weight compared to leucocyte concentrations of women (229 nmol/10^9 leucocytes) who gave birth to infants weighing above the 90^{th} percentile for birth weight.[114] Moreover, animal studies have classified zinc deficiency as teratogenic because it is capable of disturbing normal embryonic development.[115] However, there is insufficient evidence to conclude that zinc is a teratogen or related to birth weight in humans.[115]

Other studies have concluded zinc supplementation during pregnancy to be ineffective for modifying pregnancy and birth outcomes. A randomized double-blind placebo-controlled trial examined a subgroup of women (n = 580) in Alabama who were primarily low income African American and who were below the median zinc intake for the entire population at the time of enrollment (~16 weeks gestation).[116] The authors found supplementation with 25 mg of zinc per day to be significantly positively associated with infant growth and weight.[116] After adjusting for maternal BMI and gestational age, participants in the zinc supplement group gave birth to babies with significantly increased weight (128 g more) and head circumference (0.49 cm).[116] Using this same cohort,[116] but examining the 3,448 women who were not invited to the randomized control trial, one study compared women who consumed concentrations of zinc in the lowest quartile and women who consumed zinc in the top three quartiles and found no significant differences in birth weight, birth length, head circumference, fetal growth restriction or preterm delivery.[111] Likewise, a randomized control trial conducted on Iranian women examined the effect of 15 mg of zinc beginning in the second trimester and lasting until delivery.[113] The authors found no significant differences in birth weight, birth length, gestational age, macrosomia, preeclampsia or spontaneous abortion.[113]

Another randomized control trial consisting of periconceptional supplementation with Lomapharm, a micronutrient supplement consisting of 15 vitamins and trace elements including vitamin B_2 (1.4 mg), vitamin B_6 (1.9 mg), vitamin B_{12} (2.6 mg), folic acid (400 μg) and zinc (15 mg), found no difference in birth weight, birth length, head circumference or placental weight between the two groups.[117] Finally, zinc supplementation may only be beneficial if a deficiency is present; thus, a complete understanding of dietary profile and its impact on birth weight is crucial.[115]

1.2 Birth Weight

Size and weight at birth are determined by a variety of factors including nutrition during pregnancy.[118] Two indicators of problematic fetal growth rate are small for gestational age (SGA), in which an infant weighs below the 10th percentile for gestational age,[119] and large for gestational age (LGA), in which an infant weighs above the 90th percentile for gestational age.[120] Both of these conditions can be detrimental to offspring health; however, the role of maternal nutrition, particularly that of methyl donors, is unclear.

1.2.1 Other Maternal Dietary Factors and Birth Weight

The principal factor determining intrauterine growth rate is the supply of nutrients from the placenta to the fetus, which depends on placental morphology, size and blood supply.[121] Both placental weight and size are correlated with offspring birth weight[122,123] and intrauterine growth restriction (IUGR) is associated with placental inadequacy.[124] Similarly, maternal undernutrition, specifically insufficient protein intake, has led to decreased placental weight and efficiency and consequently led to reduced birth weight

and IUGR.[115,125–127] In the majority of studies conducted on white Europeans or European Americans, diets high in saturated and trans fats, sodium or refined sugar were negatively associated with birth size whereas diets high in fruits, vegetables and whole grains were positively associated with birth size.[128,129] Presently, research demonstrates that a diet primarily filled with whole foods such as fruit, vegetables, whole grains, lean meat and low-fat dairy, all rich in dietary methyl donors, is most effective at delivering an offspring of appropriate birth weight.[115]

1.2.2 Other Determinants of Birth Weight

Birth weight and size for gestational age are known to be associated with maternal characteristics such as pre-pregnancy BMI, gestational weight gain, maternal height and birth weight, preeclampsia, gestational diabetes, age, race, smoking, coffee consumption and maternal occupation requiring heavy lifting.[130–134] Paternal birth weight and BMI have also been shown to be predictors of offspring birth weight in some studies.[134,135] Pre-pregnancy BMI and gestational weight gain are important factors for predicting offspring birth weight. In fact, researchers found maternal obesity to be the principal risk factor for conceiving a large for gestational age infant[136] and demonstrated its capability of modifying the *in utero* environment.[115] Additionally, women who gain excess weight during pregnancy (i.e. above the guidelines proposed by the Institute of Medicine[137]) are more likely to give birth to LGA infants.[138,139] However, obese women (BMI $\geq$ 30 kg/m^2) who gain less than the recommended amount or lose weight during pregnancy are more likely to give birth to small for gestational age infants.[140,141] Finally, modification strategies to achieve optimal gestational weight gain seem to reduce the risk for large for gestational age infants, yet are ineffective at preventing small for gestational age births.[115]

Maternal birth weight is an important predictor of offspring birth weight and perinatal morbidity. Two studies examined the Swedish Birth Registry and found that women born LGA were 2.6 times more likely to give birth to offspring that were LGA[136] and women born SGA were three times more likely to give birth to SGA infants.[142] In fact, a Norwegian cohort found that for every additional 100 g of maternal birth weight, infant birth weight increased by 28 g on average.[143] Additionally, mortality rates were three times higher in babies born 2,500 to 2,999 g if their mother was born large (≥ 4,000 g) when compared to if their mother was born between 2,500 and 2,999 g.[143] However, mothers who weighed less than 2,000 g at birth were at greatest risk of perinatal loss in their own pregnancies.[143] Finally, illuminating the role maternal dietary patterns and socio-demographic characteristics play on infant birth weight is crucial.

1.2.3 Future Health of Low and High Birth Weight Infants

A poor intrauterine environment increases the risk of developing chronic diseases later in life. Low birth weight is associated with poor cognition in childhood,[144] low educational achievement[145] and an increased risk for diabetes[146] and high blood pressure in adulthood.[147,148] In particular, low birth weight has been shown to increase the risk for neurodevelopmental disorders such as autism and schizophrenia,[149–151] yet remains unrelated to certain mental conditions such as depression and anxiety.[152] This individuality of the outcome may partly be due to differences in epigenetic changes.[153,154] Indeed, weight at birth is an important predictor of health and achievement later in life.[155] Increasing birth weight amongst lower weight babies leads to causative increases in height, educational attainment and income in adulthood.[155,156] Infants born large for gestational age have an increased risk for developing illnesses in adulthood including

cardiovascular disease, prostate and breast cancer, type 1 and type 2 diabetes and obesity.[157–165] In conclusion, being born SGA or LGA increases the risk of developing metabolic and neurodevelopmental disorders.[166] However, the relationship between birth weight and maternal dietary patterns remains inconclusive as the overall quality of evidence is low.[167]

1.3 Fetal Programming and Epigenetics

Embryogenesis is a critical period that is vulnerable to environmental cues, especially nutrition.[168] The Developmental Origins of Health and Disease hypothesis argues that environmental influences play an essential role in fetal programming and can lead to permanent changes in metabolism and subsequently, to changes in susceptibility to adult diseases.[1] Nutrition is a major environmental factor capable of inducing *in utero* modification.[168] In fact, the long-term impact of the early life environment on predicting disease risk has been shown to be due, in part, to epigenetic modifications.[169] Epigenetic changes refer to heritable differences in gene expression irrespective of variations in gene sequence, such as DNA methylation, histone modification and chromatin remodelling.[3] The epigenome encompasses all chemical alterations of a cell involved in regulating gene expression.[168] It is becoming apparent that epigenetic changes constitute a major link between early environmental exposure, including nutrition, and disease development later in life.[3]

As previously mentioned, dietary methyl donors including methionine, folate and choline, as well as cofactors including vitamins B_2, B_6 and B_{12} and zinc are all involved in the one-carbon metabolism process whereby they play a crucial role in DNA methylation (Figure 1). In fact, maternal consumption of these methyl donors has led to

increased DNA methylation and differential gene expression in animal studies.[109] The challenge with epigenetic regulation via nutrition is that epigenetic changes are tissue-specific and differentially affected at distinct stages of development.[109] Therefore, specific nutritional differences such as high dietary methyl donor intake, will likely only affect gene expression at certain life stages, in specific tissues and at certain gene regions.[109] Nevertheless, the previously perceived notion that all dietary supplementation is beneficial may lead to unintentional epigenetic modifications in humans.[109] Therefore, specific changes in the one-carbon metabolism cycle during the periconceptional period can lead to long-term consequences for adult offspring health.[110]

1.3.1 DNA Methylation

Dietary methyl donors have a crucial impact on methylation of the DNA, which is the addition of methyl groups to the cytosine residues of the cytosine-guanosine (CpG) dinucleotides.[1] CpG islands occur near promoters in roughly half of the genes in the genome, namely the housekeeping genes.[170] These islands contain many CpG dinucleotides and are primarily unmethylated.[170] However, methylation of CpG islands near promoters can silence gene expression by inducing chromatin condensation.[171] Since most human transcription factors have CG-rich binding sites, methylation of these regions can inhibit the factors' abilities to access the DNA, thus repressing transcription of the genes.[171] Differences in one-carbon metabolism during pregnancy can significantly affect DNA methylation in the offspring leading to long-term consequences for health.[110] During pregnancy, when DNA methylation patterns are undergoing development and maturation, any nutritional factor that affects the transfer of methyl groups to DNA can result in permanent changes to CpG methylation.[172] Additionally, it has been shown that

nutritional supplementation during pregnancy can increase methylation of specific genes and therefore permanently alter gene expression.[109]

1.3.2 Results from Animal Studies

It has been shown that diets deficient in methyl donors lead to global DNA hypomethylation in rodents.[168] The *agouti* mouse model is a classic example demonstrating the importance of fetal programming and changes in epigenetics.[168] The mouse *agouti* (A^{vy}) alleles regulate the production of black pigments in single hair follicles.[173] Homozygous, black females were mated with heterozygous mottled yellow or pseudoagouti males to produce black and various *agouti* phenotypes of mice.[173] Black female mice were fed a spectrum of diets containing different concentrations of folic acid, vitamin B_{12}, betaine, choline, zinc and methionine up to two weeks before the first date mating and during pregnancy.[173]

The mice that were fed a diet low in methyl donors gave birth to offspring with hypomethylation upstream of the *agouti* gene, resulting in yellow hair colour. The yellow mice are known to have obesity, excess insulin circulating in the blood, a greater vulnerability to cancer and a shorter lifespan.[174] In contrast, the pregnant mice that were fed a diet rich in methyl donors gave birth to offspring with hypermethylation at the *agouti* gene, resulting in a darker coat colour. These darker, pseudoagouti mice are known to be lean, healthy and have a longer lifespan.[174] Thus, significant changes in A^{vy} methylation can be seen through fairly modest alterations in maternal dietary methyl donors and cofactor supplementation.[109] Although this study shows the benefits of high dietary methyl donor supplementation in mice, the results cannot be extrapolated to

humans whereby the effects of low and high dietary methyl consumption during pregnancy remain largely unknown.

Offspring born to Wistar rats that consumed a high multivitamin intake during pregnancy and were weaned to an obesogenic diet, showed greater food intake, body weight, glucose intolerance and heightened blood pressure than rats that did not consume a vitamin-rich diet during pregnancy.[175] It is not clear, however, which vitamins or minerals had a causal effect. Furthermore, a study conducted by Penailillo *et al.*[73] (2015) on Wistar rats found that female offspring whose mothers consumed a diet high in folic acid (8 mg/kg) had a 6% higher birth weight than female offspring whose mothers consumed a diet low in folic acid (1 mg/kg).[73] Differential methylation patterns were also observed in males and females emphasizing a sex-specific effect of folic acid supplementation on DNA methylation.[73] Other studies have found similar findings resulting in higher offspring birth weight due to high levels of prenatal supplementation with folic acid.[176,177]

1.3.3 Results from Human Studies

In humans, a classic example of fetal programming by nutrition is shown by changes in the health of offspring whose mothers were pregnant during the Dutch Hunger Winter. This famine arose in the Netherlands in 1944 and illustrates how the timing of nutritional restriction during pregnancy is meaningful in predicting future disease risk. Individuals whose mothers experienced famine in early pregnancy had a greater risk of coronary heart disease[178] and obesity later in life.[179] However, those whose mothers experienced famine later in pregnancy gave birth to smaller birth weight infants and exhibited a greater prevalence of insulin resistance and hypertension.[3] In comparison to

their unexposed siblings, individuals exposed to the famine prenatally had hypomethylation of the gene IGF2, which plays an important role in growth and development.[180] Therefore, the timing of restricted fetal nutrition may be important in predicting adverse outcome risk.

Additionally, factors involved in one-carbon metabolism are important for predicting DNA methylation patterns in the infant. For instance, a multi-ethnic cohort found DNA methylation at several differentially methylated regions to be associated with maternal folate consumption and offspring birth weight.[58] In fact, increases in maternal folate consumption were significantly related to increases in infant birth weight as well as higher methylation at two regions (PLAGL1 and MEG3).[58] Additionally, hypermethylation at four regions (H19, PEG10/SGCE and PLAGL1) and hypomethylation at two regions (MEG3 and NNAT) were associated with increased weight at birth.[58] The authors concluded that the relationship between maternal folate intake and infant birth weight was mediated by the differential methylation at MEG3.[58]

Another study conducted in the UK found folic acid supplementation after 12 weeks of gestation to be associated with a higher level of methylation in IGF2 in the offspring when compared to offspring whose mothers did not continue folic acid supplementation after 12 weeks of gestation.[181] In fact, folic acid supplement use up to 12 weeks of gestation did not appear to alter DNA methylation in the offspring.[181] Conversely, another study found children born to mothers who consumed the recommended 400 μg of folic acid per day periconceptionally had 4.5% greater methylation of IGF2 when compared to children born from mothers who did not consume folic acid in the periconceptional period.[182] The authors found that hypermethylation at

IGF2 correlated with lower birth weights.[182] Therefore, maternal consumption of dietary methyl donors during pregnancy can have significant effects on DNA methylation and birth weight in the offspring.

1.4 Study Rationale

The Developmental Origins of Health and Disease paradigm argues that nutrition and other environmental factors play an essential role in fetal programming.[183,184] The significance of this research is to ultimately gain a better understanding of the critical role maternal nutrition, particularly that of methyl donors, plays during pregnancy and its subsequent impact on infant birth weight. As both low and high birth weights can have significant long-lasting effects on the child, determining the role of dietary methyl donor consumption during pregnancy is important. Additionally, because dietary methyl donors and cofactors are involved in the complex and interwoven pathways of one-carbon metabolism, identifying patterns of intake in relation to birth weight is crucial. Finally, limited studies have assessed the impact of maternal dietary patterns during pregnancy and their subsequent effects on the fetus. Thus, this study was designed to answer the following research question: What is the association between patterns of dietary methyl donor intake during pregnancy and offspring birth weight in the 3D (Découvrir, Développer, Devenir) study?

1.5 Hypothesis

Low and high dietary methyl donor intake during pregnancy leads to a greater frequency of small for gestational age and large for gestational age infants in the 3D study.

1.6 Objectives

1) To identify the patterns of dietary methyl donor intake in women during the second trimester of pregnancy.

2) To determine the association between dietary methyl donor patterns and birth weight, including small and large for gestational age at birth.

Chapter 2: Methodology

This chapter provides a brief outline of the methods used to recruit patients and collect all data pertinent to the research question. The statistical analyses including normality of the distributions, outlier analysis, principal components analysis, covariate and interaction term identification are presented. The use of Detection of Informative Combined Effects (DICE) software in the statistical program R for further interaction term analysis is described. Finally, general linear and logistic regression models used to test the relationship between dietary methyl donors and birth weight, small and large for gestational age are presented.

