FOLATE, VITAMIN B12 AND NEONATE BIRTH WEIGHT

THE ASSOCIATION OF MATERNAL FOLATE AND VITAMIN B12 CONCENTRATIONS DURING PREGNANCY WITH NEONATE BIRTH WEIGHT IN SOUTH ASIANS AND WHITE EUROPEANS LIVING IN CANADA: START, FAMILY AND CHILD BIRTH COHORTS

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A thesis submitted in fulfillment of the requirements for the degree of Master of Medical Science in the Faculty of Health Sciences Graduate Studies (Nutrition and Metabolism)

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TITLE: The Association of Maternal Folate and Vitamin B12 Concentrations During Pregnancy with Neonate Birth Weight in South Asians and White Europeans Living in Canada: START, FAMILY and CHILD Birth Cohorts

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Lay Abstract

Infant birth weight is an indicator of health and disease risk in adult life. The mother's vitamin intake can influence the weight of the infant. This research aimed to study whether the mother's folate and vitamin B12 status is related to infant birth weight. Dietary and supplemental data along with blood samples from South Asian and white European pregnant women living in Canada were collected during the second trimester. The mother's dietary, supplemental and total folate and vitamin B12 intakes were not related to infant birth weight. In South Asian mothers, higher milk intake was related to higher birth weight and in white Europeans, higher egg intake was related to lower infant birth weight. Higher vitamin B12 in the blood was related to lower infant birth weight in South Asians. More research is needed to determine the relationship between folate and vitamin B12 with infant birth weight.

Abstract

Background Folate and vitamin B12 have interdependent metabolic functions that are essential for neonate growth outcomes (i.e. birth weight) based on studies from India. The objective of this research was to evaluate the association of maternal folate and vitamin B12 concentrations with neonate birth weight in South Asian (SA) and white European (WE) populations.

Methods In this cross-sectional analysis of prospective cohort studies, maternal and neonatal data were collected during the second trimester from 3758 mother-child dyads living in Canada. Maternal diet and supplement use were assessed using a validated food frequency questionnaire. Biochemical indicators were analyzed in a subset of SA mothers. Birth weight was measured within 72 hours of delivery. All regression analyses were performed unadjusted and with adjustment for identified covariates.

Results Maternal folate and vitamin B12 (dietary, supplemental and total) were not associated with neonate birth weight in SA and WE pregnant women. Higher consumption of milk products by SA women was associated with higher birth weight (β=0.06; p=0.01), whereas higher consumption of egg by WE women was associated with lower birth weight (β=-0.19; p<0.01). Folate and vitamin B12 deficiency in the SA subgroup was 13.7% and 17.8%, respectively. Maternal serum vitamin B12 status was inversely associated with birth weight (β=-0.16; p=0.03).

Conclusions Folate and vitamin B12 may be proxies for poor nutritional status. Therefore, folate and vitamin B12 may have an association with neonate birth weight in a less developed area (i.e. India) rather than in a highly developed area (i.e. Canada).

Highly developed countries have an adequate intake of folate and vitamin B12 and thus a higher nutritional baseline status. These findings complement current research on folate and vitamin B12 concentrations with birth weight in well-nourished populations.

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Lists of Abbreviations

START = South Asian Birth Cohort

FAMILY = Family Atherosclerosis Monitoring in Early Life

CHILD = Canadian Healthy Infant Longitudinal Development

SA = South Asian

WE = white European

Hcy = homocysteine

MMA = methylmalonic acid

THF = tetrahydrofolate

PG = polyglutamate

MG = monoglutamate

5MTHF = 5-methyl-tetrahydrofolate

MTHFR = methylenetetrahydrofolate reductase

TCII = transcobalamin II

FFQ = food frequency questionnaire

SHARE = Study of Health Assessment and Risk Evaluation

BMI = body mass index

GDM = gestational diabetes mellitus

OR = odds ratio

EAR = estimated average requirement

RDA = recommended dietary allowance

Declaration of Academic Achievement

This thesis has been prepared in fulfillment of the requirement for the degree of Master of Science in Medical Sciences (Metabolism & Nutrition). I prepared this thesis under the direction and supervision of Dr. Sonia Anand. Dr. Anand acted as the primary supervisor in all aspects of this research project and provided feedback to the proposal, study design and thesis drafts. Dr. Anand and I provided substantial contributions to the conception and design of this study. Dr. de Souza, and Dr. Britz-McKibbon, served as supervisory committee members. Dr. de Souza provided training and feedback with the study design and statistical methods/tests used in this thesis. Dr. de Souza and Dr. Britz-McKibbon provided feedback on the research proposal and thesis draft. I performed all statistical analyses, in consultation with Dr. de Souza as well as drafted the thesis manuscript.

Chapter 1: Literature Review

1.1 Introduction

Fetal development during gestation may have health consequences extending into adulthood. Genetic factors, environmental exposures, and maternal health behaviours influence fetal development. Barker proposed that adverse fetal nutrition during pregnancy increases susceptibility to the metabolic syndrome (Edwards, 2017). Maternal undernutrition and overnutrition increases the fetus's risk of future health complications such as obesity, insulin resistance and diabetes (Krishnaveni et al, 2014). Therefore, it is important for pregnant women to consume the required micronutrients in adequate amounts to sustain a normal fetal growth trajectory (Relton et al, 2005; Ahmed et al, 2011).

Pregnancy is a period of increased metabolic demand (i.e. a higher requirement of energy, protein and micronutrients) required to sustain the high demands of the growing fetus and the mother (Gadgil et al, 2014 and Brown, 2017). B vitamins are cofactors in energy metabolism and as such, the requirement of these vitamins increases proportionally to the increase in energy during pregnancy. Changes in folate and vitamin B12 metabolism occurs to ensure a continuous supply of nutrients are available to the growing fetus and the uteroplacental organs, despite intermittent maternal dietary intake. A high circulating folate concentration is required during pregnancy due to the increased demand of the fetus, increased folate catabolism, increased folate excretion, decreased folate absorption, hormonal influence on folate metabolism and adverse effects of low

folate intake (Butte, 2000). Folate requirements during pregnancy increases by 147% to build and maintain maternal stores and to meet the needs of the growing fetus. Furthermore, serum vitamin B12 concentrations progressively decline during pregnancy, and it is therefore crucial to maintain vitamin B12 status through dietary means. To meet the demands of the fetus and increased maternal metabolic needs a 40% increase in vitamin B12 is recommended (Ladipo, 2000).

Folate and vitamin B12 are essential nutrients as they cannot be synthesized de novo and must be obtained through external means (Greenberg et al, 2011; Quay et al, 2015). Folate and vitamin B12 play a critical role in nucleic acid metabolism, cell growth and proliferation (Wadhwani et al, 2016). During pregnancy, folate and vitamin B12 requirements are higher than usual, to support rapid cell proliferation and tissue growth of the uterus, placenta, and fetus and the expanding maternal blood volume (Shane, 2008). As such, the importance of an adequate supply of folate and vitamin B12 is well documented in the literature (Fall et al, 2003; Sande et al, 2013; Chandyo et al, 2017).

Folate is higher in plant-based diets as high sources of folate include green leafy vegetables, fruits and fortified flour. In South Asians (SA) living in Canada, higher maternal adherence to a plant-based diet was associated with a higher neonate birth weight; while a higher adherence to a plant-based diet in White Europeans (WE) was associated with a lower neonate birth weight (Zulyniak et al, 2017). The reason for this discrepancy is not known. However, this finding is important as SA neonates typically have a lower birth weight than WE neonates (de Wilde et al, 2013).