2.1 Data Collection

The Integrated Research Network in Perinatology of Québec and Eastern Ontario (IRNPQEO) is a collaborative group of experts who aim to elucidate the impact of different exposures during the pre and postnatal period. The multidisciplinary team, who developed the 3D multicenter longitudinal study, involves researchers from McGill University, University of Montréal, Laval University, University of Sherbrooke and the University of Ottawa. In total, 2,456 pregnant women were recruited in the cohort study, 1,612 of whom completed detailed three-day food records. Families were recruited from the nine sites participating in this study: The Centre Hospitalier Universitaire (CHU) St. Justine, the CHU of Montréal (St. Luc Hospital), the CHU of Sherbrooke, the CHU of Québec, the CHU of McGill, Royal Victoria Hospital, Sacré-Cœur Hospital, St. Mary's Hospital Center, the Jewish General Hospital of Montréal. Women had to be between 8 and 14 weeks pregnant, between 18 and 50 years of age, fluent in either English or French and be monitored and give birth in one of the hospitals listed above to be accepted

into the study. Women were excluded from the study if any of the following situations applied to them: planned to donate or bank their cord blood, engaged in intravenous drug use, had renal disease or altered renal function, tested positive for HIV, had collagen vascular disease, cancer, a severe hematologic disorder or multiple gestations.

Eligible women were monitored during each trimester, at birth and her child was assessed three months, one and two years after delivery. During these visits, women had to complete socio-demographic and lifestyle questionnaires, provide biological samples and allow anthropometric measurements, blood pressure and glucose tolerance to be assessed. Participants also had to authorize the access to personal medical records for the mother, father and child. Demographics and socioeconomic status were assessed using questionnaires. Health history and personal habits, such as coffee, tea, cigarette and alcoholic beverage intake in each trimester were self-reported at each trimester. Ethnicity and pre-pregnancy weight were self-reported. Maternal height was measured at the first prenatal visit. Pre-pregnancy BMI (kg/m^2) was determined using measured height in metres and self-reported weight in kilograms.

2.2 Dietary Assessment

From May 2010 to September 2012, 1,612 pregnant women completed detailed three-day food records, which documented all food and beverage intake for two weekdays and one weekend day during the second trimester of pregnancy. Study personnel instructed participants on how to complete a three-day food record and return the diary within one week of completion. The food records were photocopied at each centre, sent to the CHU St. Justine and subsequently to the University of Ottawa for analysis. The chronicled food and beverage intake from the diary was entered into a

nutrient analysis program, ESHA Food Processor™ (version 10.13.1.0, ESHA Research, Oregon) using foods primarily listed in the 2010 Canadian Nutrient File (CNF) database. Three trained registered dietitians (RD) completed data entry under the supervision of an experienced research coordinator (also an RD).

Dietary methyl donor intake during gestation was derived by averaging the three-day food and beverage intake into mean micronutrient daily intake using the ESHA Food Processor™ for the following nutrients: methionine, choline, zinc, vitamins B_2, B_6, B_{12}, and B_9 (folate). The Canadian Nutrient File (CNF) is incomplete (< 50%) for choline, however it was included in analyses since the CNF documented the principal sources of choline (liver and eggs);[13] thus, it is likely that the missing values are from foods that contain minimal or negligible amounts of the nutrient. In addition, each participant's methyl donor intake was divided by their caloric intake and then multiplied by 1,000 to obtain dietary methyl donor intake per 1,000 kcal for each individual. Vitamin, mineral and nutritional supplement intake three months prior to becoming pregnant and during the second and third trimesters have been documented using the mother medication log. However, the information from these documents was not available at the time the data was analyzed; thus, supplement intake was not assessed.

2.3 Outcomes Assessment

Small and large for gestational age (SGA and LGA, respectively) were determined using gestational age and weight at birth. SGA was defined as an infant weighing below the estimated 10th percentile as defined by the SOGC.[185] LGA was defined as an infant weighing above the estimated 90th percentile for gestational age. The contemporary standard provided by Kramer *et al.*[120] (2001) was used. It is sex-specific

and was established using early ultrasound estimates for various gestational ages from the Canadian national linked file for singleton births and infant deaths from 1994 to 1996.

2.4 Statistical Analyses

Statistical analyses were performed using Statistical Package for the Social Sciences® (SPSS version 23.0) to identify patterns of dietary methyl donor intake as well as determine if there were associations between patterns of dietary methyl donor intake and birth weight, small and large for gestational age. The program R (version 3.2.3) was used to determine parsimonious models for predicting birth weight, SGA and LGA and the package, Detection of Informative Combined Effects (DICE), in R was used to identify two-way and three-way interactions between predictor variables and covariates.

2.4.1 Univariate Analyses

Initially, univariate analyses were performed on all continuous predictor (folic acid, folate, dietary folate equivalent, vitamins B_2, B_6, B_{12}, choline, methionine and zinc) and outcome (birth weight) variables. The mean, standard deviation, skewness, kurtosis and Shapiro-Wilk P-values were computed (Table I: Appendix B). Histograms, Q-Q plots and box plots were used to visually assess normality as well as the presence of outliers. Univariate analyses were also performed on calorie-adjusted dietary methyl donors (Table II: Appendix B). The Shapiro-Wilk test of normality assumes a null hypothesis that the sample came from a normally distributed population. The null hypothesis was rejected for all variables ($p < .001$). Nevertheless, upon visually inspecting the histograms and Q-Q plots, birth weight and all dietary methyl donors, with the exception of vitamin B_{12}, appeared to be normally distributed (Figure 2: Appendix B; Figure 3: Appendix B).

Additionally, the central limit theorem implies that large sample sizes (> 30 or 40) that violate the assumptions of normality are not likely to be problematic in parametric testing.[186]

Absolute values of skewness and kurtosis were computed and an absolute skewness value > 2 or an absolute kurtosis value > 7 were used as reference values when assessing non-normality.[187] Vitamin B_{12} was the only variable with absolute skewness (5.8 ± 0.07) and kurtosis (57.8 ± 0.13) values above the reference values. In terms of vitamin B_{12}, 23 observations exceeded ±2.58 standard deviations (SD) from the mean. When these observations were removed, the histogram, Q-Q plot and box plot all appeared to be similar to those depicting a normal distribution (Figure 4: Appendix B). The 23 outliers were evaluated separately to identify any demographic or socioeconomic differences in these women, but no significant differences were observed (Table III: Appendix B). To ensure a uniform sample size, these women were removed from all subsequent analyses.

Preterm Infants

Seventy-six (76) preterm infants were identified in the sample. The chi-square test of independence and independent samples t-tests were used to determine if women who had preterm infants differed socioeconomically or demographically from women who had infants at term. No significant differences ($p \geq .05$) in age, income, marital status, pre-pregnancy BMI, parity or maternal birthplace were observed (Table IV: Appendix B). However, women who were not educated past secondary school were more than twice (Odds Ratio 2.72 (1.34, 5.54)) as likely to have an infant born preterm than women with higher education. Additionally, women who were diagnosed with gestational

diabetes during pregnancy were twice (2.15 (1.15, 4.03)) as likely to give birth to a preterm infant. Smokers were 88% (1.88 (1.06, 3.35)) more likely to give birth to a preterm infant than those who did not smoke during pregnancy. Furthermore, independent samples t-tests showed no significant differences in neither mean dietary methyl donor intake (Table V: Appendix B) nor in calorie-adjusted dietary methyl donor intake (Table VI: Appendix B) between women who had preterm infants and women who had term infants. Nevertheless, due to differences in socioeconomic, physiological and lifestyle factors between women giving birth to preterm and term infants, preterm infants were removed from subsequent analyses.

2.4.2 Adequate Intake Based on DRIs

Mean intakes of dietary methyl donors (folic acid, dietary folate equivalent, vitamins B_2, B_6, B_{12}, choline, methionine and zinc) were computed and assessed for adequate intake, hence meeting the DRIs during pregnancy. The EAR cut-point method is used when assessing adequate nutrient intake in normally distributed populations.[13] Individuals consuming values below the EAR are assumed to be at risk for inadequate intake of the nutrient, while those consuming values at or above the EAR are assumed to have adequate intake. The EARs were used for dietary folate equivalent, vitamins B_2, B_6, B_{12} and zinc and the AI was used for choline. Because there is insufficient information to determine an EAR value for choline, individuals who consumed nutrient values at or above the AI for choline were assumed to be meeting nutrient demands; however, no inference about inadequacy can be made about individuals who consumed nutrient values below the AI.[13] Methionine adequacy was not assessed because the EAR includes cysteine and is weight-dependent. Cysteine intake was not measured and participant

weight was not calculated at the time the food record was completed. Demographic and socioeconomic differences in women who were consuming above or below the estimated average requirement for these nutrients was examined.

2.4.3 Principal Components Analysis

Principal components analysis (PCA) was performed to determine the relationship amongst methyl donors. PCA was performed as a variable-reduction technique to determine if any of the dietary methyl donors could be reduced to a smaller set of variables or "components" that would explain most of the variance previously explained by the original variables. A minimum eigenvalue of 1.0 was used to define a component. Spearman's correlation matrix was computed to determine if any of the dietary methyl donors were correlated with one another (Table VII: Appendix B). Several of the correlation coefficients were high ($r > 0.5$); therefore, it was assumed the factors were correlated. Because oblique rotation assumes the factors are correlated, Direct Oblimin was used. Factor Analysis was computed three separate times: with folic acid and all other dietary methyl donors, with dietary folate equivalent and all other dietary methyl donors and with folate and all other dietary methyl donors.

The assumptions for PCA (use of continuous variables, linear relationship between variables, sampling adequacy, suitability for data reduction, absence of significant outliers) were assessed and did not appear to be violated. The Bartlett's Test for Sphericity assumes the null hypothesis that the correlation matrix is an identity matrix meaning the variables are orthogonal and cannot be reduced into factors. When tested, the null hypothesis was rejected ($p < .05$) for each of the models. The Kaiser-Meyer-Olkin Measure (KMO) assesses sampling adequacy whereby it compares the partial

correlation between two variables with the observed correlation values. The KMO for each model was greater than 0.83, above the proposed value of 0.5; therefore, the variables were suitable for dimension reduction.

2.4.4 Covariates

Putative covariates (maternal age, pre-pregnancy BMI, household income, maternal education, parity, maternal birthplace (born in Canada or not), smoking, gestational diabetes mellitus, gestational age at delivery and infant sex) were tested to determine if they were significantly related to birth weight, SGA, LGA or one of the dietary methyl donors. Covariates were assessed for significance with either a predictor or an outcome variable using general linear models and logistic regressions (Table VIII: Appendix B). Variables found to be significant predictors of either birth weight, SGA or LGA were the following: pre-pregnancy BMI, maternal education, parity, smoking, gestational age at delivery and infant sex.

The categorization of covariates was determined using Fisher's Least Significant Difference (LSD) post-hoc tests (not shown). Household income and mother born in Canada or not were significantly associated with at least one predictor variable and were included in the model. Although marital status was significant with at least one predictor variable, it was not included in the final model as most (96%) of the participants were in a relationship and any differences in dietary intake are likely mediated by other socioeconomic factors.[188,189] Maternal age was significantly associated with both folic acid and folate, but not with dietary folate equivalent or with any other predictors. Hence, it was included solely in the models when folic acid and folate were included as

predictors. Having been diagnosed with gestational diabetes mellitus during pregnancy was not significant with any predictor or outcome variables.

2.4.5 Interactions

Interactions between covariates and dietary methyl donors were tested using hierarchically well-formulated regression models predicting birth weight, SGA and LGA models. Multiplicative interactions between predictor variables and covariates on birth weight were determined using unadjusted general linear models (Table IX: Appendix B). When assessing significance in predicting birth weight, significant ($p < .05$) two-way interactions between vitamin B_{12} intake and maternal birthplace (Figure 5: Appendix B) as well as vitamin B_{12} intake and smoking (Figure 6: Appendix B) were found. Additionally, gestational age at delivery significantly ($p < .05$) interacted with vitamin B_6 intake and choline intake, respectively.

Spearman's correlation matrix was used to identify relationships between continuous and interval covariates (maternal age, gestational age at delivery, pre-pregnancy BMI, maternal education, household income and parity) and dietary methyl donors. Interaction terms suspected of being collinear ($r > 0.5$) along with putative interactions between dietary methyl donors and dichotomous covariates (infant sex, maternal birthplace and smoking) were tested in unadjusted logistic regression models predicting SGA and LGA (Table X: Appendix B; Table XI: Appendix B). Two-way interactions between folate and sex as well as between vitamin B_6 and sex were found to be significant ($p < .05$) in the model predicting SGA. Likewise, two-way interactions between folate and maternal birthplace as well as choline and smoking were found to be

significant ($p < .05$) in the model predicting LGA. Therefore, these respective interactions were forced in the final SGA and LGA models.

The software DICE in the statistical program R was also used to identify all significant multiplicative and multi-term interaction terms. DICE was used to examine all possible two-way and three-way interactions between covariates and predictor variables (with DFE as the folate form included in the model) and the program R was used to develop the most parsimonious models to predict birth weight, SGA and LGA. First, continuous predictor variables were centered (i.e. the mean was subtracted from each case) to mediate multicollinearity effects and facilitate the interpretation of the interaction terms. Next, significant interaction terms were computed for birth weight. Two-way significant interaction terms were as follows: vitamin B_6 and maternal age ($p = .001$), choline and BMI ($p = .02$), methionine and maternal age ($p = .01$), zinc and income ($p = .04$), gestational age and infant sex ($p = .01$) and gestational age and maternal birthplace ($p = .04$). Several significant three-way interaction terms were found as well (not shown). Similarly, when computing interaction terms between predictor and covariates in SGA and LGA models, several significant two-way and three-way interaction terms were identified (not shown). Significant two-way and three-way interaction terms were included in the R-computed models predicting birth weight, SGA and LGA.

2.4.6 General Linear Models and Logistic Regressions

General linear models and logistic regressions were used to test the relationship between dietary methyl donors separately on birth weight, SGA and LGA in both SPSS and R. Subsequent analyses incorporated significant covariates and interaction terms into the models. The analyses computed in SPSS were tested in three separate models: The

first model was unadjusted in which the dietary methyl donor was incorporated as the sole predictor of birth weight. The next model included the dietary methyl donor and adjusted for significant covariates (pre-pregnancy BMI, gestational age at delivery, maternal birthplace, household income, maternal education, parity and smoking). Finally, the third model adjusted for all dietary methyl donors, covariates and previously determined interaction terms for each respective outcome. Folic acid, folate and dietary folate equivalent were tested individually and in separate models. Additionally, the statistical software R was used to model the relationships between predictor and outcome variables whereby no terms were forced into the models predicting birth weight, SGA or LGA. However, up to three-way interaction terms were computed.

The assumptions of linear regression (use of continuous variables, linear relationship between variables, no significant outliers, independence of observations and homoscedasticity) were tested and none of the variables (dietary methyl donors or birth weight) appeared to violate any of these assumptions. A plot of the residuals was computed from the fully adjusted model containing DFE (Figure 7: Appendix B). Data points are symmetrically distributed and tend to cluster along the y-axis zero line suggesting the variance around the regression line is similar for all values of the predictor variables. The assumptions of logistic regression (independence of observations and a linear relationship between an independent variable and log odds) were assessed and none of the variables (dietary methyl donors, SGA or LGA) appeared to violate any of the assumptions.

Chapter 3: The Association Between Dietary Methyl Donor Intake and Infant Birth Weight

The aim of this chapter is to provide a detailed account of the results for the first and second objectives. Firstly, the maternal and neonatal characteristics of the study population are presented. Secondly, the patterns of dietary methyl donor intake are explained including demographic and socioeconomic differences in adequate and inadequate nutrient consumers. Finally, the association of dietary methyl donor intake and resulting patterns of intake with birth weight, small for gestational age and large for gestational age are presented.