Understanding the potential differences in the influence of maternal folate and vitamin B12 intake in different ethnic groups will enhance our understanding of the association between maternal folate, vitamin B12, and neonate birth weight. With this view, the present study was designed to explore the association between maternal folate, maternal vitamin B12 and neonatal birth weight in a Canadian birth cohort study. This thesis answers 3 exploratory questions:

1) What is the association between maternal dietary, supplemental and total folate and vitamin B12 and neonate birth weight in SA and WE populations?
2) What is the association of folate and vitamin B12-rich food groups (i.e. green leafy vegetables, fruits and milk) consumption with neonate birth weight in SA and WE populations?
3) What is the association between maternal biochemical indicators of folate and vitamin B12 status (i.e. serum vitamin B12) and neonate birth weight?

1.2 Folate and Vitamin B12

1.2.1 Folate

1.2.1a Folate Forms

Folate or vitamin B9 is a water-soluble B vitamin naturally present in certain foods, added to grain-based products, and also taken as a dietary supplement. “Dietary folate” refers to the form of folate naturally found in food, known as tetrahydrofolate (THF), which usually has additional glutamate residues, making it a polyglutamate (PG). “Folic acid” refers to the oxidized monoglutamate (MG) form of folate that is synthesized

and added to foods through fortification or sold as supplements (Figure 1.1) (Bailey & Caudill, 2012; Greenberg et al, 2011). "Total folate" encompasses both dietary folate and folic acid.

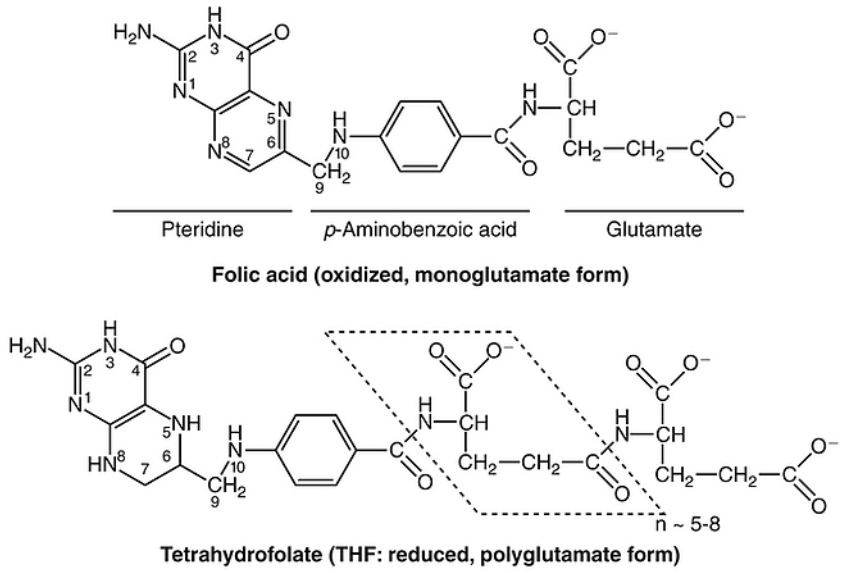

Figure 1.1: Chemical structure of dietary folate and folic acid

1.2.1b Folate Function

Folate is involved in four key metabolic cycles at the cellular level: 1) biosynthesis of purines and thymidylate, precursors of DNA and RNA synthesis; 2) synthesis of methionine from homocysteine (Hcy); 3) synthesis of S-adenosylmethionine 4) catabolism of serine and glycine (Shane, 2008). During pregnancy, there is an increased requirement for folate in order to accommodate the rapid cell proliferation, tissue growth of the uterus and placenta, growth of the fetus, and expansion of maternal blood volume (Shane, 2008).

1.2.1c Folate Digestion & Absorption

Dietary folate predominantly occurs in the PG form and is cleaved into the MG form prior to absorption (Figure 1.2). Hydrolysis of PG folate primarily occurs in the proximal jejunum, catalyzed by the brush border enzyme glutamate carboxypeptidase II. In order for absorption to occur, glutamate carboxypeptidase II cleaves the terminally linked glutamate residues from the PG folate into a MG. In humans, the total body concentration of folate is approximately 15-30 mg based on the liver tissue concentration (Bailey & Caudill, 2012; Shane, 2008).

The primary folate transport mechanism in the intestines are the transmembrane carriers. The reduced folate carrier is a bi-directional transmembrane protein that is saturable with a high affinity for folates but a low affinity for folic acid. Protein coupled folate transporter is a transmembrane unidirectional transport protein that plays a major role in intestinal folate absorption. It is expressed in the brush border of the small intestine, functions optimally in an acidic pH environment, and has a high affinity for both folate and folic acid (Bailey & Caudill, 2012). The transport of folate via the circulation into the intestinal cells is mediated by protein coupled folate transporters. However, cellular uptake into the peripheral tissues primarily utilizes the reduced folate carrier. As dietary folate and folic acid pass through the intestinal mucosa, a large proportion is metabolized to 5-methyl-tetrahydrofolate (5MTHF) (Bailey & Caudill, 2012; Shane, 2008). Another folate transport mechanism found at the cell level are the folate-binding protein mediated systems such as folate receptors.