3.1 Results

3.1.1 Characteristics of the Study Population

Of the 2,456 eligible pregnant women involved in the 3D study, 1,612 of them completed three-day food records. Demographic characteristics (Table 1) of the population show that the mean age of participants was 32, the majority were in a relationship, nulliparous, born in Canada, non-smokers, did not have gestational diabetes and were of normal weight according to Health Canada's categories for BMI.[190] Socioeconomic characteristics (Table 1) show that the majority of women were educated beyond secondary school and living in a household earning $80,000 or more annually. Neonatal characteristics (Table 2) show that there were roughly equal male and female births, that most were born at term and were in the normal range for birth weight (2,500 g – 4,000 g) as defined by the Centers for Disease Control and Prevention.[191] Approximately 6% of infants were born preterm (< 37 weeks), 9% were born SGA and

7% were born LGA. After removing participants with missing values of the following: missing or incomplete food diaries, maternal age, education, household income, pre-pregnancy BMI, smoking type, gestational diabetes, gestational age at delivery, infant sex, birth weight or if they were deemed as misreporters by using the Goldberg method,[192] a final subset of 1,233 women was used for this analysis.

3.1.2 Patterns of Dietary Methyl Donor Intake

Dietary Methyl Donor Intake According to Dietary Reference Intakes

The means and standard deviations of dietary methyl donor intake from the three-day food records of the study participants along with DRIs for pregnant women were analyzed (Table 3). From food sources alone, most women appeared to be meeting the recommended intake (i.e. above the EAR) during pregnancy for vitamin B_2, vitamin B_6, vitamin B_{12} and zinc. Most women were below the EAR for dietary folate equivalent (520 µg/day) and below the AI for choline (450 mg/day). Weight-dependent methionine EAR values were not calculated and thus identifying if women were obtaining adequate amounts of methionine could not be determined.

Dietary Methyl Donor Intake in Reference to Demographic and Socioeconomic Factors

The chi-square test of independence was used to determine if women who had adequate or inadequate methyl donor intakes differed according to the following dichotomous variables: maternal birthplace, marital status, maternal education, household income, parity, smoking and gestational diabetes. The independent samples t-test was used to determine if women who consumed adequate versus inadequate amounts of methyl donors differed by age or pre-pregnancy BMI. As most women consumed

adequate amounts of vitamin B_2 and vitamin B_{12} and inadequate amounts of choline, differences between adequate and inadequate consumers for these nutrients were not analysed.

When determining the differences in demographic and socioeconomic factors for women who consumed adequate versus inadequate amounts for dietary folate equivalent (Table 4), vitamin B_6 (Table 5) and zinc (Table 6), significant differences were observed for maternal birthplace and education. Women who were born in Canada were 48% (1.48 (1.11, 1.96)) more likely to consume adequate amounts of dietary folate equivalent, 68% (1.68 (1.28, 2.20)) more likely to consume adequate amounts of vitamin B_6 and twice as likely (2.07 (1.57, 2.74)) to consume adequate amounts of zinc when compared to women who were not born in Canada. Furthermore, women who were educated beyond secondary school were 68% (1.68 (1.01, 2.80)) more likely to consume adequate amounts of vitamin B_6. Women with significantly higher pre-pregnancy BMIs (0.8 ± 0.5, $p = .01$) consumed less (below the EAR) vitamin B_6 than women with lower pre-pregnancy BMIs.

Principal Components Analysis

Principal components analysis was computed three times: with folic acid, folate and dietary folate equivalent along with vitamins B_2, B_6, B_{12}, choline, methionine and zinc (Table 7). When folic acid was incorporated into the model, two components were extracted: one that contained folic acid and one that did not. The first component had an eigenvalue of 3.9, explained 56% of the variance, did not contain folic acid, but rather vitamins B_2, B_6, B_{12}, choline, methionine and zinc. All variables were highly correlated ($r > 0.76$) with the factor. The second extracted component had an eigenvalue of 1.1, explained 15% of the remaining variance and consisted of folic acid, vitamin B_2 and

vitamin B_6. Folic acid was highly correlated ($r = 0.98$) with the factor, whereas vitamin B_2 ($r = 0.28$) and vitamin B_6 ($r = -0.13$) were not. When folate was incorporated into the model, one component with an eigenvalue of 4.2 explaining 61% of the variance was extracted. All dietary methyl donors were highly correlated ($r > 0.75$) with the factor, with folate being slightly less correlated ($r = 0.62$). Similarly, when dietary folate was incorporated into the model, one component with an eigenvalue of 4.1 explaining 59% of the variance was extracted. All of the variables were highly correlated ($r > 0.75$) with the factor although slightly less for dietary folate equivalent ($r = 0.50$). As previously noted, the synthetic form of the vitamin, folic acid, is found in enriched flour, cornmeal and pasta whereas the natural form, folate is found mainly in fruits, vegetables and legumes. Thus, the differences in correlations may reflect varying food sources of folate and folic acid.

3.1.3 Dietary Methyl Donor Patterns and Birth Weight

The relationships between dietary methyl donor patterns and birth weight, small for gestational age and large for gestational age were tested using principal components regression. By using this method, we were able to test if patterns of dietary methyl donor intake had any effect on infant birth weight while avoiding any effect multicollinearity would have had on the results.[193] The four regression coefficients extracted from principal components analysis were employed as predictor variables in linear regression models to assess their predictive value of infant birth weight (Table 8). Both models containing either folate or dietary folate equivalent yielded positive regression coefficients that significantly predicted birth weight. Conversely, neither of the two

regression coefficients produced from the model containing folic acid were significant predictors of birth weight.

Similar results were obtained when dietary methyl donors were tested separately as predictors of infant birth weight using general linear models (Table 9). Unlike folic acid, folate and dietary folate equivalent were both positively associated with weight at birth. In fact, for every 1 microgram increase in folate or DFE, infant birth weight increased anywhere from 0.2 to 0.4 g on average. Zinc and vitamin B_{12} were significant predictors of birth weight in the covariate-adjusted and the interaction-adjusted models, respectively. When adjusting for covariates, every 1 milligram increase in zinc predicted a 7.9 g ± 3.7 increase in birth weight. However, after adjusting for interaction terms and other dietary methyl donors, this association was no longer significant. Nevertheless, for every 1 microgram increase in vitamin B_{12} an increase of 43.3 g ± 19.7 in infant birth weight was predicted. No calorie-adjusted dietary methyl donors were significant predictors of birth weight in any of the three unadjusted, covariate-adjusted or interaction-adjusted models (Table 10).

The program R was used to determine the most parsimonious model for predicting birth weight. Without forcing any predictors or covariates into the model, the resultant calculation was as follows whereby "age" signifies gestational age and "*" denotes an interaction:

y = age + parity + pre-pregnancy BMI + infant sex + zinc + age*zinc + education*income

Zinc was the only dietary methyl donor incorporated into the model along with its interaction with gestational age at birth. These results are consistent in the SPSS-derived models whereby vitamin B_{12} and zinc appear to be the most important nutrients for predicting infant birth weight.

3.1.4 Dietary Methyl Donor Patterns and Small for Gestational Age

The four regression coefficients extracted from principal components analysis were used as predictor variables in logistic regression models to examine the relationship between patterns of dietary methyl donor intake and small for gestational age (Table 8). Of the two components extracted from folic acid, only the regression coefficient from the one without folic acid was significant in predicting SGA risk. Additionally, the other two regression coefficients extracted from components that contained either folate or DFE were significant predictors of SGA in the infant. Overall, these regression coefficients reduced the risk of small for gestational age by roughly 20% (0.8 (0.6, 0.9)).

The relationships between each dietary methyl donor and small for gestational age were assessed using logistic regressions (Table 11). Folate and dietary folate equivalent were significant predictors of SGA in the covariate-adjusted model. However, their odds ratios were nearly equivalent to 1.0, suggesting a null effect. Zinc and vitamin B_2 significantly reduced the risk for small for gestational age in both unadjusted and covariate-adjusted models by roughly 9% and 34%, respectively. Methionine significantly reduced the risk for SGA, but after adjusting for covariates, this significance was lost. Overall, incremental increases in vitamin B_2, vitamin B_6, methionine and zinc appeared to reduce the risk for SGA anywhere from 10 – 60% depending on the methyl donor and model. However, vitamin B_6 was the only nutrient that remained significant

after adjusting for all covariates, dietary methyl donors and interaction terms and was associated with a 62% (0.38 (0.16, 0.91)) reduced risk for SGA. None of the calorie-adjusted dietary methyl donors were significant predictors of small for gestational age in any of the three unadjusted, covariate-adjusted or interaction-adjusted models (Table 12).

The program R was used to determine the most parsimonious model predicting SGA. Without forcing any predictors or covariates into the model, the resulting calculation was as follows, whereby "diabetes" signifies gestational diabetes, "age" signifies maternal age, "birthplace" signifies maternal birthplace and "*" denotes an interaction:

$$y = \text{parity} + \text{smoking} + \text{diabetes} + \text{DFE} + \text{age} + \text{methionine} + \text{zinc} + \text{birthplace} + \text{education} + \text{smoking*diabetes} + \text{diabetes*DFE} + \text{diabetes*age} + \text{DFE*age} + \text{diabetes*methionine} + \text{age*zinc} + \text{age*birthplace} + \text{zinc*birthplace} + \text{zinc*education} + \text{diabetes*DFE*age} + \text{age*zinc*birthplace} + \text{education*vitamin } B_2$$

Dietary folate equivalent, methionine and zinc along with the interaction between vitamin B_2 and maternal education were the dietary methyl donors with the most predictive value of small for gestational age. These nutrients were also involved in several significant interactions with socio-demographic characteristics. These results are consistent with the SPSS-derived models and imply dietary folate equivalent, vitamins B_2, B_6, methionine and zinc to be the most important dietary methyl donors for predicting small for gestational age risk.

3.1.5 Dietary Methyl Donor Patterns and Large for Gestational Age

As with small for gestational age, the regression coefficients from principal components analysis were used as predictor variables in logistic regressions to assess their predictive value of large for gestational age (Table 8). However, none of the regression coefficients from the extracted components significantly predicted large for gestational age. The relationships between each individual dietary methyl donor and large for gestational age were assessed using unadjusted, covariate-adjusted and interaction-adjusted logistic regressions (Table 13). Both methionine and choline were significant predictors of LGA in the covariate-adjusted and interaction-adjusted models, respectively. Interestingly, for every 0.1 mg increase in choline intake during pregnancy, the risk of giving birth to a LGA infant increased by 10%. Of the calorie-adjusted dietary methyl donors, vitamin B_6, methionine and zinc were all significantly associated with large for gestational age in the unadjusted and covariate-adjusted models (Table 14). Overall, calorie-adjusted vitamin B_6, methionine and zinc appeared to reduce the risk of LGA anywhere from 20% to 85% depending on the nutrient and model. However, significance was lost in all three variables when adjusting for interaction terms and other dietary methyl donors.

The program R was used to determine the most parsimonious model predicting LGA. Without forcing any predictors or covariates into the model, the resultant calculation was as follows whereby “birthplace” signifies maternal birthplace, “age” signifies gestational age and “*” denotes an interaction:

y = parity + methionine + BMI + birthplace + choline + zinc + parity*methionine + parity*BMI + methionine*BMI + methionine*birthplace + methionine*smoking + birthplace*smoking + smoking*choline + methionine*zinc + BMI*zinc + parity*methionine*BMI + methionine*birthplace*smoking + methionine*BMI*zinc + smoking*age

Choline, methionine and zinc were the only nutrients incorporated into the model predicting large for gestational age. These results are consistent with the SPSS-derived models and imply vitamin B_6, choline, methionine and zinc to be the most important dietary methyl donors for reducing the risk large for gestational age.

Overall, dietary methyl donor intake during pregnancy appears to affect infant birth weight, including small and large for gestational age risk. Zinc seems to be the most important nutrient for attaining optimal birth weight, as it was positively associated with infant birth weight as well as a reduced risk for both small and large for gestational age. Both vitamin B_6 and methionine intake reduced the risk for small and large for gestational age infants. Dietary folate equivalent and folate, but not folic acid, were important for reaching adequate birth weight and preventing small for gestational age. Vitamin B_{12} significantly increased infant birth weight, yet had no effect on small or large for gestational age whereas choline intake appeared to increase the risk for large for gestational age. Finally, it is clear that patterns of dietary methyl donor intake along with their interactions with socio-demographic characteristics are important in predicting infant birth weight, including small and large for gestational age.

Table 1. Demographic and Socioeconomic Characteristics (n = 1,332)

Variable	Mean (SD)	n (%)
Age	31.7 (4.29)	
Pre-pregnancy BMI (kg/m^2)	24.7 (5.48)	
Underweight BMI< 18.5		41 (3.1)
Normal Weight BMI 18.5 – 24.9		824 (61.9)
Overweight BMI 25.0 – 29.9		281 (21.1)
Obese BMI ≥ 30.0		186 (14.0)
Marital Status		
Married or Common Law[a]		1277 (95.9)
Single-Parent Family		55 (4.1)
Maternal Education		
Secondary School or Less		77 (5.8)
College/Technical Diploma/CEGEP[b]		335 (25.1)
University-Undergraduate Degree[c]		557 (41.8)
University-Post-Graduate Degree (Master's/PhD)[c]		364 (27.3)
Household Annual Income ($)		
< 30,000		99 (7.4)
30,000 – 59,999		226 (17.0)
60,000 – 79,999		235 (17.6)
80,000 – 99,999		300 (22.5)
≥ 100,000		473 (35.5)
Parity		
0		776 (58.2)
1		417 (31.3)
≥ 2		139 (10.5)
Born in Canada		
Yes		971 (72.9)
No		361 (27.1)
Smoking		
Never		855 (64.2)
Stopped Before Pregnancy		306 (23.0)
Current		171 (12.8)
Gestational Diabetes		
Yes		1209 (90.8)
No		123 (9.2)

[a] Includes women living with a partner.
[b] CEGEP: *Collège d'enseignement général et professionnel* or General and Vocational College.
[c] Attended or completed degree or diploma.

Table 2. Neonatal Characteristics (n = 1,332)

Variable	Mean (SD)	n (%)
Sex		
Female		664 (49.8)
Male		668 (50.2)
Gestational Age (weeks)	39.0 (0.04)	
Preterm		
< 37 weeks		76 (5.7)
≥ 37 weeks		1256 (94.3)
Birth Weight (g)	3360.9 (14.2)	
Small for Gestational Age		
Yes		120 (9.0)
No		1212 (91.0)
Large for Gestational Age		
Yes		93 (7.0)
No		1239 (93.0)

Table 3. Daily Micronutrient Intake During Pregnancy from Food Sources (n = 1,233)

Nutrient	**EAR**	**RDA**	**UL**	**Mean (SD)**	**Inadequate[f] n (%)**	**Adequate[f] n (%)**
Folic Acid (µg)	--	--	1000[c]	113.6 (60.2)[d]	--	--
DFE (µg)	520	600	--	462.6 (133.9)	848 (68.8)	385 (31.2)
Vitamin B_2 (mg)	1.2	1.4	--	2.3 (0.6)	20 (1.6)	1213 (98.4)
Vitamin B_6 (mg)	1.6	1.9	100	1.9 (0.5)	358 (29.0)	875 (71.0)
Vitamin B_{12} (µg)	2.2	2.6	--	4.7 (1.8)	55 (4.5)	1178 (95.5)
Choline (mg)	--	450[b]	3500	194.7 (62.8)	--	--
Methionine (g)[a]	--	--	--	1.7 (0.5)[e]	--	--
Zinc (mg)	9.5	11	40	11.8 (3.2)	296 (24.0)	937 (76.0)

[a] The DRIs for methionine include cysteine and are weight-dependent.
[b] Adequate Intake (AI) value. RDA value has not been established for choline, thus cannot be used to predict inadequacy risk.
[c] The Tolerable Upper Intake Level (UL) for folate pertains to synthetic forms consumed from supplements and/or fortified foods.
[d] Values pertain to the synthetic form of folate (i.e. folic acid) found in fortified foods.
[e] Mean value of methionine intake regardless of individual body weight.
[f] Adequate and inadequate intake were based on participants consuming levels above or below the EAR for each nutrient.

Source: Health Canada, Dietary Reference Intake (DRIs) established by the Institute of Medicine's Food and Nutrition Board.[13] Values listed were established for women between the ages of 19 and 50 years of age during pregnancy.

Table 4. Differences in Socioeconomic Factors in Women with Adequate vs. Women with Inadequate DFE Intake from Food Sources (n = 1,233)

Variable	**Inadequate[d] n (%)**	**Adequate[f] n (%)**	**Chi-Square T-Test P-Value**	**Inadequacy Risk Odds Ratio**
Age (years)	31.8 (4.4)[e]	31.6 (4.2)[e]	.46	--
Pre-pregnancy BMI (kg/m^2)	24.4 (4.9)[e]	25.0 (5.8)[e]	.08	--
Maternal Birthplace			.007	1.48 (1.11, 1.96)
Canada	603 (71.1)	302 (78.4)		
Outside Canada	245 (28.9)	83 (21.6)		
Marital Status			.17	--
Married and Common Law[a]	818 (96.5)	365 (94.8)		
Single-Parent Family	30 (3.5)	20 (5.2)		
Maternal Education			.85	--
Secondary School or Less	44 (5.2)	21 (5.5)		
College/University[b]	804 (94.8)	364 (94.5)		
Household Income ($)			.65	--
< 30,000	60 (7.1)	30 (7.8)		
≥ 30,000	788 (92.9)	355 (92.2)		
Parity			.44	--
0	498 (58.7)	217 (56.4)		
1+	350 (41.3)	168 (43.6)		
Smoking			.33	--
Yes	100 (11.8)	53 (13.8)		
No[c]	748 (88.2)	332 (86.2)		
Gestational Diabetes			.42	--
Yes	78 (9.2)	30 (7.8)		
No	770 (90.8)	355 (92.2)		

[a] Includes women living with a partner
[b] Includes women who attended or completed college, technical diploma, CEGEP, University undergraduate degree or University post-graduate degree (Master's/PhD)
[c] Includes women who stopped smoking before becoming pregnant
[d] Consumption below the EAR for DFE
[e] Reported values are the mean and standard deviation
[f] Consumption at or above the EAR for DFE

Table 5. Differences in Socioeconomic Factors in Women with Adequate vs. Women with Inadequate Vitamin B_6 Intake from Food Sources (n = 1,233)

Variable	**Inadequate[d] n (%)**	**Adequate[f] n (%)**	**Chi-Square T-Test P-Value**	**Inadequacy Risk Odds Ratio**
Age (years)	31.6 (4.5)[e]	31.8 (4.2)[e]	.44	--
Pre-pregnancy BMI (kg/m^2)	25.2 (5.6)[e]	24.4 (5.1)[e]	.01	--
Maternal Birthplace			.0002	1.68 (1.28, 2.20)
Canada	236 (65.9)	669 (76.5)		
Outside Canada	122 (34.1)	206 (23.5)		
Marital Status			.15	--
Married or Common Law[a]	339 (94.7)	844 (96.5)		
Single-Parent Family	19 (5.3)	31 (3.5)		
Maternal Education			.047	1.68 (1.01, 2.80)
Secondary School	26 (7.3)	39 (4.5)		
College/University[b]	332 (92.7)	836 (95.5)		
Household Income ($)			.10	--
< 30,000	33 (9.2)	57 (6.5)		
≥ 30,000	325 (90.8)	818 (93.5)		
Parity			.48	--
0	202 (56.4)	513 (58.6)		
1+	156 (43.6)	362 (41.4)		
Smoking			.21	--
Yes	51 (14.2)	102 (11.7)		
No[c]	307 (85.8)	773 (88.3)		
Gestational Diabetes			.21	--
Yes	37 (10.3)	71 (8.1)		
No	321 (89.7)	804 (91.9)		

[a] Includes women living with a partner
[b] Includes women who attended or completed college, technical diploma, CEGEP, University undergraduate degree or University post-graduate degree (Master's/PhD)
[c] Includes women who stopped smoking before becoming pregnant
[d] Consumption below the EAR for vitamin B_6
[e] Reported values are the mean and standard deviation
[f] Consumption at or above the EAR for vitamin B_6

Table 6. Differences in Socioeconomic Factors in Women with Adequate vs. Women with Inadequate Zinc Intake from Food Sources (n = 1,233)

Variable	**Inadequate[d] n (%)**	**Adequate[f] n (%)**	**Chi-Square T-Test P-Value**	**Inadequacy Risk Odds Ratio**
Age (years)	31.8 (4.5)[e]	31.7 (4.2)[e]	.61	--
Pre-pregnancy BMI (kg/m^2)	24.4 (4.7)[e]	24.7 (5.4)[e]	.42	
Maternal Birthplace			< .001	2.07 (1.57, 2.74)
Canada	183 (61.8)	722 (77.1)		
Outside Canada	113 (38.2)	215 (22.9)		
Marital Status			.50	--
Married or Common Law[a]	282 (95.3)	901 (96.2)		
Single-Parent Family	14 (4.7)	36 (3.8)		
Maternal Education			.91	--
Secondary School	16 (5.4)	49 (5.2)		
College/University[b]	280 (94.6)	888 (94.8)		
Household Income ($)			.10	--
< 30,000	28 (9.5)	62 (6.6)		
≥ 30,000	268 (90.5)	875 (93.4)		
Parity			.86	--
0	173 (58.4)	542 (57.8)		
1+	123 (41.6)	395 (42.2)		
Smoking			.06	--
Yes	46 (15.5)	107 (11.4)		
No[c]	250 (84.5)	830 (88.6)		
Gestational Diabetes			.24	--
Yes	21 (7.1)	87 (9.3)		
No	275 (92.9)	850 (90.7)		

[a] Includes women living with a partner
[b] Includes women who attended or completed college, technical diploma, CEGEP, University undergraduate degree or University post-graduate degree (Master's/PhD)
[c] Includes women who stopped smoking before becoming pregnant
[d] Consumption below the EAR for zinc
[e] Reported values are the mean and standard deviation
[f] Consumption at or above the EAR for zinc

Table 7. Components Extracted from Principal Components Analysis with Direct Oblimin Rotation (n = 1,233)

Folate Form[a]	**Component Extracted**	**Eigenvalue**	**Variance Explained**[b] **(%)**	**Correlation with Component**[c]	
Folic Acid (μg)	1	3.9	56.2	Methionine	0.85
				Zinc	0.84
				Vitamin B_2	0.82
				Vitamin B_{12}	0.80
				Choline	0.77
				Vitamin B_6	0.76
Folic Acid (μg)	2	1.1	15.2	Folic Acid	0.98
				Vitamin B_2	0.28
				Vitamin B_6	-0.13
Folate (μg)	1	4.2	60.7	Zinc	0.84
				Vitamin B_2	0.84
				Methionine	0.82
				Choline	0.78
				Vitamin B_6	0.77
				Vitamin B_{12}	0.75
				Folate	0.62
DFE (μg)	1	4.1	58.9	Vitamin B_2	0.86
				Zinc	0.85
				Methionine	0.83
				Choline	0.77
				Vitamin B_{12}	0.76
				Vitamin B_6	0.75
				DFE	0.50

[a] Form of folate employed in the Principal Components Analysis model.
[b] Percentage of variance explained by the component extracted from the model.
[c] Correlation of each variable in each component with the extracted component.

Table 8. Principal Components Analysis Regression: Extracted Components Tested as Significant Predictors of Birth Weight, Small and Large for Gestational Age (n = 1,233)

Component	**Birth Weight B ± SE**	**t-score P-Value**	**SGA[c] Odds Ratio**	**Wald Test P-Value**	**LGA[d] Odds Ratio**	**Wald Test P-value**
Without Folic Acid[a] (μg)	22.2 ± 12.7	.08	0.76 (0.62, 0.94)	.01	0.86 (0.68, 1.08)	.20
Folic Acid (μg)	15.6 ± 12.7	.22	0.92 (0.75, 1.12)	.41	1.16 (0.94, 1.44)	.16
Folate (μg)	25.3 ± 12.7	.046	0.76 (0.61, 0.93)	.01	0.87 (0.69, 1.09)	.23
DFE[b] (μg)	25.3 ± 12.7	.046	0.76 (0.62, 0.94)	.01	0.88 (0.70, 1.11)	.28

[a] Two components were extracted when folic acid was included in the principal components analysis. This is the first component extracted that contained all dietary methyl donors except folic acid.
[b] Dietary folate equivalent
[c] Small for gestational age
[d] Large for gestational age

Table 9. Relationship Between Dietary Methyl Donor Intake During Pregnancy and Infant Birth Weight (n = 1,233)

Variable	B_1 ± SE	P_1	B_2 ± SE	P_2	B_3 ± SE	P_3
Folic Acid (μg)	0.3 ± 0.2	.21	0.1 ± 0.2	.59	0.1 ± 0.2	.52
Folate (μg)	0.4 ± 0.14	.01	0.3 ± 0.1	.008	0.3 ± 0.2	.04
DFE[a] (μg)	0.2 ± 0.1	.01	0.2 ± 0.1	.04	0.2 ± 0.1	.09
Vitamin B_2 (mg)	31.2 ± 21.4	.15	26.4 ± 19.7	.18	-41.0 ± 34.5	.24
Vitamin B_6 (mg)	30.0 ± 27.0	.27	32.9 ± 24.6	.18	-1333.8 ± 1025.8	.19
Vitamin B_{12} (μg)	11.1 ± 7.2	.12	10.3 ± 6.5	.11	43.3 ± 19.7	.03
Choline (mg)	0.3 ± 0.2	.10	0.3 ± 0.2	.16	-9.5 ± 8.0	.23
Methionine (g)	29.1 ± 28.2	.30	24.8 ± 25.5	.33	-43.3 ± 40.7	.29
Zinc (mg)	7.5 ± 4.0	.06	7.9 ± 3.7	.03	8.5 ± 6.0	.16

[a] Dietary folate equivalent
P_1 = unadjusted
P_2 = adjusted for covariates (pre-pregnancy BMI, maternal age, gestational age at birth, infant sex, maternal birthplace, household income, maternal education, parity and smoking)
P_3 = adjusted for covariates, other dietary methyl donors and interactions (maternal birthplace x vitamin B_{12}, smoking x vitamin B_{12}, gestational age at birth x vitamin B_6 and gestational age at birth x choline)

The regression coefficients and P-values for the fully-adjusted (B_3 ± SE, P_3) models for vitamin B_2, B_6, B_{12}, choline, methionine and zinc are from the model containing dietary folate equivalent as the folate form. In other words, when computing fully-adjusted general linear models for these variables, dietary folate equivalent, not folate or folic acid, was the folate form incorporated into the model. Maternal age was adjusted for only in the models containing folic acid and folate.

Table 10. Relationship Between Calorie-Adjusted Dietary Methyl Donor Intake During Pregnancy and Infant Birth Weight (n = 1,233)

Variable	B_1 ± SE	P_1	B_2 ± SE	P_2	B_3 ± SE	P_3
Folic Acid (μg)	0.2 ± 0.5	.75	-0.1 ± 0.4	.74	-0.02 ± 0.5	.97
Folate (μg)	0.4 ± 0.4	.29	0.5 ± 0.3	.14	0.6 ± 0.4	.12
DFE[a] (μg)	0.3 ± 0.3	.31	0.1 ± 0.2	.56	0.2 ± 0.2	.40
Vitamin B_2 (mg)	-18.7 ± 64.7	.77	-5.3 ± 58.5	.93	-77.2 ± 79.3	.33
Vitamin B_6 (mg)	-68.5 ± 69.1	.32	-23.2 ± 63.0	.71	-1741.0 ± 2364.6	.46
Vitamin B_{12} (μg)	8.9 ± 16.7	.60	10.3 ± 15.1	.50	83.8 ± 43.7	.06
Choline (mg)	0.1 ± 0.5	.79	0.1 ± 0.5	.86	-13.0 ± 18.5	.48
Methionine (g)	-56.4 ± 69.8	.42	-34.5 ± 63.6	.59	-86.5 ± 86.6	.32
Zinc (mg)	-0.3 ± 11.8	.98	6.1 ± 10.7	.57	11.1 ± 13.5	.41

[a] Dietary folate equivalent
P_1 = unadjusted
P_2 = adjusted for covariates (pre-pregnancy BMI, maternal age, gestational age at birth, infant sex, maternal birthplace, household income, maternal education, parity and smoking)
P_3 = adjusted for covariates, other dietary methyl donors and interactions (maternal birthplace x vitamin B_{12}, smoking x vitamin B_{12}, gestational age at birth x vitamin B_6 and gestational age at birth x choline)

The regression coefficients and P-values for the fully-adjusted (B_3 ± SE, P_3) models for vitamin B_2, B_6, B_{12}, choline, methionine and zinc are from the model containing dietary folate equivalent as the folate form. In other words, when computing fully-adjusted general linear models for these variables, dietary folate equivalent, not folate or folic acid, was the folate form incorporated into the model. Maternal age was adjusted for only in the models containing folic acid and folate.

Table 11. Relationship Between Dietary Methyl Donor Intake During Pregnancy and Small for Gestational Age at Birth (n = 1,233)

Variable	Odds Ratio$_1$	P_1	Odds Ratio$_2$	P_2	Odds Ratio$_3$	P_3
Folic Acid (µg)	0.999	.40	0.999	.46	0.999	.69
	(0.995, 1.00)		(0.995, 1.002)		(0.995, 1.10)	
Folate (µg)	0.998	.07	0.997	.02	0.996	.13
	(0.995, 1.00)		(0.995, 1.00)		(0.992, 1.03)	
DFE[a] (µg)	0.999	.06	0.998	.04	0.999	.43
	(0.997, 1.00)		(0.997, 1.00)		(0.997, 1.001)	
Vitamin B_2 (mg)	0.68	.03	0.66	.03	0.96	.91
	(0.47, 0.97)		(0.46, 0.96)		(0.50, 1.84)	
Vitamin B_6 (mg)	0.61	.03	0.55	.01	0.38	.03
	(0.39, 0.94)		(0.35, 0.86)		(0.16, 0.91)	
Vitamin B_{12} (µg)	0.89	.06	0.89	.07	0.98	.84
	(0.79, 1.01)		(0.79, 1.01)		(0.81, 1.18)	
Choline (mg)	0.998	.17	0.997	.12	1.00	.73
	(0.994, 1.00)		(0.99, 1.00)		(0.996, 1.01)	
Methionine (g)	0.62	.04	0.65	.07	1.27	.52
	(0.39, 0.99)		(0.41, 1.04)		(0.61, 2.67)	
Zinc (mg)	0.91	.01	0.91	.005	0.92	.17
	(0.85, 0.98)		(0.84, 0.97)		(0.82, 1.04)	

[a] Dietary folate equivalent
P_1 = unadjusted
P_2 = adjusted for covariates (pre-pregnancy BMI, maternal age, gestational age at birth, infant sex, maternal birthplace, household income, maternal education, parity and smoking)
P_3 = adjusted for covariates, other dietary methyl donors and interactions (folate x infant sex and vitamin B_6 x infant sex)

The regression coefficients and P-values for the fully-adjusted ($B_3 \pm SE$, P_3) models for vitamin B_2, B_6, B_{12}, choline, methionine and zinc are from the model containing dietary folate equivalent as the folate form. In other words, when computing fully-adjusted logistic regression models for these variables, dietary folate equivalent, not folate or folic acid, was the folate form incorporated into the model. Maternal age was adjusted for only in the models containing folic acid and folate.