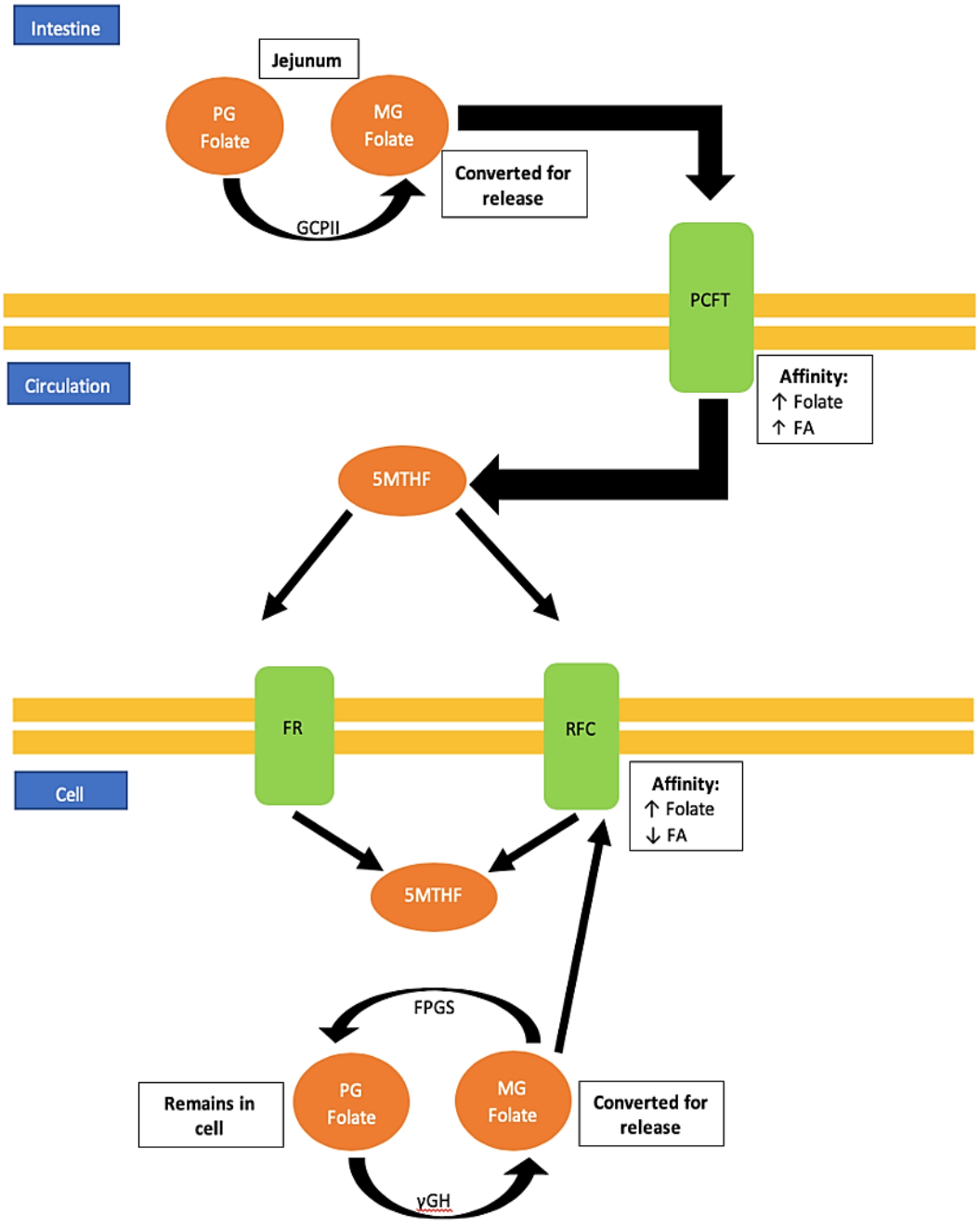

Figure 1.2: A schematic overview of folate absorption and transport from the intestinal mucosa, via the circulation and into the cell
Abbreviations (PG: polyglutamate; MG: monoglutamate; GCPII: glutamate carboxypeptidase II; PCFT: protein-coupled folate transporter; 5MTHF: 5-methyl-tetrahydrofolate; FR: folate receptor; RFC: reduced folate carrier; FPGS: folylpolyglutamate synthase; γGH: γ-glutamyl hydrolase

1.2.1d Folate Metabolism & Bioavailability

Folic acid is metabolized into dihydrofolate and then reduced to THF in the mucosal cells by dihydrofolate reductase (Figure 1.3). Following this, THF, the active coenzyme form of folate, is converted into methylfolate by the enzyme methylenetetrahydrofolate reductase (MTHFR). This conversion is required to use methylfolate in the one-carbon transfer reaction needed for purine and pyrimidine synthesis during DNA and RNA assembly, DNA methylation and to regulate Hcy metabolism (Greenberg et al, 2011). When a high dose of folic acid is consumed (>10 μM), absorption occurs by a non-saturable diffusion like process, leading to the appearance of unmetabolized folate in circulation. Furthermore, unmetabolized folic acid can also be found in the circulation if the metabolic capacity of dihydrofolate reductase is exceeded (Bailey & Caudill, 2012; Shane, 2008).

After entering the circulation, folate primarily circulates as 5MTHF and is transported to the liver. In circulation, both low and high affinity proteins bind to folate, of which ~50% binds loosely to albumin. Folate receptors and high affinity binding proteins engage in folate uptake via the endocytosis process. Here, folate is metabolized into the PG form for storage, intracellular usage or release back into the blood or bile, after reduction to a MG form (Figure 1.3). (Bailey & Caudill, 2012).

In the cells, methionine synthase demethylates 5MTHF into THF and folylpolyglutamate synthetase converts it back to a PG. Folate retained in the tissues in the PG form does not cross the cell membrane due to its charge and binding mechanisms to enzymes. Therefore, most tissue folate is in the PG form. However, tissues are unable

to store additional folate beyond that required for metabolic function. Therefore, in order to be released from the tissues, folate is reconverted into the MG form (Figure 1.2; Figure 1.3) (Bailey & Caudill, 2012; Shane, 2008).

Folate plays a crucial role in the one-carbon metabolism for physiological nucleic acid synthesis, cell division, regulation of gene expression, amino acid metabolism and neurotransmitter synthesis. Folate coenzymes function in the donation and acquisition of one-carbon units during metabolic reactions (Bailey & Caudill, 2012).

Bioavailability refers to the efficiency of folate utilization, including the physiological and biochemical processes involved in intestinal absorption, transport, metabolism and excretion. Unlike folic acid, dietary folate sources exhibit variable and incomplete bioavailability (Bailey & Caudill, 2012). The bioavailability of dietary folate when consumed on an empty stomach is 100%. However, the bioavailability of dietary folate and folic acid consumed with food is limited. It is estimated that 50% of dietary folate and 85% of folic acid is bioavailable (Bailey & Caudill, 2012; Shane, 2008).

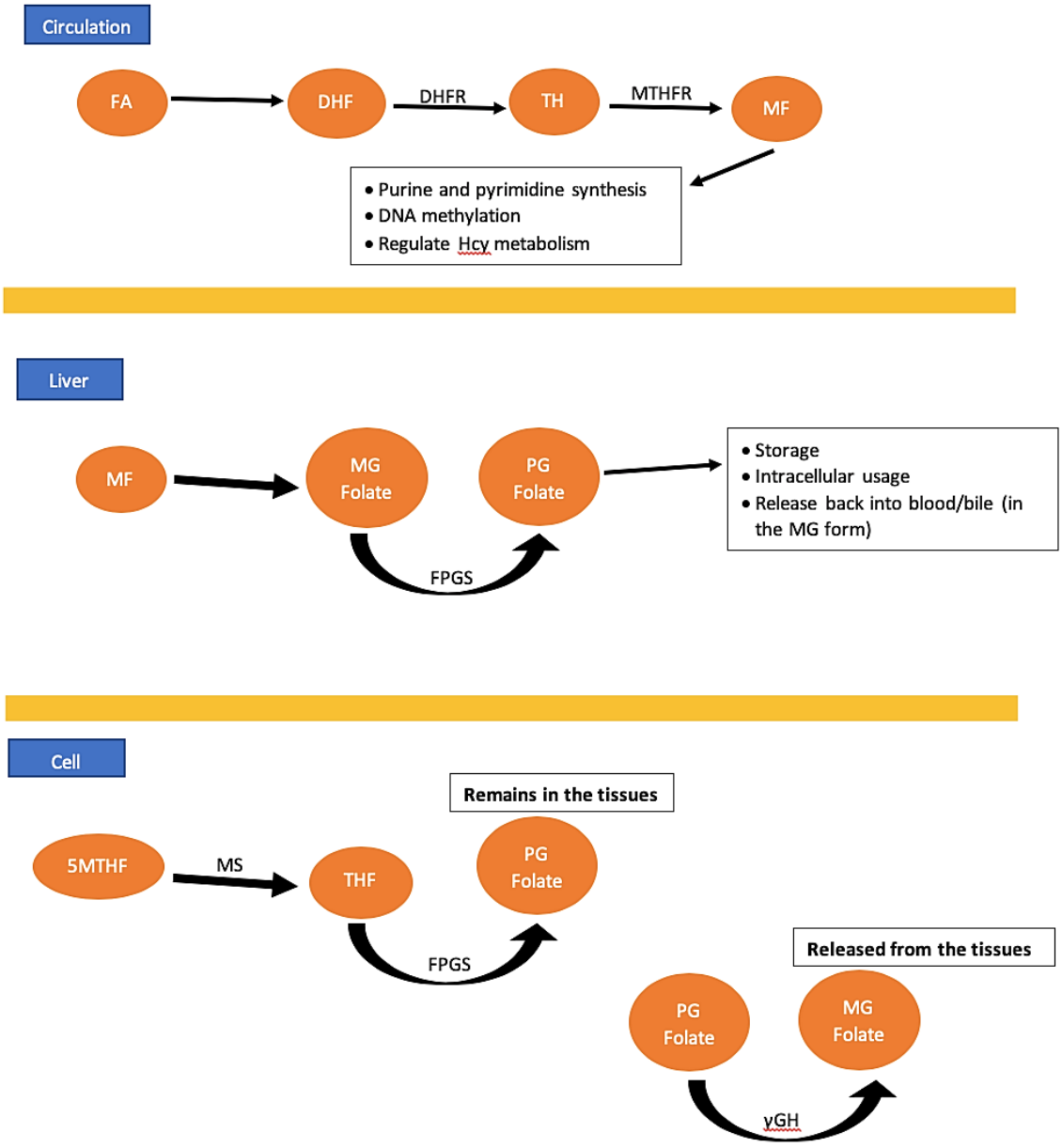

Figure 1.3: A systematic overview of folate metabolism from the circulation to the liver tissue and into the cell
Abbreviations (FA: folic acid; DHF: dihydrofolate; DHFR: dihydrofolate reductase. THF: tetrahydrofolate, MTHFR: methylenetetrahydrofolate reductase; Hcy: homocysteine; MF: methylfolate; MG monoglutamate; PG: polyglutamate; FPGS: folylpolyglutamate synthase; 5MTHF: 5-methyl-tetrahydrofolate; MS: methionine synthase; γGH: γ-glutamyl hydrolase)

1.2.2 Vitamin B12

1.2.2a Vitamin B12 Form

Vitamin B12 also known as cobalamin is a water-soluble vitamin consisting of a central cobalt atom surrounded by a ring structure (Figure 1.4) (Banerjee & Ragsdale, 2003).