Table 12. Relationship Between Calorie-Adjusted Dietary Methyl Donor Intake During Pregnancy and Small for Gestational Age at Birth (n = 1,233)

Variable	Odds Ratio$_1$	P_1	Odds Ratio$_2$	P_2	Odds Ratio$_3$	P_3
Folic Acid (µg)	0.999	.74	1.00	.94	0.998	.61
	(0.991, 1.01)		(0.992, 1.01)		(0.989, 1.01)	
Folate (µg)	0.998	.55	0.997	.35	0.993	.19
	(0.993, 1.00)		(0.991, 1.00)		(0.983, 1.00)	
DFE[a] (µg)	0.998	.45	0.999	.48	0.998	.47
	(0.994, 1.00)		(0.994, 1.00)		(0.994, 1.00)	
Vitamin B_2 (mg)	0.49	.19	0.55	.27	0.69	.61
	(0.17, 1.41)		(0.19, 1.59)		(0.16, 2.88)	
Vitamin B_6 (mg)	0.55	.29	0.48	.21	0.31	.21
	(0.18, 1.68)		(0.16, 1.52)		(0.05, 1.96)	
Vitamin B_{12} (µg)	0.85	.24	0.87	.32	0.99	.95
	(0.64, 1.12)		(0.66, 1.15)		(0.67, 1.45)	
Choline (mg)	0.999	.82	0.999	.84	1.00	.52
	(0.991, 1.01)		(0.991, 1.01)		(0.99, 1.01)	
Methionine (g)	0.49	.21	0.63	.42	1.36	.70
	(0.16, 1.50)		(0.21, 1.999)		(0.29, 6.34)	
Zinc (mg)	0.82	.06	0.82	.06	0.83	.15
	(0.67, 1.01)		(0.67, 1.01)		(0.64, 1.07)	

[a] Dietary folate equivalent
P_1 = unadjusted
P_2 = adjusted for covariates (pre-pregnancy BMI, maternal age, gestational age at birth, infant sex, maternal birthplace, household income, maternal education, parity and smoking)
P_3 = adjusted for covariates, other dietary methyl donors and interactions (folate x infant sex and vitamin B_6 x infant sex)

The regression coefficients and P-values for the fully-adjusted ($B_3 \pm SE$, P_3) models for vitamin B_2, B_6, B_{12}, choline, methionine and zinc are from the model containing dietary folate equivalent as the folate form. In other words, when computing fully-adjusted logistic regression models for these variables, dietary folate equivalent and not folate or folic acid, was the folate form incorporated into the model. Maternal age was adjusted for only in the models containing folic acid and folate.

Table 13. Relationship Between Dietary Methyl Donor Intake During Pregnancy and Large for Gestational Age at Birth (n = 1,233)

Variable	Odds Ratio$_1$	P_1	Odds Ratio$_2$	P_2	Odds Ratio$_3$	P_3
Folic Acid (μg)	1.00	.22	1.00	.66	1.00	.72
	(0.999, 1.01)		(0.997, 1.004)		(0.997, 1.005)	
Folate (μg)	1.00	.77	1.00	.99	1.00	.06
	(0.997, 1.00)		(0.998, 1.003)		(1.00, 1.01)	
DFE[a] (μg)	1.00	.47	1.00	.77	1.00	.34
	(0.999, 1.002)		(0.999, 1.002)		(0.999, 1.003)	
Vitamin B_2 (mg)	0.93	.70	0.85	.41	0.92	.82
	(0.64, 1.36)		(0.57, 1.26)		(0.47, 1.83)	
Vitamin B_6 (mg)	0.62	.06	0.64	.08	0.66	.26
	(0.38, 1.02)		(0.39, 1.06)		(0.33, 1.35)	
Vitamin B_{12} (μg)	0.96	.52	0.95	.49	1.06	.52
	(0.84, 1.09)		(0.84, 1.09)		(0.88, 1.28)	
Choline (mg)	0.999[b]	.61	0.999[b]	.47	1.10[b]	.03
	(0.995, 1.00)		(0.995, 1.002)		(1.01, 1.21)	
Methionine (g)	0.62	.07	0.58	.047	0.54	.14
	(0.37, 1.05)		(0.34, 0.99)		(0.23, 1.24)	
Zinc (mg)	0.97	.37	0.96	.28	1.02	.81
	(0.90, 1.04)		(0.89, 1.03)		(0.90, 1.15)	

[a] Dietary folate equivalent
[b] OR reported is for each 0.1 mg increase in choline intake
P_1 = unadjusted
P_2 = adjusted for covariates (pre-pregnancy BMI, maternal age, gestational age at birth, infant sex, maternal birthplace, household income, maternal education, parity and smoking)
P_3 = adjusted for covariates, other dietary methyl donors and interactions (folate x maternal birthplace and choline x smoking)

The regression coefficients and P-values for the fully-adjusted ($B_3 \pm SE$, P_3) models for vitamin B_2, B_6, B_{12}, choline, methionine and zinc are from the model containing dietary folate equivalent as the folate form. In other words, when computing fully-adjusted logistic regression models for these variables, dietary folate equivalent and not folate or folic acid, was the folate form incorporated into the model. Maternal age was adjusted for only in the models containing folic acid and folate.

Table 14. Relationship Between Calorie-Adjusted Dietary Methyl Donor Intake During Pregnancy and Large for Gestational Age at Birth (n = 1,233)

Variable	Odds Ratio$_1$	P_1	Odds Ratio$_2$	P_2	Odds Ratio$_3$	P_3
Folic Acid (μg)	1.00	.67	0.999	.87	0.996	.37
	(0.99, 1.01)		(0.991, 1.01)		(0.986, 1.00)	
Folate (μg)	0.997	.38	0.999	.84	1.01	.09
	(0.991, 1.00)		(0.993, 1.01)		(0.999, 1.01)	
DFE (μg)	0.999	.78	0.999	.66	0.49	.68
	(0.995, 1.00)		(0.994, 1.00)		(0.10, 2.41)	
Vitamin B_2 (mg)	0.46	.19	0.40	.13	0.39	.38
	(0.14, 1.48)		(0.12, 1.30)		(0.08, 1.94)	
Vitamin B_6 (mg)	0.15	.005	0.19	.02	0.39	.23
	(0.04, 0.56)		(0.05, 0.76)		(0.08, 1.84)	
Vitamin B_{12} (μg)	0.86	.34	0.88	.40	1.23	.30
	(0.63, 1.17)		(0.64, 1.20)		(0.83, 1.82)	
Choline (mg)	0.995	.32	0.995	.32	1.02	.06
	(0.986, 1.00)		(0.986, 1.00)		(0.999, 1.04)	
Methionine (g)	0.15	.005	0.14	.005	0.18	.07
	(0.04, 0.57)		(0.04, 0.56)		(0.03, 1.12)	
Zinc (mg)	0.79	.04	0.79	.049	0.92	.59
	(0.63, 0.99)		(0.63, 0.999)		(0.69, 1.23)	

[a] Dietary folate equivalent
P_1 = unadjusted
P_2 = adjusted for covariates (pre-pregnancy BMI, maternal age, gestational age at birth, infant sex, maternal birthplace, household income, maternal education, parity and smoking)
P_3 = adjusted for covariates, other dietary methyl donors and interactions (folate x maternal birthplace and choline x smoking)

The regression coefficients and P-values for the fully-adjusted ($B_3 \pm SE$, P_3) models for vitamin B_2, B_6, B_{12}, choline, methionine and zinc are from the model containing dietary folate equivalent as the folate form. In other words, when computing fully-adjusted logistic regression models for these variables, dietary folate equivalent and not folate or folic acid, was the folate form incorporated into the model. Maternal age was adjusted for only in the models containing folic acid and folate.

3.2 Discussion

Adequate nutrition during pregnancy is crucial to ensure healthy growth and development of the fetus.[153,169] In particular, the growing fetus obtains diet-derived methyl donors, which serve to establish DNA methylation patterns, exclusively through maternal nutrition.[2] Adequate consumption of methyl donors and cofactors may be an important driver of optimal birth weight in the infant. However, the majority of women in our study were consuming sums of folate and choline from diet alone below the recommended amounts. Assessing key determinants of adequate dietary methyl donor intake during pregnancy, especially in the Canadian immigrant population, is warranted.

3.2.1 Patterns of Dietary Methyl Donor Intake

Micronutrient Intake During Pregnancy

The majority of women in our study were consuming amounts at or above the estimated average requirement for vitamins B_2, B_6, B_{12}, methionine and zinc from food sources. In contrast, 69% and 99% of women did not meet the respective EAR and AI recommendations for dietary folate and choline. Our results are consistent with other Canadian studies in which many women were meeting the EARs for vitamins B_6, B_{12} and zinc, but had intakes of DFE and choline below their respective EAR and AI values. In fact, women in the APrON and PREFORM studies had median vitamin B_6 intake of approximately 1.8 mg/day and median vitamin B_{12} intakes of 4.0 μg/day and 4.7 μg/day, respectively, suggesting most women met or superseded the RDAs from food sources alone.[63,65] Likewise, participants in the Prenatal Health Project averaged 10 mg/day of dietary zinc intake thereby surpassing the 9.5 mg/day estimated average requirement.[62] Nevertheless, Canadian women may be at risk for low dietary intake of folate and

choline. Forty-one percent and 87% of women in the PREFORM cohort consumed dietary folate equivalent and choline below recommended amounts.[63] From food sources alone, women participating in the APrON study averaged 300 μg/day of dietary folate whereas women participating in the Prenatal Health Project and PREFORM studies consumed roughly 473 μg/day and 483 μg/day, respectively, of DFE, well below the 520 μg EAR.[62,63,65]

The low intake of folate from food sources demonstrates the importance of folic acid supplementation during pregnancy to ensure adequate intake. However, the 450 mg/day AI measure for choline should be revisited: There is insufficient scientific evidence to determine an EAR value and the Canadian Nutrient File is roughly 50% complete for choline; therefore, many foods in the database have not yet been analyzed for choline content. Ultimately, folate and choline are important for normal brain development in the fetus and significant proportions of the population are consuming diets low in one or both of these nutrients.[194] Consequently, it is important to identify the factors mediating adequate and inadequate intake of dietary methyl donors during pregnancy.

Factors Affecting Micronutrient Intake During Pregnancy

A variety of factors play key roles in prenatal care and dietary intake during pregnancy.[195] Several studies have reported the influence of socioeconomic status on diet quality whereby people of affluence are more likely to consume a diet rich in nutrients.[196] Although the findings of our study did not report any differences in adequate nutrient intake for low-income (< $30,000) women, maternal education level significantly shaped nutrient intake. Women who were educated beyond secondary school were 68% more

likely to consume adequate amounts of vitamin B_6 from food sources. An American study found similar results whereby diet quality was lowest amongst less educated adults.[197] In fact, the authors found that adults with less than a high school education consumed more saturated fats and sodium compared to adults with higher education.[197] Consequently, educated women may have more nutritional knowledge as well as a greater capacity to translate their understanding into dietary practice.[197]

Indeed, not only do diet and lifestyle patterns appear to be intertwined with socioeconomic status, they are also related to unhealthy weight gain and obesity.[198] Our findings indicate a relationship between education level, dietary intake and being overweight. In particular, we found women were significantly heavier, on average, if they were not educated past secondary school (26.4 kg/m^2) or consumed vitamin B_6 below the EAR (25.2 kg/m^2) in comparison to if they were more educated (24.5 kg/m^2) or consumed adequate amounts of vitamin B_6 from food sources (24.4 kg/m^2). Thus, the association between dietary vitamin B_6 consumption and BMI may have been mediated by education whereby less educated women were more likely to consume low nutrient, high-calorie diets that contribute to weight gain.[199]

In addition to socioeconomic factors, maternal birthplace may dictate the consumption of dietary methyl donors. Although the diet-disease relationships outlining immigrant health status in Canada have not been adequately addressed, several studies have shown that immigrants are often healthier prior to arriving in Canada as their health deteriorates a few years after immigrating.[200,201] In fact, women who are settled (10 or more years) immigrants to Canada are more likely to have poor health status when

compared to Canadian-born women.[202] While the deterioration in immigrant health is likely due to a variety of factors, it is partly mediated by dietary acculturation.[203,204]

Our study found that women who were not born in Canada were significantly less likely to consume adequate (> EAR) amounts of dietary folate, vitamin B_6 and zinc when compared to women who were born in Canada. Similar results were found in a study comparing pregnant US-born Mexican women with Mexico-born women residing in America. Among women who were born in Mexico, greater time spent in the United States was associated with lower folate and zinc intake suggesting consumption of a less nutritious diet.[205] In fact, evidence suggests that dietary intake of immigrant women comprises mainly of processed foods with high fat, sugar and salt intake: hallmarks of the Western diet.[205,206]

This discrepancy in nutrient intake between immigrants and Canadian-born women may be explained by other unexamined factors such as racial and/or ethnic differences and food insecurity. Ethnicity, culture and religion have been found to be important predictors of diet quality including diet-disease relationships.[207] For instance, data from the US national survey found that education and income levels explained racial and ethnic differences in diet quality.[198] Moreover, measures of food insecurity including inaccessible traditional foods, financial inability and lack of transportation were recognized as risk factors for poor diet quality in Canadian immigrants.[204] Although these factors were not addressed, they may have played important roles in perturbing micronutrient intake in Canadian immigrant women.

Overall, roughly a third (29%) of participants were below the EAR for vitamin B_6 and were more likely to be born outside of Canada, less educated and overweight. Since

vitamin B_6 deficiency is usually accompanied by insufficiencies in other B-complex vitamins,[208] elucidating factors inhibiting adequate intake is crucial. Ultimately, we conclude that there are a variety of factors affecting dietary intake during pregnancy. These results indicate that barriers to adequate dietary folate, vitamin B_6 and zinc intake, namely in the Canadian immigrant population, need be addressed. Additionally, our findings confirm that food sources alone may be insufficient to provide adequate intake of these nutrients in certain populations; accordingly, a deeper understanding of supplementation during pregnancy, especially in the immigrant population, is warranted.

Principal Components Analysis

Three separate models were tested in principal components analysis: the first with folic acid, in which two components were extracted, the second with folate and the third with dietary folate equivalent. Regardless of folate form, each model extracted a component whereby methionine, zinc and vitamin B_2 were highly correlated (> 0.82) with the component. These nutrients are commonly found in meat, fish, seafood as well as milk and other dairy products with few sources acquired from fruits and vegetables. The nutrients that correlated less with the component, though highly still (> 0.75), were vitamin B_6, vitamin B_{12} and choline. Common sources of vitamin B_{12} and choline include eggs, meat, poultry and dairy products whereas vitamin B_6 is also found in fish as well as in some fruits and vegetables. Unlike the other nutrients, the main sources of naturally-occurring folate do not come from animal products, but rather dark green vegetables and dried legumes. Finally, as aforementioned, folic acid is fortified in pasta, cornmeal and white flour.

Spearman's correlation matrix revealed numerous correlations between dietary methyl donors (Table VII: Appendix B). In particular, we found vitamin B_2 and vitamin B_{12} intake during pregnancy to be highly correlated ($r = 0.69$). Thus, it is possible that women consuming high levels of vitamin B_2 were also consuming high levels of vitamin B_{12} and vice versa. Since the greatest sources of vitamin B_2 and B_{12} come from animal products, it is conceivable that this correlation outlines a dietary relationship with respect to meat, fish, poultry and dairy consumption. Additionally, folic acid was weakly correlated with the other micronutrients and was negatively correlated with vitamins B_6 and B_{12}. In the folic acid extracted component, folic acid explained most of the variance and was highly (0.98) correlated with the component. This high correlation as well as the overall weak correlations between folic acid and vitamins B_2, B_6 and B_{12} suggests that a higher intake of enriched grain is associated with a lower intake of animal products. These results are consistent with both folate and dietary folate equivalent models whereby the folate form in each model correlated weakest with the component.

In conclusion, dietary intake of folic acid and folate do not appear to correlate as strongly with the rest of the nutrients. This may suggest that dietary consumption of dark green vegetables, dried legumes and enriched grain products is less associated with consumption of animal products (meat, fish, poultry and dairy). Therefore, it can be inferred that some women had lower intakes of animal products and higher intakes of fortified grains, legumes and green vegetables. Overall, the adequate consumption of dietary methyl donors and cofactors from food sources varied based on pre-pregnancy BMI, maternal birthplace and education. Finally, elucidating a deeper comprehension of these associations and their impact on birth weight is needed.