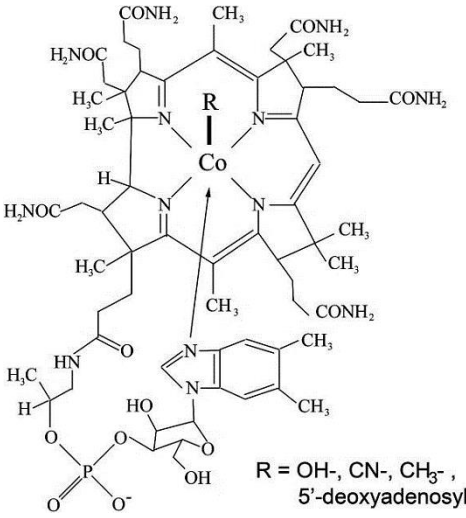

Figure 1.4: Chemical structure of vitamin B12

1.2.2b Vitamin B12 Function

Vitamin B12 is a cofactor for two enzymes, methylmalonyl-CoA mutase and methionine synthase. Vitamin B12 functions as a coenzyme for the methylmalonyl-CoA mutase in the odd chain fatty acid and energy metabolism in the mitochondria and for methionine synthase in conjunction with MTHFR in the remethylation of Hcy in the cytosol (Banerjee & Ragsdale, 2003). Vitamin B12 is a co-enzyme necessary for lipid, protein, carbohydrate and Hcy metabolism, erythropoiesis and DNA and RNA synthesis (Ozturk et al, 2015).

1.2.2c Vitamin B12 Digestion & Absorption

Vitamin B12 digestion begins in the mouth as saliva contains a vitamin B12 binding protein called haptocorrin (Figure 1.5). Vitamin B12 is sometimes released in the stomach as a result of the acidic environment (Shane, 2008). The vitamin B12 bound to haptocorrin is carried into the duodenum. The gastric parietal cells release 2-4 μg of a specific vitamin B12 binding protein known as intrinsic factor. However, intrinsic factor does not bind to vitamin B12 until the stomach acid is neutralized and haptocorrin is removed from vitamin B12 by digestive enzymes known as proteases (Quadros, 2010; Bailey & Caudill, 2012). The intrinsic factor-vitamin B12 complex is carried into the ileum and taken up by a transmembrane protein, the intrinsic factor-receptor. Intrinsic factor then degrades and vitamin B12 is released into the cytosol. The gut epithelial cells then release vitamin B12 as a complex attached to a protein known as transcobalamin II (TCII). The vitamin B12 absorption process occurs over a span of 3 to 4 hours. The TCII-vitamin B12 complex is secreted into the portal blood and delivered to the liver and the rest of the tissues. The major form of vitamin B12 in the plasma is methylcobalamin, whereas in the liver, vitamin B12 is in the form of 5'-deoxyadenosylcobalamin. The TCII complex is tasked with carrying absorbed vitamin B12, as it is the primary serum transport protein for vitamin B12. TCII-vitamin B12 is transported to various tissues based on a receptor mediated endocytosis process, in which TCII is recognized by the receptors. Following endocytosis, the TCII-vitamin B12 complex is degraded and the free vitamin B12 is transported into the cytosol (Bailey & Caudill, 2012).

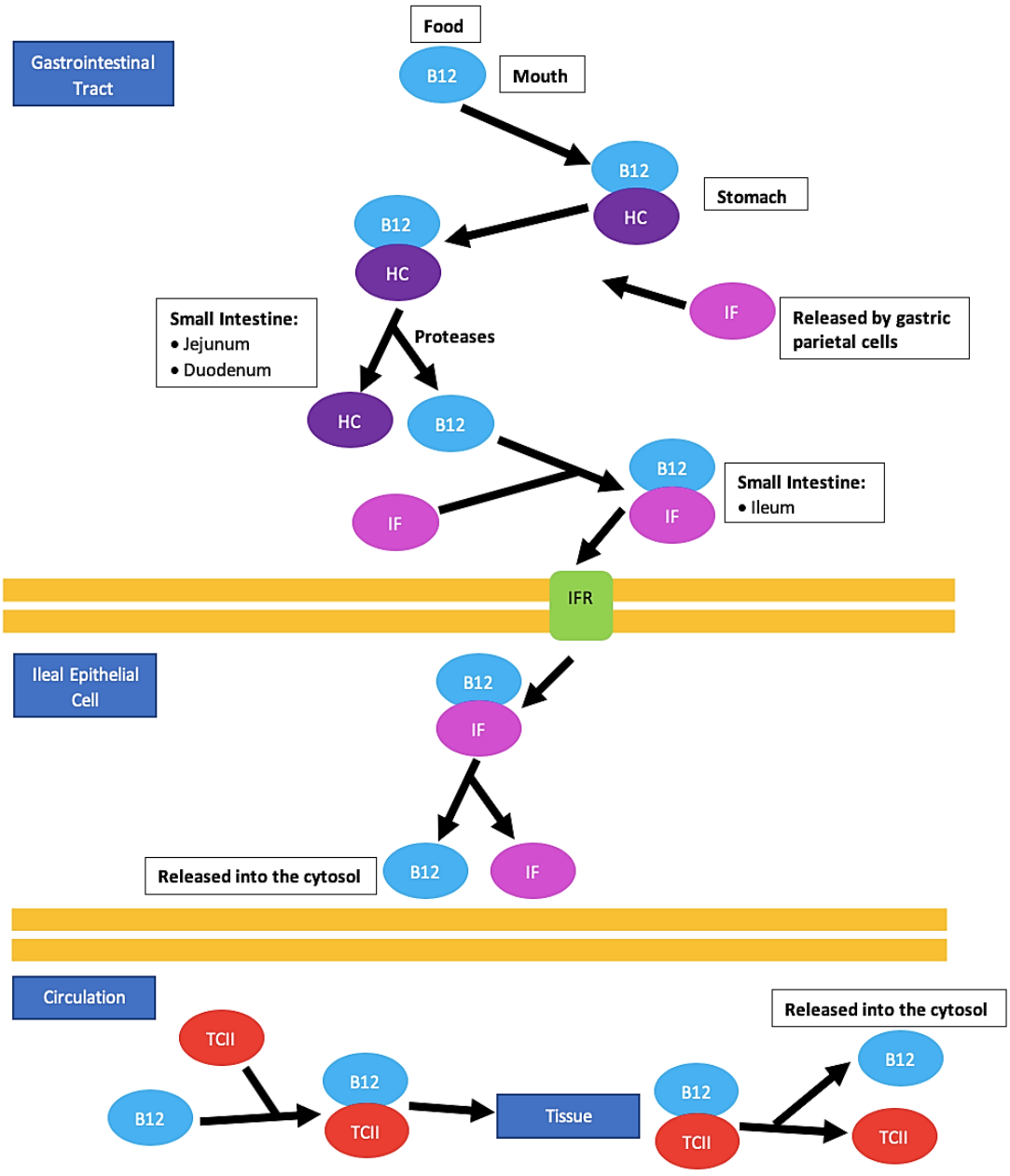

Figure 1.5: A schematic overview of vitamin B12 absorption and transport from the gastrointestinal tract into the tissues, via the circulation
Abbreviations (B12: vitamin B12; HC: haptocorrin; IFR: intrinsic factor receptos; TCII: transcobalamin II)