3.2.2 Patterns of Dietary Methyl Donor Intake on Birth Weight

Birth Weight

Infant birth weight is mediated by a variety of factors including maternal nutrition. Our study demonstrates the importance of dietary folate, vitamin B_{12} and zinc intake during pregnancy for optimal infant birth weight. The folate and DFE-containing components extracted from PCA as well as individual dietary methyl donors, folate, DFE, vitamin B_{12} and zinc, were significantly associated with birth weight. Previous studies exhibited similar results whereby maternal diets higher in folate and zinc were associated with increased weight at birth.[46,111,116] The relationship between maternal vitamin B_{12} consumption and infant birth weight is less understood, as some studies found a significant relationship,[209,210] while others reported no effect.[211,212] However, many studies examining the role of vitamin B_{12} intake during pregnancy were conducted in India where a large portion of the population are ovo-lacto vegetarians with low meat consumption.[213] Therefore, the overall lower vitamin B_{12} and socioeconomic statuses of women reported in these studies does not necessarily reflect the nutrient status of women in this cohort.

However, the relationship between vitamin B_{12} intake from food sources and infant birth weight differed based on whether or not the mother was born in Canada (Figure 5: Appendix B). Firstly, mean vitamin B_{12} and DFE intake were significantly higher in women born in Canada compared to immigrants (not shown). Of course, this may be related to racial or ethnic differences in dietary intake whereby immigrant women consumed a diet with significantly less animal product and fortified grains than commonly consumed in the traditional Western diet.[214] Secondly, in Canadian-born

women, vitamin B_{12} intake was positively associated with birth weight. Yet, for women born outside of Canada, vitamin B_{12} intake was negatively associated with birth weight. This finding is contrary to the results of several studies whereby vitamin B_{12} intake was protective of low birth weight.[209,210] The negative association of vitamin B_{12} intake on infant birth weight may be explained by a difference in methylation pattern whereby vitamin B_{12}, which is a cofactor for the enzyme, methionine synthase, acts to alter DNA methylation at certain gene regions and consequently reduce birth weight.[215,216]

The interaction between smoking and vitamin B_{12} on infant birth weight has previously been explored whereby smoking during pregnancy has been shown to negatively affect maternal vitamin B_{12} status.[209,211] We found vitamin B_{12} intake during pregnancy to be positively associated with birth weight in smokers, yet remain unrelated to birth weight in non-smokers (Figure 6: Appendix B). This may be due to a mechanism whereby vitamin B_{12} stores are depleted in women who smoke, thus an increased intake in vitamin B_{12} serves to mediate the negative effects of smoking on infant birth weight.

Finally, the R-derived model determined that zinc was the only nutrient important for predicting birth weight. In fact, even the interaction between zinc and gestational age was a significant predictor included in the model. Similar results have been reported whereby zinc intake was related to a reduction in preterm birth[217] and low birth weight.[116,218] Overall, folate, vitamin B_{12} and zinc appear to be the most important nutrients acquired from food sources in relation to infant birth weight. Further analyses into the relationship between vitamin B_{12} intake in the Canadian immigrant population as well as in smokers is warranted. Finally, to ensure adequate intake, supplementation of these nutrients may be important in certain populations.

Small for Gestational Age

Previous epidemiological studies have associated dietary patterns during pregnancy with the prevention of adverse neonatal health outcomes including small for gestational age. In fact, a previous study noted that consumption of fish, carbohydrate-rich foods and folic acid supplementation around the time of conception was associated with a decreased risk of SGA at birth.[219] Although our study examined dietary intake during the second trimester, similar results were observed whereby the first component extracted from each model was significantly associated with SGA. In fact, the regression coefficients from these three components all predicted a 24% decreased risk in small for gestational age. The common nutrients in these extracted components are methionine, zinc, choline and vitamins B_2, B_6 and B_{12}. The results indicate that diets rich in these nutrients may reduce the risk of SGA.

However, once adjusting for all significant covariates and interaction terms, vitamin B_6 was the only nutrient to significantly decrease the odds of SGA. Also, after adjusting for calorie intake, no significant results were observed. The R-derived model was multi-factorial and included the nutrients DFE, methionine, zinc and vitamin B_2 along with many socio-demographic factors. Our findings are consistent with previous studies that found significant variations in dietary pattern based on education, age, smoking, parity and ethnicity.[220] Therefore, the risk of having an infant small for gestational age is likely mediated by a multitude of factors, both dietary and socio-demographic.

Large for Gestational Age

Previous studies have reported no effect of dietary pattern on LGA risk.[130] However, after adjusting for covariates, other dietary methyl donors and interaction terms, we found choline to be significantly associated with LGA whereby for every 0.1 mg increase in choline, the risk of LGA increased by 10% (1.10 (1.01, 1.21)). The R-derived model calculated similar results in which choline, methionine and zinc were found to be significant predictors of large for gestational age amongst several socio-demographic characteristics. Our findings are consistent with other studies noting multiparous, non-smoking women with high pre-pregnancy BMI are at a greater risk of giving birth to an infant who is large for gestational age.[221] We also found maternal birthplace to be an important predictor of large for gestational age, which may be due to differences in dietary pattern or lifestyle choices among ethnicities. Other studies have reported ethnicity to be an important predictor in which the risk for LGA is reduced in women of Afro-Caribbean and South Asian descent.[221] Nevertheless, the role of ethnicity on large for gestational age is left to be confirmed by future studies.

In conclusion, several dietary methyl donors and cofactors as well as socio-demographic factors appeared to be important for governing infant birth weight, small and large for gestational age risk. The null effect of calorie intake on birth weight, SGA and LGA was surprising. Contrary to our presumption, it can be hypothesized that women who consumed more calories, did not consume more dietary methyl donors, but rather less nutritious high-calorie foods. Overall, our results indicate that maternal nutrition plays an important part in predicting infant birth weight and hence, deficiencies in micronutrient consumption via food sources during pregnancy should be addressed.

3.2.3 Strengths and Limitations

The results of our study should be interpreted with respect to its strengths and limitations. The principal strengths of this study are the large sample size, prospective nature, the use of food records to measure dietary intake, the use of medical charts to obtain infant birth weight as well as Canadian-based standards for measuring SGA and LGA. By using the Canadian Nutrient File in ESHA Food Processor™ a more representative view of Canadian staple foods was obtained. In addition, the 3D study was multi-centered therefore incorporated various urban groups of the Québec population.

Despite the strengths of our study, there were some limitations. Firstly, although three-day food records have been shown to be one of the most valid tools to assess diet during pregnancy when compared to circulating markers, a common problem involves categorizing an individual's regular diet because of within-person variations of daily consumption.[222,223] Despite the fact that participants were instructed on how to complete the three-day food record, some problems with this method included missing information such as preparation method, description of food item, serving size amount, recipe or mixed food ingredients.[224] Additionally, the Canadian Nutrient File database supplies the average amounts of nutrients present in the food, however the exact nutrient composition of a particular food can vary based on location, season and methodologies.[222] Furthermore, dietary intake was measured only in the second trimester. Repeated measures of intake during each trimester may have encapsulated a more complete picture of dietary intake during pregnancy. Nevertheless, food records are considered the gold standard of food assessment for individuals, since they do not rely on memory and enable more details to be recorded.[225] Finally, although supplemental intake of women during

pregnancy was not examined, previous studies have found dietary impact alone to be associated with infant birth weight.[48,115] Nonetheless, a complete understanding of total dietary methyl donor intake and its impact on birth weight should be revisited with complete information.

Another limitation lies in the diagnosis of small and large for gestational age. Even though the growth curves proposed by Kramer *et al.*[120] (2001) are sex-specific and population-based, they are not ethnically nor racially diverse. The ultrasound technology used to determine gestational age and anthropometric measures is not a direct measurement of fetal size.[226] Furthermore, the cross-sectional nature of this reference fails to provide two or more sequential measurements of the fetus, thus undermining the definition of growth: the increase in size over time.[120] This dampens the generalizability of the findings as they may not be relevant to the entire Canadian population.

Finally, socioeconomic and demographic characteristics of participants were self-reported in questionnaires and ethnicity was not addressed. Moreover, DNA methylation was not measured, leaving the epigenetic effect of dietary methyl donors and cofactors on infant birth weight to be confirmed by future studies. In conclusion, these limitations could weaken some associations, but are unlikely to completely deter any general relationships found; this is especially true, in particular, for nutrients that cannot be found in supplements such as methionine and choline. Lastly, our results add to the scientific literature findings that diet quality and natural sources of nutrients may have an independent effect on infant birth weight.[48,115]

Chapter 4: Conclusion

Using the three-day food records provided by the 3D study, our research examined the intakes of dietary methyl donors and cofactors during the second trimester of pregnancy in a Québec cohort of Canadian women. Patterns of intake were examined and assessed in relation to infant birth weight, small and large for gestational age. In this final chapter, a summary of the findings as well as concepts for future research are proposed.

4.1 Summary of Findings

Overall, our hypothesis was verified: low and high consumptions of dietary methyl donor intake during pregnancy was associated with a greater prevalence of small and large for gestational age infants. The majority of women were below the respective EAR and AI values for dietary folate (69%) and choline (99%), supporting the need for prenatal folic acid supplementation. Given the importance of choline for maintaining a healthy pregnancy, examining the risks and benefits of including choline in prenatal supplementation is warranted. Additionally, it may be advisable for the scientific community to revisit the Adequate Intake measure for choline since it was first defined in 1998 and drawn from limited data.[13] Canadian immigrant women were significantly less likely to obtain adequate amounts of dietary folate, vitamin B_6 and zinc. In addition, women who were not educated past secondary school were at an increased risk of obtaining inadequate vitamin B_6, which was also associated with a higher pre-pregnancy BMI. Vitamin B_{12} intake during pregnancy appeared to mediate the negative effects of smoking on birth weight, yet adversely affected birth weight in Canadian immigrants.

Infant birth weight was significantly associated with a variety of factors including maternal nutrition. After adjusting for covariates and interaction terms, vitamin B_6 significantly reduced the risk for SGA and choline significantly increased the risk for LGA. However, after adjusting for calorie intake, none of the dietary methyl donors or cofactors appeared to have any effect on birth weight, small or large for gestational age, suggesting a possible relationship with other unidentified factors.

4.2 Future Directions

Future research should be aimed at determining adequate amounts of dietary methyl donor and cofactor intake from food sources and supplements to ensure that birth weight is in the normal range. Developing policies and intervention strategies to ensure adequate consumption of dietary methyl donors and cofactors during pregnancy, especially in the Canadian immigrant population, is crucial. Next, examining the influence of timing in nutritional modification may be important to illuminate a deeper understanding of the role dietary methyl donors play in fetal programming. Finally, detailing the impact of maternal nutrition, particularly that of methyl donors, on DNA methylation in the fetus as well as subsequent changes in birth weight are warranted.

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Appendix A: Research Ethics Board (REB) Approvals and Permissions

The following Research Ethics Board (REB) Approvals are attached:

1. **Research Ethics Board at CHU St. Justine**
Protocol Number: 3679

This ethics approval was granted to Dr. William Fraser, the principal investigator of the 3D study developed by the Integrated Research Network of Perinatology of Québec and Eastern Ontario (IRNPQEO).

2. **The University of Ottawa: Health Sciences and Science Research Ethics Board**
File Number: H03-15-19

DÉCISION DU COMITÉ SCIENTIFIQUE

Projet No : 3679 **Chercheur principal : FRASER William**

Titre du projet : L'effet de la prise de suppléments de multivitamine prénatale sur la réponse placentaire du stress

Décision

- ■ Accepté; transmis au Comite d'éthique à la recherche pour l'évaluation éthique
- ☐ Accepté avec modifications mineures demandées; transmis au Comité d'éthique à la recherche pour l'évaluation éthique; soumettre réponses seulement après l'évaluation éthique
- ☐ En suspens avec modifications; doit être revu par les évaluateurs
  - ☐ Refusé; des corrections majeures doivent être apportées avant de resoumettre le projet
  - ☐ Refusé : informations insuffisantes pour juger de la faisabilité du projet
- ☐ Protocole non-conforme/incomplet; les documents suivants sont manquants :

Commentaires du comité :

Évaluateurs 1 et 2

Ce projet propose de déterminer l'apport en vitamines et minéraux de femmes enceintes québécoises prenant des suppléments prénataux quotidiens de multivitamines.
Il s'agit d'une étude ancillaire basée sur la cohorte IRNPQ / 3D, projet financé par les IRSC et pour lequel une banque de données a déjà été approuvée par le comité d'éthique.
Le but du présent projet est seulement d'avoir accès au journal alimentaire d'un échantillon des femmes du projet 3D.
Une deuxième partie expérimentale du projet (réponse placentaire au stress) est effectuée à Ottawa mais ne concerne pas la cohorte 3D. Le titre de la présente demande peut prêter à confusion pour cette raison
Il s'agit d'une étude purement descriptive basée sur un questionnaire alimentaire standardisé et validé.
L'échantillon sera aléatoire (333 femmes parmi 2500 environ), basé sur un calcul de puissance correctement détaillé.
Je ne vois pas de problème scientifique particulier concernant cette partie du projet. Dans la mesure où la banque de données 3D a déjà été approuvée, il m'aurait semblé correct de ne pas repasser par tout le processus du comité d'éthique pour ce simple examen des journaux alimentaires. Il semble toutefois que cette demande provienne du comité d'éthique à la recherche à Ottawa.
Formulaire de soumission; point 9c. Taille de l'échantillon

- ….De ce nombre, combien seront mineurs ; « Tous » devrait être inscrit dans majeurs

Le 12 mars 2015

Docteur William Fraser
5757 Decelles
suite:120

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

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OBJET: Titre du projet: L'impact des apports en donneurs de groupements méthyle durant la grossesse sur le poids à la naissance, l'étude 3D

No. de dossier: 4115

Responsables du projet: William Fraser M.D., **Demande d'accès de la banque IRNPQEO**
Chercheuse principale: Bénédicte Fontaine-Bisson, Université d'Ottawa.
Collaboratrice: Lise Dubois, Université d'Ottawa

Cher Docteur,

Votre demande d'utilisation des données provenant de la Biobanque IRNPQEO, dans le cadre du projet cité en rubrique. est approuvée par le comité d'éthique de la recherche pour la période du **27 février 2015 au 27 février 2016**. Notez que pour une collaboration avec un (ou plusieurs) tiers (institutions ou entreprises privées) impliquant des transferts de fonds et/ou données et/ou matériel biologique, une entente (contrat) doit être conclue avec le Bureau des ententes de recherche (BER).

Recevez, Cher Docteur, nos salutations distinguées.

Me Geneviève Cardinal
Présidente du Comité d'éthique de la recherche

GC/sa
c.c. : BER

3175, Côte-Sainte-Catherine
Montréal (Québec)
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Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Ethics Approval Notice

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Bénédicte	Fontaine-Bisson	Health Sciences / Others	Supervisor
Meghan	McGee	Health Sciences / Others	Student Researcher

File Number: H03-15-19

Type of Project: Master's Thesis – Secondary Use of Data

Title: The Association Between Differing Dietary Methyl Donor Intake During Pregnancy and Birth Weight in the 3D study

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
03/26/2015	03/25/2016	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A

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Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement (2010) and other applicable laws and regulations in Ontario, has examined and approved the ethics application for the above named research project. Ethics approval is valid for the period indicated above and subject to the conditions listed in the section entitled "Special Conditions / Comments".

During the course of the project, the protocol may not be modified without prior written approval from the REB except when necessary to remove participants from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the project (e.g., change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, including consent and recruitment documentation, should be submitted to the Ethics Office for approval using the "Modification to research project" form available at: http://research.uottawa.ca/ethics/submissions-and-reviews.

Please submit an annual report to the Ethics Office four weeks before the above-referenced expiry date to request a renewal of this ethics approval. To close the file, a final report must be submitted. These documents can be found at: http://research.uottawa.ca/ethics/submissions-and-reviews.