1.2.2d Vitamin B12 Metabolism & Bioavailability

During methionine synthesis, the methyl-cobalt bond undergoes heterolytic cleavage and the methionine synthase is activated. Methionine is an essential amino acid and a methyl donor to s-adenosylmethionine via the activity of methyltransferase (Figure 1.6). S-adenosylmethionine is a required methyl source for creatine and phospholipids, neurotransmitters, DNA, and RNA synthesis, and protein methylation. Once the methyl group is donated, s-adenosylhomocysteine is created which is then cleaved by s-adenosylhomocysteine-hydrolase to form Hcy and adenosine. Hcy can then be remethylated to methionine or condensed with serine into cystathionine by a vitamin B6 dependant enzyme known as cystathionine beta-synthase. Methionine can be synthesized by two different enzymes, vitamin B12-dependant methionine synthase as well as betaine-homocysteine methyltransferase (Erickson, 1960; Matthews et al, 2008; Bailey & Caudill, 2012). Essentially, a methyl group is taken from 5-methyl-tetrahydrofolate by methionine synthase and added to Hcy. The methyl-vitamin B12 bound to methionine synthase is demethylated during the Hcy reaction but is remethylated during the 5-methyl-tetrahydrofolate reaction. Therefore, methionine metabolism is dependent on vitamin B6, folate and vitamin B12 (Bailey & Caudill, 2012).

The production of 5-methyl-tetrahydrofolate by methylenetetrahydrofolate reductase is an important functional and regulatory step in the conversion of Hcy to methionine. The folate-dependant remethylation process is catalyzed by methionine synthase and requires vitamin B12 and 5-methyl-tetrahydrofolate. In this reaction, a methyl group from 5-methyl-tetrahydrofolate is transferred to vitamin B12 and then

sequentially to Hcy, in order to create methionine. The involvement of both folate and vitamin B12 with methionine synthase is the biological reason the metabolisms of both vitamins are so closely intertwined (Bailey & Caudill, 2012; Wadhwani et al, 2016).

The bioavailability of vitamin B12 depends on the amount in the diet, however it averages 50%. The half-life of the transcobalamin II-vitamin B12 complex in the plasma is approximately 6 minutes. Transcobalamin II delivers 4 nmol/day of vitamin B12 to the tissues. A high proportion, estimated to be 2-3 mg, of vitamin B12 is stored in the liver. It is estimated, that the whole-body turnover rate for vitamin B12 is 0.1%/day (Bailey & Caudill, 2012).

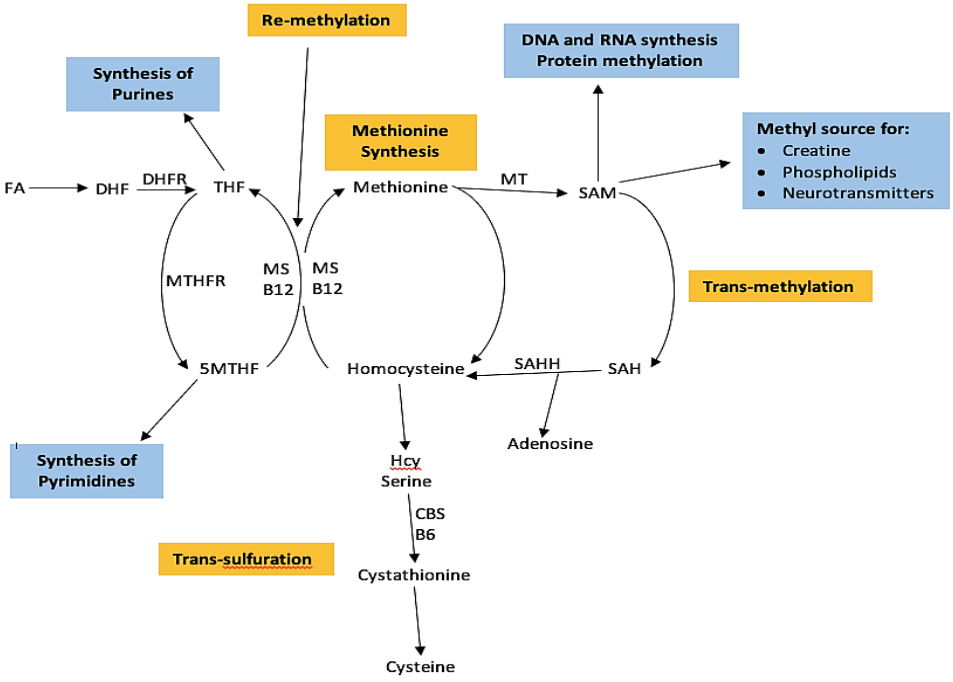

Figure 1.6: A schematic overview showing the interrelation between folate, vitamin B12 and Hcy metabolism
Abbreviations (FA: folic acid; DHF: dihydrofolate; DHFR: dihydrofolate reductase; THF: tetrahydrofolate; MTHFR: methylenetetrahydrofolate reductase; 5MTHF: 5-methyl-tetrahydrofolate; MS: methionine synthase; B12: vitamin B12; Hcy: homocysteine; CBS: cystathionine beta-synthase; B6: vitamin B6; MT: methyltransferase; SAM: s-adenosylmethionine; SAH: s-adenosylhomocysteine; SAHH: s-adenosylhomocysteine hydrolase)

1.2.3 Recommendations

The Institute of Medicine has established dietary reference intakes, estimated average requirement (EAR), recommended dietary allowance (RDA), adequate intake and the tolerable upper intake level for folate and vitamin B12. EAR is the daily intake value that is estimated to meet the requirement of half the population in a life-stage and gender group. RDA is the average daily dietary intake that is sufficient to meet the requirement of almost all healthy individuals (97-98%) in a life stage and gender group (Food & Nutrition Board, 1998). Table 1.1 reports the RDA and EAR for both folate and vitamin B12 (National Institute of Health, 1998).

The EAR for folate during pregnancy is 520 μg compared to the 320 μg recommended for adults (>19 years). For pregnant women, regardless of age, the RDA is 600 μg compared to the 400 μg recommended for adults (Shane, 2008). The folic acid recommendation to reduce the risk of neural tube defects is 400 μg/day, in addition to the folate consumed through the diet. The tolerable upper limit of folic acid intake for adults is 1000 μg; this limit was set based on the concern of masking vitamin B12 deficiency rather than folic acid toxicity. There is no tolerable upper limit for dietary folate (Shane, 2008).

The EAR for vitamin B12 during pregnancy is 2.2 μg/day compared to the 2.0 μg recommended for adults. The RDA for pregnant women is 2.6 μg/day, while the RDA for adults is 2.4 μg/day of vitamin B12. There is no tolerable upper limit for vitamin B12 as no toxicity at a high dose has been reported (Shane, 2008).

Table 1.1: The RDA and EAR for folate and vitamin B12

	Folate		Vitamin B12	
	RDA (µg)	**EAR (µg)**	**RDA (µg)**	**EAR (µg)**
Adult (Men/Women)	400	320	2.4	2.0
Pregnancy	600	520	2.6	2.2
Lactating	500	450	2.8	2.4

Abbreviations - RDA: Recommended Dietary Allowance, EAR: Estimate Average Requirement
National Institute of Health (2019)

1.2.4 Folate & Vitamin B12 Deficiency

Pregnant women are at a high risk for developing folate deficiency due to the increased demand of folate during pregnancy for DNA synthesis and one-carbon transfer reactions. Pregnancy complications associated with folate deficiency include placental abruption, preeclampsia, spontaneous abortion, still birth and fetal growth restriction (Scholl & Johnson, 2000). A well-established neonatal effect of maternal folate deficiency is neural tube defects in which the neural folds of the developing embryo fail to fuse, leading to embryological malformations of the central nervous system (Wadhwani et al, 2016). Moreover, folate deficiency is associated with decreased DNA synthesis leading to immature erythropoietic precursors. This results in increased red blood cell (RBC) volume known as megaloblastic anemia, characterized by an accumulation of large abnormal nucleated erythrocytes in the bone marrow (Bailey & Caudill, 2012). The deficiency in folate causes defective synthesis of thymidine resulting in the misincorporation of uracil into the DNA, causing double-stranded DNA breaks.

Cell division is impaired resulting in a drop in white cells and platelets (Bailey & Caudill, 2012).

If vitamin B12 is deficient, folate becomes "trapped" in the 5MTHF form and THF is not created for the formation of 5,10-methylenetetrahydrofolate, which is required for thymidylate, so DNA synthesis is disrupted (Bailey & Caudill, 2012). The autoimmune disease pernicious anemia is the most common cause of severe malabsorption of vitamin B12 (National Institutes, 2011), where a chronic autoimmune atrophic gastritis develops with antibodies to gastric parietal cells. Almost 50% of afflicted individuals also develop antibodies to intrinsic factor. As such, there is a complete loss of intrinsic factor function and therefore vitamin B12 malabsorption ensues. Also, a defect in the methionine metabolism pathway or a deficiency in vitamin B12 or folate can cause severe hyperhomocysteinemia, as the conversion process to methionine is disrupted.

1.2.5 Folate & Vitamin B12 Imbalance

High maternal folate and low vitamin B12 during pregnancy is a major concern, especially in developed countries where folate fortification is mandated, such as in Canada (Fayyaz et al, 2014; Visentin et al, 2016). High maternal folate status achieved through consumption of fortified foods plus supplementation, may mask vitamin B12 deficiency, which untreated, may result in adverse outcomes for both the mother and child. An imbalance between folate and vitamin B12 is associated with lower neonatal birth weight and anthropometric measurements, adiposity, as well as insulin resistance (Sukumar et al, 2016). In 12 studies of women with a high prevalence of vitamin B12

deficiency, folate deficiency was only reported in <10% of the study populations. This may be due to increased awareness of the importance of folic acid supplementation to prevent neural tube defects, and public health efforts to ensure adequate dietary folate status during pregnancy.

A cross-sectional study from Pune, India (n = 49 pregnant women) reported that a higher ratio of folate to vitamin B12 was associated with lower birth weight (r = -0.512; p=0.009) and birth length (r = -0.424; p=0.034), head circumference (r = -0.469; p=0.018) and chest circumference (r = -0.514; p=0.009). The ratio of folate to vitamin B12 was positively and significantly correlated to Hcy (r=0.349; p=0.014). Perturbing the folate:vitamin B12 ratio, either by increasing folate or decreasing vitamin B12 may increase Hcy concentrations (Gadgil et al, 2014). Therefore, it is important to address vitamin B12 status alongside improving folate status in the population.

1.3 Biochemical Indicators of Folate & Vitamin B12

There are two major biomarkers of folate status: RBC folate (circulating direct biomarker) and plasma Hcy (functional biomarker). Similarly, there are three major biomarkers of vitamin B12: serum vitamin B12 (circulating direct biomarker), plasma methylmalonic acid (MMA) (functional biomarker) and plasma Hcy (functional biomarker) (Finkelstein et al, 2015). The cut-off values for these direct and functional biomarkers in pregnancy are uncertain, therefore, cut-off values for adults have been used for pregnancy as a substitute by many studies.

1.3.1 Red Blood Cell Folate

RBC folate concentration is an indicator of long-term folate status because erythrocytes have a lifespan of ~ 120 days. In contrast, serum folate is an indicator of recent folate intake (WHO, 2015). In the circulation, folate is not taken up by the mature erythrocytes, therefore, RBC folate concentrations reflect folate absorbed during erythropoiesis (Bailey & Caudill, 2012; WHO, 2015). There is a direct relationship between total folate and RBC folate concentrations (i.e. circulating direct biomarker). Folate deficiency is defined as an RBC folate concentration <320 nmol/L (MacFarlane et al, 2011).

1.3.2 Serum Vitamin B12

Serum vitamin B12 concentrations fluctuate minimally with daily intake because body stores are relatively large. Therefore, serum vitamin B12 is a good reflection of overall vitamin B12 status (Gammon et al, 2012). There is a direct relationship between total vitamin B12 and serum vitamin B12 concentrations (i.e. circulating direct biomarker). B12 deficiency is defined as a serum vitamin B12 concentration <148 pmol/L (MacFarlane et al, 2011).

1.3.3 Plasma Homocysteine

Plasma Hcy is a naturally occurring amino acid (Walker et al, 1999). Hcy is a functional indicator of both folate and vitamin B12 deficiency because Hcy concentrations reflect changes in metabolic function as seen in Figure 1.5. Hcy is predominantly catabolized by cystathionine beta synthase into cystathionine via the trans-sulfuration pathway (Megahed & Taher, 2005). In adults, Hcy toxicity is prevented by the

trans-sulfuration pathway, in which Hcy is metabolized and excreted in the urine as sulfate. However, the key enzyme, cystathionase, is absent in the fetal liver prior to birth. Therefore, the fetus depends on the methionine cycle for Hcy detoxification as both folate and vitamin B12 are used to methylate Hcy into methionine. A deficiency in folate and/or vitamin B12 increases the risk that Hcy will accumulate to a toxic level (Lindblad et al, 2005; Walker et al, 1999; Bailey & Caudill, 2012). There is therefore an inverse relationship between folate and vitamin B12 and Hcy concentrations (i.e. functional biomarker). Nevertheless, Hcy is not a specific indicator of folate or vitamin B12 status as it is also influenced by other nutrient deficiencies caused by genetic abnormalities and renal insufficiency. Various cut-off values have been used to define high Hcy, but common reference cut-off values range from >10 to >16 μmol/L (Bailey & Caudill, 2012; MacFarlane et al, 2011).

1.3.4 Plasma Methylmalonic Acid

Vitamin B12 deficiency can be differentiated from folate deficiency by the presence of elevated plasma MMA (Miner et al, 1997). In vitamin B12 deficiency, MMA reflects impaired methylmalonyl-CoA mutase activity (Selhub et al, 2007). Essentially, reduced adenosyl-vitamin B12 concentrations lead to reduced activity of methylmalonyl-CoA mutase and increased methylmalonyl-CoA (Figure 1.6). This increases methylmalonyl-CoA cleavage by methylmalonyl-CoA hydrolase to coenzyme A and MMA. As a result, MMA concentrations increase in the blood (McMullin et al, 2001). There is therefore an inverse relationship between vitamin B12 and MMA concentrations (i.e. functional biomarker). A plasma MMA concentration >370 nmol/L is considered

high and is also indicative of vitamin B12 deficiency (McFarlane et al, 2011; Jeruszka-Bielak et al, 2017).