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

Kim Thompson
Protocol Officer for Ethics in Research
For Daniel Lagarec, Chair of the Health Sciences and Sciences REB

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Appendix B: Supplemental Statistical Analyses

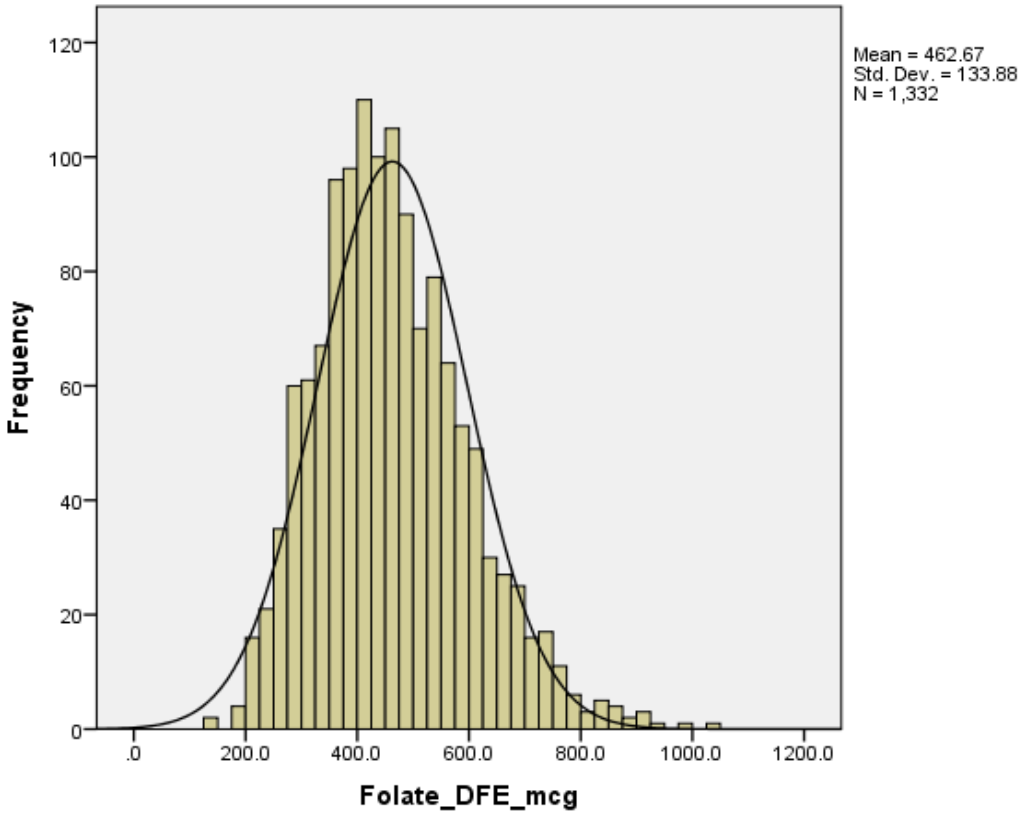

Figure 2. Histogram of Dietary Folate Equivalent Superimposed with Normal Distribution (n = 1,332)

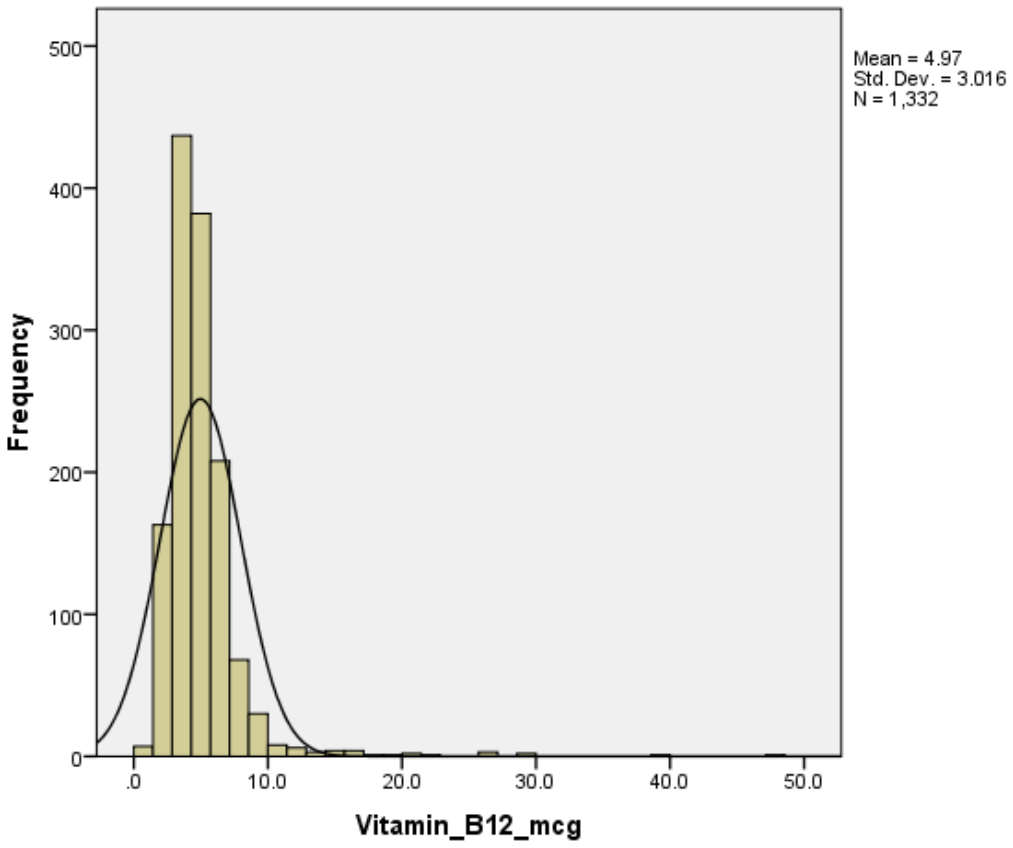

Figure 3. Histogram of Vitamin B_{12} Superimposed with Normal Distribution (n = 1,332)

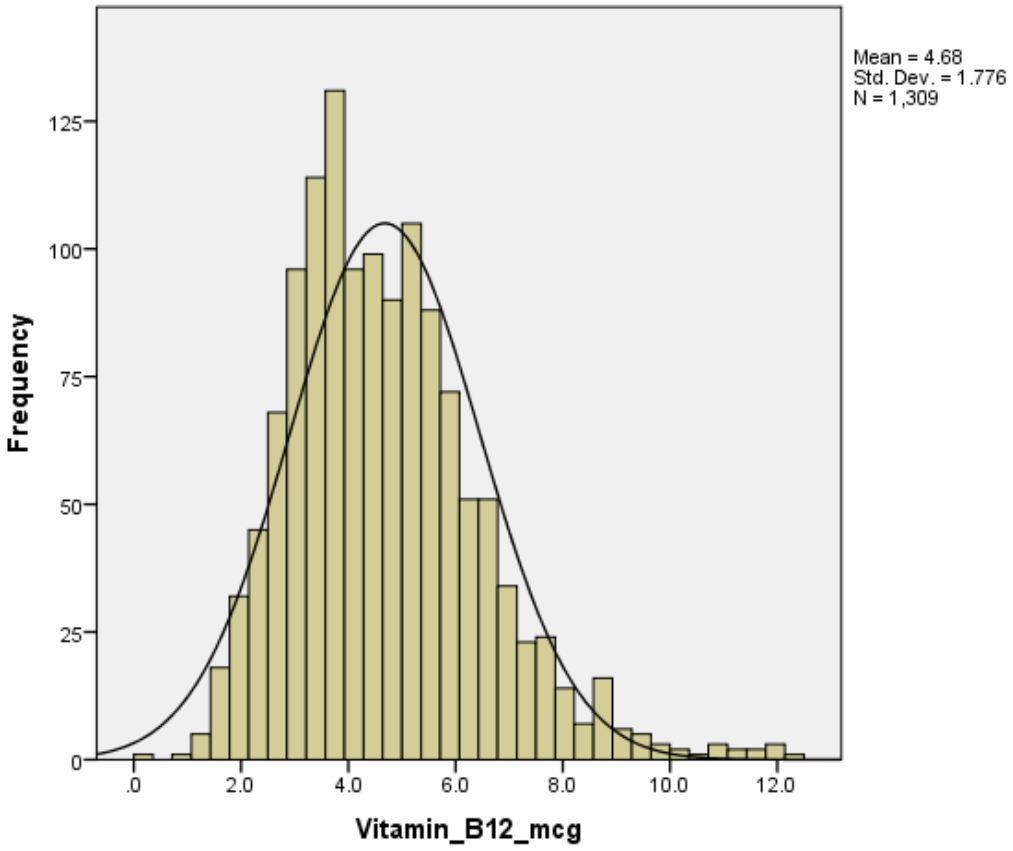

Figure 4. Histogram of Vitamin B_{12} without Outliers Superimposed with Normal Curve (n = 1,309)

Outliers were defined as exceeding ±2.54 standard deviations from the mean.

Table I. Descriptive Statistics of Predictor Variables and Birth Weight (n = 1,332)

Variable	**Mean ± SE**	**SD**	**Skewness ± SE**	**Kurtosis ± SE**	**Shapiro-Wilk P-Value**
DFE[a] (µg)	462.6 ± 3.67	133.88	0.60 ± 0.07	0.46 ± 0.13	< .001
Folate (µg)	271.6 ± 2.47	90.31	0.95 ± 0.07	1.84 ± 0.13	< .001
Folic Acid (µg)	113.5 ± 1.65	60.24	0.67 ± 0.07	0.54 ± 0.13	< .001
Vitamin B_2 (mg)	2.3 ± 0.02	0.61	0.97 ± 0.07	2.58 ± 0.13	< .001
Vitamin B_6 (mg)	1.9 ± 0.01	0.48	0.51 ± 0.07	0.42 ± 0.13	< .001
Vitamin B_{12} (µg)	5.0 ± 0.08	3.02	5.80 ± 0.07	57.84 ± 0.13	< .001
Vitamin B_{12} (µg) *without outliers*	4.7 ± 0.05	1.78	0.86 ± 0.07	1.25 ± 0.13	< .001
Choline (mg)	194.7 ± 1.72	62.81	0.71 ± 0.07	1.14 ± 0.13	< .001
Methionine (g)	1.7 ± 0.01	0.46	0.52 ± 0.07	0.86 ± 0.13	< .001
Zinc (mg)	11.8 ± 0.09	3.16	0.70 ± 0.07	1.18 ± 0.13	< .001
Birth Weight (g)	3360.9 ± 14.18	517.44	-0.59 ± 0.07	2.96 ± 0.13	< .001
Birth Weight (g) *without preterm*	3417.6 ± 12.54	444.44	0.23 ± 0.07	0.49 ± 0.14	< .01

[a] Dietary folate equivalent

Table II. Descriptive Statistics of Calorie-Adjusted Predictors (n = 1,233)

Variable	**Mean ± SE**	**SD**	**Skewness ± SE**	**Kurtosis ± SE**	**Shapiro-Wilk P-Value**
Folic Acid (µg)	52.2 ± 0.75	26.2	0.58 ± 0.07	0.63 ± 0.14	< .001
Folate (µg)	124.3 ± 1.02	35.7	0.94 ± 0.07	1.90 ± 0.14	< .001
DFE[a] (µg)	212.2 ± 1.41	49.5	0.49 ± 0.07	0.74 ± 0.14	< .001
Vitamin B_2 (mg)	1.0 ± 0.01	0.2	0.63 ± 0.07	0.67 ± 0.01	< .001
Vitamin B_6 (mg)	0.9 ± 0.01	0.2	0.64 ± 0.07	0.89 ± 0.14	< .001
Vitamin B_{12} (µg)	2.1 ± 0.02	0.8	1.3 ± 0.07	3.9 ± 0.14	< .001
Choline (mg)	88.8 ± 0.68	23.9	0.52 ± 0.07	0.42 ± 0.14	< .001
Methionine (g)	0.78 ± 0.01	0.2	0.52 ± 0.07	0.74 ± 0.14	< .001
Zinc (mg)	5.4 ± 0.03	1.1	0.99 ± 0.07	3.28 ± 0.14	< .001

[a] Dietary folate equivalent

Table III. Differences in Demographic and Socioeconomic Characteristics of Women who Consumed > ±2.58 SD of Vitamin B_{12}

Variable	**Standard Points**[d] **n (%)**	**Outliers**[f] **n (%)**	**Chi-Square or T-Test P-Value**
Age (years)	31.7 (4.3)[e]	33.3 (4.0)[e]	.07
Pre-pregnancy BMI (kg/m^2)	24.7 (5.4)[e]	25.2 (7.4)[e]	.67
Maternal Birthplace			.19
Canada	957 (73.1)	14 (60.9)	
Outside Canada	352 (26.9)	9 (39.1)	
Marital Status			.32
Married or Common Law[a]	1,254 (95.8)	23 (100)	
Single-Parent Family	55 (4.2)	0	
Maternal Education			.55
Secondary School	75 (5.7)	2 (8.7)	
College/University[b]	1,234 (94.3)	21 (91.3)	
Income			.57
< 30,000	98 (7.5)	1 (4.3)	
≥ 30,000	1,211 (92.5)	22 (95.7)	
Parity			.80
0	762 (58.2)	14 (60.9)	
1+	547 (41.8)	9 (39.1)	
Smoking			.55
Yes	169 (12.9)	2 (8.7)	
No[c]	1,140 (87.1)	21 (91.3)	
Gestational Diabetes			.93
Yes	121 (9.2)	2 (8.7)	
No	1,188 (90.8)	21 (91.3)	

[a] Includes women living with a partner
[b] Includes women who attended or completed college, technical diploma, CEGEP, University undergraduate degree or University post-graduate degree (Master's/PhD)
[c] Includes women who stopped smoking before becoming pregnant
[d] n = 1,309
[e] Reported values are the mean (standard deviation) for respective populations
[f] n = 23

Table IV. Differences in Demographic and Socioeconomic Characteristics in Women who had Infants Born Preterm and Those who had Infants Born Term (n = 1,309)

Variable	**Preterm[d]** **n (%)**	**Term[f]** **n (%)**	**Chi-Square or T-Test P-Value**	**Odds Ratio[g]**
Age (years)	31.3 (4.1)[e]	31.8 (4.3)[e]	.34	--
Pre-pregnancy BMI (kg/m^2)	26.3 (8.1)[e]	24.6 (5.3)[e]	.07	--
Maternal Birthplace			.34	--
Canada	52 (68.4)	905 (73.4)		
Outside Canada	24 (31.6)	328 (26.6)		
Marital Status			.29	--
Married or Common Law[a]	71 (93.4)	1,183 (95.9)		
Single-Parent Family	5 (6.6)	50 (4.1)		
Maternal Education			.006	2.72 (1.33, 5.54)
Secondary School	10 (13.2)	65 (5.3)		
College/University[b]	66 (86.8)	1,168 (94.7)		
Household Income ($)			.30	--
< 30,000	8 (10.5)	90 (7.3)		
≥ 30,000	68 (89.5)	1,143 (92.7)		
Parity			.51	--
0	47 (61.8)	715 (58.0)		
1+	29 (38.2)	518 (42.0)		
Smoking			.03	1.88 (1.06, 3.35)
Yes	16 (21.2)	153 (12.4)		
No[c]	60 (78.9)	1,080 (87.6)		
Gestational Diabetes			.02	2.15 (1.15, 4.03)
Yes	13 (17.1)	108 (8.8)		
No	63 (82.9)	1,125 (91.2)		

[a] Includes women living with a partner
[b] Includes women who attended or completed college, technical diploma, CEGEP, University undergraduate degree or University post-graduate degree (Master's/PhD)
[c] Includes women who stopped smoking before becoming pregnant
[d] n = 76
[e] Reported values are the mean (standard deviation) for respective populations
[f] n = 1,233
[g] Likelihood of giving birth to an infant at term

Table V. Differences in Mean Dietary Methyl Donor Intake in Women who had Infants Born Preterm and Those who had Infants Born Term (n = 1,233)