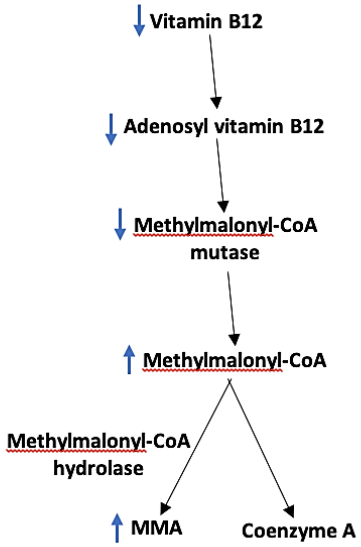

Figure 1.7: A schematic overview showing the interrelation between vitamin B12 and MMA metabolism
Abbreviations (MMA: methylmalonic acid)

1.4 Intake of Folate & Vitamin B12

1.4.1 Food Fortification in Canada

In November 1998, Canada introduced mandatory folic acid fortification for grain-based products such as flour, cereals and breads, at an amount of 150 µg of folic acid/100 g (Shane, 2008; De Wals et al, 2007; Ami et al, 2016). The objective of food fortification was to increase the daily folic acid intake by 100 µg on average (Ami et al, 2016). The rationale for implementing folic acid fortification in Canada was to decrease the occurrence of neural tube defects in the population. This program was successful - fortification resulted in a 50% reduction in neural tube defect prevalence (Shane, 2008;

De Wals et al, 2007; Ami et al, 2016). Moreover, within months of the legislative mandates, there was a notable increase in RBC folate concentrations across the female population (Greenberg et al, 2011). In Canada, vitamin B12 has also been added to cereals and soy-based meat alternative products (Dietitians of Canada, 2017).

1.4.2 Dietary Sources of Folate & Vitamin B12

Naturally occurring folate can be found in a variety of plant and animal-based food products (Appendix A (a)). Dietary folate is present at high concentrations in wheat germ, yeasts, cereals, pulses, fruits, green leafy vegetables and liver (the storage organ for folate in mammals). Folate retention during cooking varies by food item and cooking method, as folate is subject to thermal degradation and leaches into cooking water (Singh, 2018; Simpson et al, 2010).

Naturally occurring vitamin B12 can only be found in animal-based foods such as meat, poultry, fish, eggs and dairy (Appendix A (b)). Some breakfast cereals, soy and rice beverages, and soy-based meat alternative products are fortified. The fortification of meat alternative products with vitamin B12 is mandatory, but the vitamin B12 fortification of breakfast cereals is voluntary (Dietitians of Canada, 2016; Government of Canada, 2018). Therefore, vegetarians and individuals who consume low amounts of animal foods must rely on fortified foods and often supplementation in order to achieve adequate vitamin B12 concentrations (Quay et al, 2015).

1.4.3 Supplementation

Folic acid used in supplements is chemically stable and is absorbed more readily than naturally occurring folate in foods (Shane, 2008). However, after absorption into the

gastrointestinal tract and liver, folic acid must once again be converted to the metabolically active 5MTHF form in order to participate in metabolic processes as a substrate (Furness et al, 2013). In Canada, pregnant women are advised to take a prenatal multivitamin, folic acid supplement and either a B-complex or vitamin B12 supplement to ensure adequate intake for maternal support of fetal development. The Society of Obstetricians and Gynaecologists of Canada recommend that women consume multivitamins a minimum of three months prior to conception, throughout pregnancy and as long as breastfeeding is continued (Wilson et al, 2007; Ami et al, 2016). In a systematic review and meta-analysis of randomized controlled trials (n = 8 studies involving 860 participants), supplementation with folate (0.25 to 5 mg/d) increased birth weight by 2g compared with no supplementation. Similarly, in a dose-response meta-analysis, a 2-fold increase in folate intake corresponded to a 2% increase in birth weight (Fekete et al, 2012).

1.4.4 Diet & Birth Weight

Maternal plant-based diets may support fetal growth. The odds of small for gestational age was inversely associated with total consumption of fruits (aOR = 0.63) and legumes (aOR = 0.68) in a Spanish cohort (Martinez-Galiano et al, 2018). Similarly, a study from India, showed that a high consumption of fruits, milk and green leafy vegetables were positively associated with birth weight and size. In a prospective study conducted in India (n=797 rural Indian women), Rao et al (2001) measured the intake of foods from 17 food groups with a semiquantitative food frequency questionnaire (FFQ). They found that birth size was positively associated with maternal consumption of milk

products ($\beta = 6.9$; $p<0.05$) at 18 weeks of gestation as well as green leafy vegetables ($\beta = 19.1$; $p<0.001$) and fruits ($\beta = 7.4$; $p<0.01$) at 28 weeks of gestation (Rao et al, 2001).

1.5 Maternal Folate & Vitamin B12 Association with Birth Weight in Pregnant Women

Most of the studies demonstrating an association between low vitamin B12 and low birth weight are from India, where the rate of low birth weight and low vitamin B12 are high (Sukumar et al, 2016). In prospective studies, vitamin B12 deficiency is associated with low birth weight, and low maternal folate and vitamin B12 concentrations predict smaller neonatal size (Krishnadevi et al, 2014) whereas high folate status predicts higher birth weight (Relton et al, 2005; Furness et al, 2013; Yajnik et al, 2014). Therefore, the folate-vitamin B12 pathway may have a role in regulating fetal growth. A prospective observational cohort study out of India involving 1838 mother-child dyads, at <13 weeks of gestation, found that high folate in conjunction with low vitamin B12 during pregnancy was associated with small for gestational age infants in SA women (Dwarkanath et al, 2013). Yajnik et al (2008) reported that higher Hcy concentrations at 18 weeks was associated with smaller newborn size. Furthermore, infants born to women with Hcy concentrations >7.3 μmol/l weighed 110g less at birth than those born to women with Hcy <5.8 μmol/l. Compared with infants born to women with folate concentrations >25.9 nmol/l, infants born to women with folate concentrations <19.0 nmol/l had a 53-125g lower birth weight (Bergen et al, 2012). Several studies have reported that low maternal folate and vitamin B12 concentrations as well as high Hcy

concentrations are related to smaller newborn size (Muthayya et al, 2006; Scholl & Johnson, 2000; Relton et al, 2005; Yajnik et al, 2005; Krishnaveni et al, 2014). Similarly, in an Indian birth cohort, Krishnaveni et al (2014) indicated that higher maternal Hcy status in pregnancy was associated with lower birth weight of the neonate. They reported that maternal Hcy concentrations were inversely associated with all neonatal anthropometric measurements ($p < 0.05$) (Krishnaveni et al, 2014). Previous observational studies have observed positive associations of maternal folate status with high birth weight (Neggers et al, 1997; Relton et al, 2005). Wang et al (2016) reported that maternal folic acid supplementation during pregnancy significantly increased the risk of large for gestational age neonates. In a nested case-control study, (China; $n > 3000$) women with RBC folate concentrations between 400 and 570 ng/ml were found to be at a two-fold higher risk of large for gestational age neonates (OR = 2.38; 95% CI: 1.41-4.03; P=0.001), than women with RBC folate concentrations ≤400 ng/ml. There was evidence of a dose-dependent association, as women with an RBC folate concentration >570 ng/ml had a OR of 2.56 (95% CI: 1.55-4.25; P<0.001) (Xie et al, 2018).

SA women have been identified as an at-risk ethnic group for vitamin B12 deficiency (Gammon et al, 2012) because of the high frequency of vegetarianism and infrequent meat consumption by omnivores in the SA population (Sukumar et al, 2016). The systematic review conducted by Sukumar et al (2016) reported a 25% prevalence of maternal vitamin B12 deficiency during pregnancy, using data from 57 studies across 32 countries (representing 34762 pregnant women). By trimester, the rates were 21%, 19% and 29% respectively. In a retrospective cohort study of 748 pregnant Canadian women,

SA compared to WE had lower serum vitamin B12 status and higher serum MMA concentrations during early pregnancy (Schroder et al, 2017).

Hogeveen et al (2010) conducted a prospective study in the Netherlands (n = 366 pregnant women) exploring the relationship between maternal Hcy, vitamin B12, MMA and folate with neonate birth weight. They found that birth weight was related negatively to maternal Hcy ($r = -0.12$; $p= 0.03$) but not to maternal vitamin B12, MMA or folate (all $|r| <0.06$; $p >0.150$). They concluded that maternal Hcy, and related B vitamins were not related to birth weight (Hogeveen et al, 2010). Similarly, in another Norwegian cohort study of 2934 singleton WE pregnancies, no significant association between dietary folate intake, supplemental folic acid use, total dietary folate intake or plasma folate with the various birth outcomes (i.e. gestational age, neonate birth weight, head circumference, crown-heel length and small for gestational age) was found (Nilsen et al, 2010).