Mean Intake	**Preterm**[b] **Mean (SD)**	**Term**[c] **Mean (SD)**	**T-Test P-Value**
DFE (μg)	448.9 (111.2)	462.7 (135.0)	.30
Vitamin B_2 (mg)	2.2 (0.6)	2.2 (0.6)	.54
Vitamin B_6 (mg)	1.8 (0.6)	1.9 (0.5)	.76
Vitamin B_{12} (μg)	4.7 (2.0)	4.7 (1.7)	.79
Choline (mg)	193.2 (66.0)	193.2 (60.4)	.996
Methionine (g)	1.6 (0.5)	1.7 (0.4)	.43
Zinc (mg)	11.6 (3.2)	11.8 (3.1)	.64

[a] Dietary folate equivalent
[b] n = 76
[c] n = 1,233

Table VI. Differences in Calorie-Adjusted Dietary Methyl Donor Intake in Women who had Infants Born Preterm and Those who had Infants Born Term (n = 1,233)

Calorie-Adjusted Variable	**Preterm[a] Mean (SD)**	**Term[b] Mean (SD)**	**T-Test P-Value**
DFE (µg)	213.9 (47.9)	212.2 (49.5)	.78
Vitamin B_2 (mg)	1.0 (0.2)	1.0 (0.2)	.98
Vitamin B_6 (mg)	0.9 (0.2)	0.9 (0.2)	.89
Vitamin B_{12} (µg)	2.2 (0.7)	2.1 (0.8)	.67
Choline (mg)	90.2 (24.5)	88.8 (23.9)	.61
Methionine (g)	0.8 (0.2)	0.8 (0.2)	.61
Zinc (mg)	5.4 (1.0)	5.4 (1.1)	.72

[a] n = 76
[b] n = 1,233

Table VII. Spearman's Correlation Matrix Including All Dietary Methyl Donors (n = 1,233)

	Folic Acid	**DFE**	**Folate**	**Vitamin B_2**	**Vitamin B_6**	**Vitamin B_{12}**	**Choline**	**Methionine**	**Zinc**
Folic Acid	1.00	--	--	0.24	-0.27	-0.03	0.02	0.02	0.11
DFE	--	1.00	--	0.46	0.33	0.15	0.32	0.25	0.36
Folate	--	--	1.00	0.41	0.56	0.25	0.44	0.35	0.42
Vitamin B_2	0.24	0.46	0.41	1.00	0.48	0.69	0.65	0.59	0.66
Vitamin B_6	-0.27	0.33	0.56	0.48	1.00	0.43	0.51	0.58	0.59
Vitamin B_{12}	-0.03	0.15	0.25	0.69	0.43	1.00	0.51	0.65	0.65
Choline	0.02	0.32	0.44	0.65	0.51	0.51	1.00	0.54	0.52
Methionine	0.02	0.25	0.35	0.59	0.58	0.65	0.54	1.00	0.68
Zinc	0.11	0.36	0.42	0.66	0.59	0.65	0.52	0.68	1.00

Dietary methyl donors followed by their units are as follows: folic acid (μg), folate (μg), DFE (μg), vitamin B_2 (mg), vitamin B_6 (mg), vitamin B_{12} (μg), choline (mg), methionine (g), zinc (mg).

Table VIII. Reported P-values of Significant Covariates for Predicting Either an Outcome or a Predictor Variable (n = 1,233)

Covariate	**Predictor**	**Outcome**
Age (years)	Folic Acid: p =.001 Folate: p = .002	--
Maternal Birthplace	Folic Acid: p = .001 Folate: p = .002 DFE: p < .001	--
Marital Status	Folic Acid: p = .007	--
Household Income ($)	Folic Acid: p = .04 Folate: p = .001 Vitamin B_6: p = .04	--
Pre-pregnancy BMI (kg/m^2)	--	Birth weight: p < .001
Gestational Age (weeks)	--	Birth weight: p < .001
Infant Sex	--	Birth weight: p = .002
Maternal Education	--	SGA: p = .04
Parity	--	Birth weight: p < .001
Smoking	--	Birth weight: p = .04

Variables that were neither significant with birth weight, SGA, LGA, folic acid, folate nor dietary folate equivalent were tested for significance with the rest of the dietary methyl donors, but no significant predictive value was observed. Maternal age, household income, marital status, maternal birthplace and gestational diabetes mellitus were tested for significance in predicting birth weight, but were not found to be significant ($p \geq .05$).

Table IX. Significant Interactions Between Covariates and Dietary Methyl Donors on Birth Weight (n = 1,233)

Interaction Terms	**Birth Weight (P-value)**
Vitamin B_{12} x Maternal Birthplace	.03
Vitamin B_{12} x Smoking	.03
Vitamin B_6 x Gestational Age	.02
Choline x Gestational Age	.04

"x" denotes an interaction

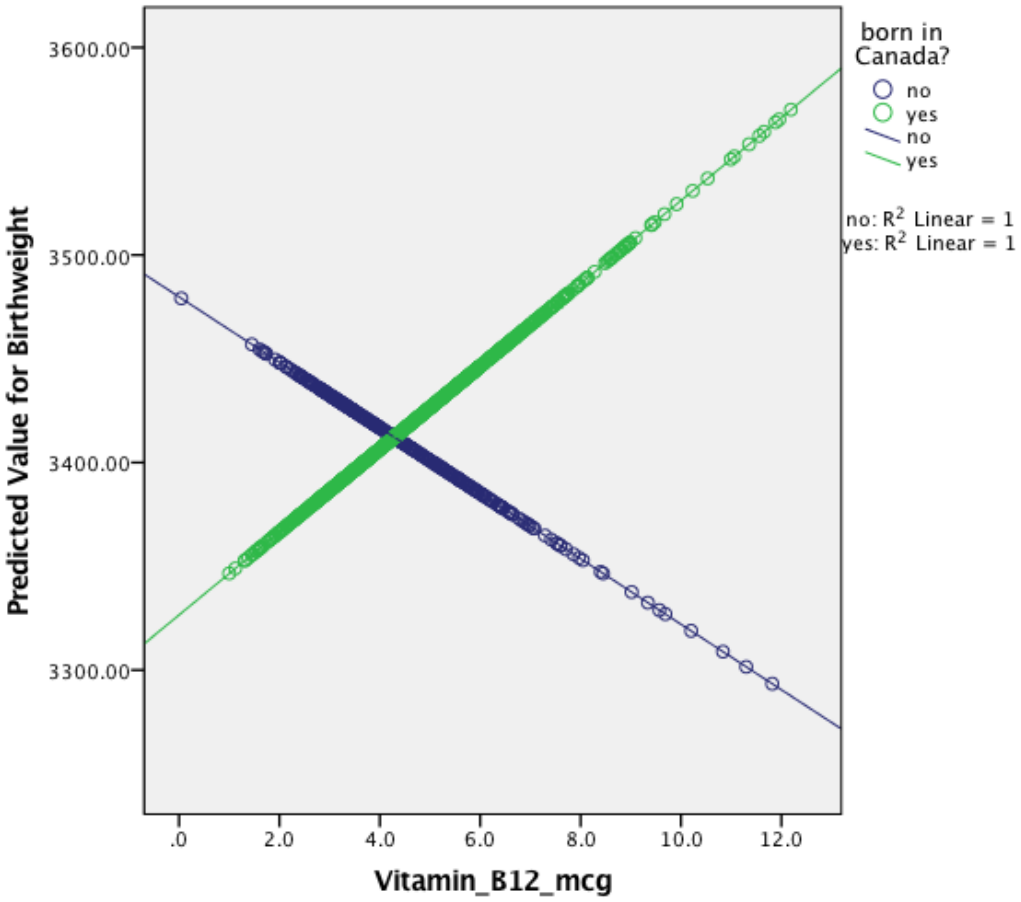

Figure 5. Interaction Plot Outlining the Interaction Between Maternal Birthplace and Vitamin B_{12}.

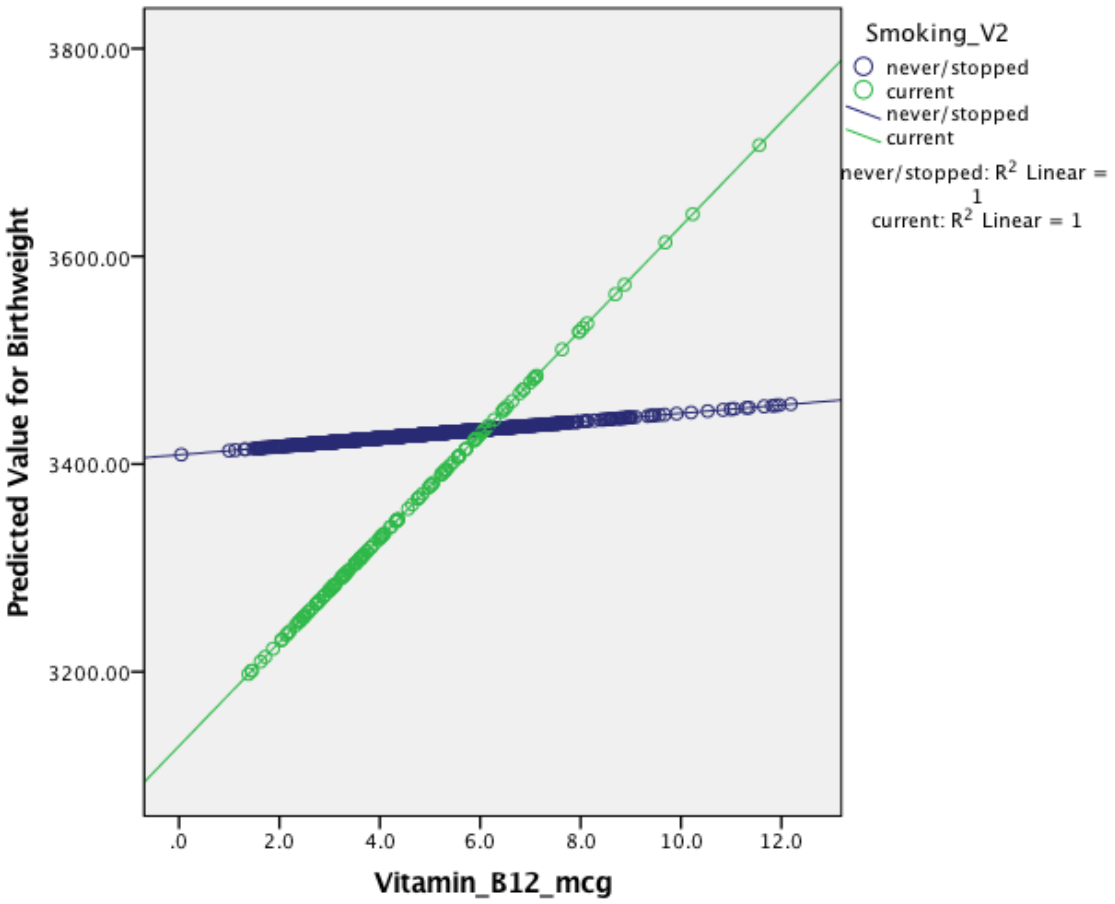

Figure 6. Interaction Plot Outlining the Interaction Between Smoking and Vitamin B_{12}.

Table X. Spearman Correlation Coefficients for Covariates and Dietary Methyl Donors and Resulting P-Values of Interaction Terms in SGA and LGA Models (n = 1,233)

Putative Interaction Term	**Correlation Coefficient**	**P-Value in SGA[a] model**	**P-Value in LGA[b] model**
Folic Acid x Maternal Age	- 0.096	.29	.62
Folic Acid x Pre-pregnancy BMI	0.127	.34	.23
Folic Acid x Maternal Education	.082	.76	.29
Folic Acid x Parity	0.09	.38	.51
Folate x Gestational Age	0.079	.34	.23
Folate x Maternal Age	0.096	.13	.65
Folate x Pre-pregnancy BMI	- 0.112	.70	.25
Folate x Maternal Education	- 0.082	.93	.31
Folate x Household Income	0.098	.09	.56
Folate x Parity	- 0.085	.82	.96
Vitamin B_6 x Pre-pregnancy BMI	- 0.105	.94	.20
Vitamin B_6 x Household Income	0.073	.61	.67
Choline x Household Income	0.094	.59	.99
Zinc x Household Income	0.089	.54	.87

"x" denotes an interaction
[a] Small for gestational age
[b] Large for gestational age

Table XI. Interactions Between Dichotomous Covariates and Dietary Methyl Donors on SGA and LGA (n = 1,233)

Interaction Term	**P-value in SGA[a] model**	**P-value in LGA[b] model**
Folic Acid x Infant Sex	.22	.52
DFE x Infant Sex	.55	.50
Folate x Infant Sex	.02	.80
Vitamin B_2 x Infant Sex	.19	.80
Vitamin B_6 x Infant Sex	.03	.77
Vitamin B_{12} x Infant Sex	.25	.79
Choline x Infant Sex	.21	.19
Methionine x Infant Sex	.09	.90
Zinc x Infant Sex	.38	.38
Folic Acid x Maternal Birthplace	.82	.60
DFE x Maternal Birthplace	.70	.06
Folate x Maternal Birthplace	.39	.02
Vitamin B_2 x Maternal Birthplace	.47	.16
Vitamin B_6 x Maternal Birthplace	.65	.69
Vitamin B_{12} x Maternal Birthplace	.92	.86
Choline x Maternal Birthplace	.82	.11
Methionine x Maternal Birthplace	.58	.33
Zinc x Maternal Birthplace	.57	.21
Folic Acid x Smoking	.89	.72
DFE x Smoking	.20	.44
Folate x Smoking	.12	.38
Vitamin B_2 x Smoking	.07	.20
Vitamin B_6 x Smoking	.08	.24
Vitamin B_{12} x Smoking	.23	.18
Choline x Smoking	.50	.02
Methionine x Smoking	.06	.47
Zinc x Smoking	.21	.08

"x" denotes an interaction
[a] Small for gestational age
[b] Large for gestational age

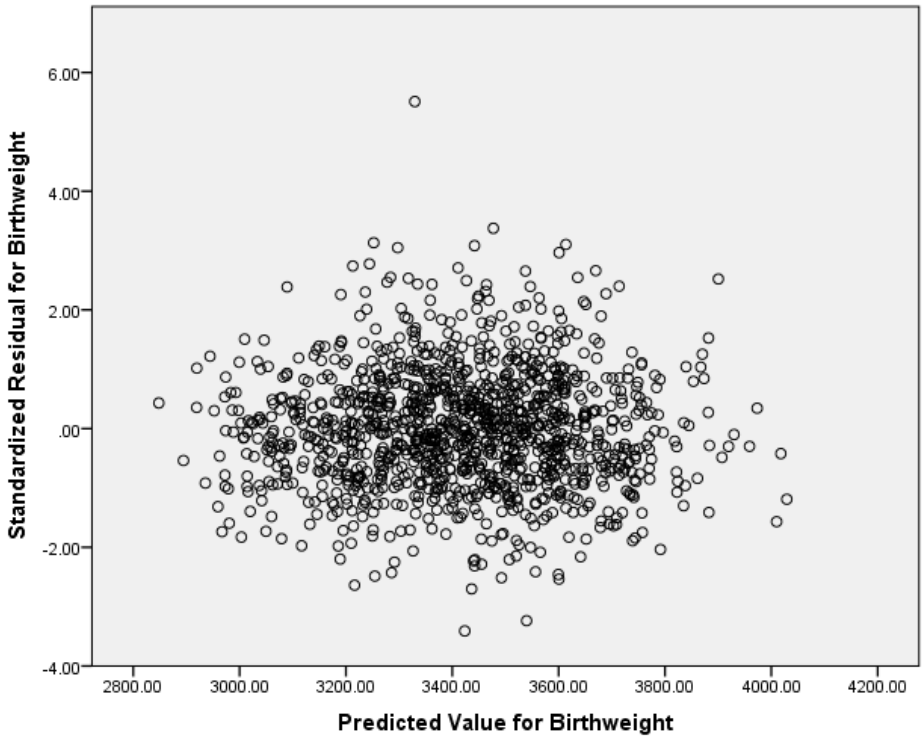

Figure 7. Residual Plot for Fully Adjusted Model of Birth Weight