Chapter 2: Study Design & Methodology

2.1 Study Design

In this cross-sectional analysis of a prospective cohort study, we used dietary and supplemental data from 3758 mother-neonate pairs. The SA participants were from the South Asian Birth Cohort (START) and the WE participants were from the Family Atherosclerosis Monitoring In Early Life (FAMILY) and Canadian Healthy Infant Longitudinal Development (CHILD) studies (Anand et al, 2016; START Study Protocol, 2016). The START study is a birth cohort composed of SA from the Region of Peel (Ontario, Canada) (Anand et al, 2013). The FAMILY study is a birth cohort composed primarily of WE from Hamilton (Ontario, Canada) (Morrison et al, 2009). The CHILD study is a multi-ethnic birth cohort recruited from 6 cities across Canada: Vancouver, Edmonton, Winnipeg, Morden, Winkler and Toronto (Subbarao et al, 2015). The demographic, social, dietary, physical and biochemical measures used for this analysis was collected during the antenatal visit at 24-28 weeks of gestation (the second trimester of pregnancy). A trained research assistant measured birth weight within 72 hours of delivery or recorded it from the birth chart (START Study Protocol, 2016). Birth weight refers to the first weight of the neonate obtained at delivery.

2.2 Study Objectives

The overarching aim of this thesis was to describe the relationship between maternal folate and vitamin B12 concentrations and neonate birth weight as illustrated in Figure 2.1. The specific research questions were as follows:

1) What was the association between maternal dietary, supplemental and total folate and vitamin B12 intake and neonate birth weight in SA and WE populations?

We hypothesized that SA, relative to WE, would have a higher maternal folate status and as such a higher folate intake would be associated with a higher neonate birth weight. Similarly, SA relative to WE would have a higher maternal vitamin B12 status and therefore, a higher vitamin B12 intake would be associated with a higher neonate birth weight. We hypothesized that SA would have a higher maternal folate and vitamin B12 status due to their increased intake of green leafy vegetables and legumes, which are high sources of folate, as well as their high and frequent consumption of milk and yogurt-based products, which are good alternatives sources of vitamin B12.

2) What was the association of folate and vitamin B12-rich food groups (i.e. green leafy vegetables, fruits and milk) consumption with neonate birth weight in SA and WE populations?

As seen in previously reported studies from India (i.e. Rao et al, 2001), we hypothesized that a high maternal consumption of green leafy vegetables, fruits etc. (i.e. higher folate) would be associated with a higher neonate birth weight. Likewise, we hypothesized that a high maternal consumption of milk and eggs (i.e. higher vitamin B12) would be associated with a higher neonate birth weight. Furthermore, we expected SA to have a

higher consumption of folate and vitamin B12-rich food groups and therefore, a higher birth weight compared to WE. Appendix B summarizes the FFQ food items included in each folate and vitamin B12-rich food category in the (a) START (b) FAMILY and (c) CHILD cohorts.

3) What was the association between maternal biochemical indicators of folate and vitamin B12 status (RBC folate, serum vitamin B12, plasma Hcy and plasma MMA) and neonate birth weight in SA?

We hypothesized that the maternal concentrations of the direct indicators of folate (RBC folate) and vitamin B12 (serum vitamin B12) status would be positively associated with neonate birth weight whereas the functional indicators (plasma Hcy and MMA) would be inversely associated with neonate birth weight.

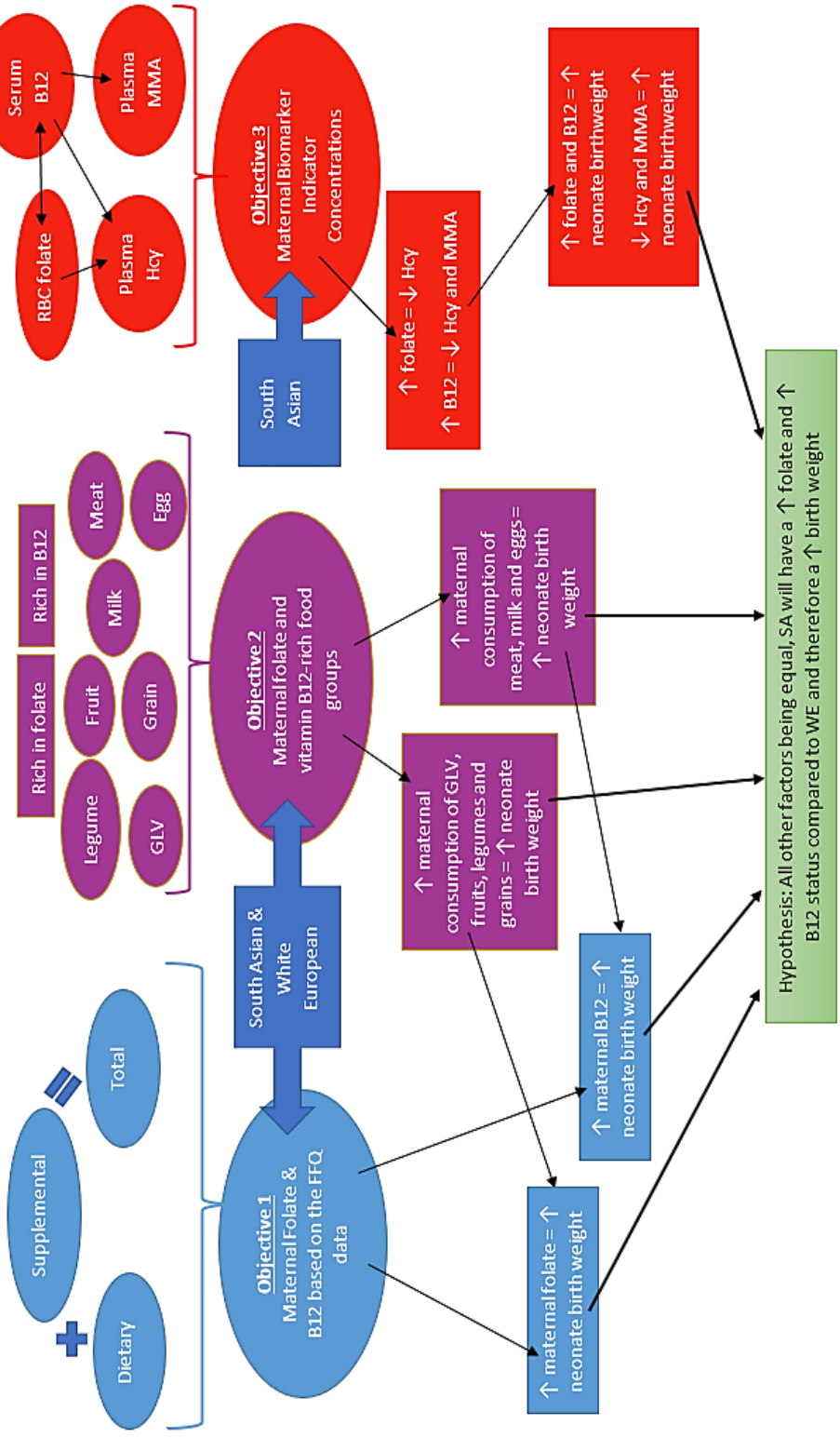

Figure 2.1: A schematic overview of the overarching aim of the thesis as well as the objectives (1-3) breakdown and hypotheses
Abbreviations (B12: vitamin B12; GLV: green leafy vegetables; RBC: red blood cell; Hcy: homocysteine; MMA: methylmalonic acid; SA; South Asians; WE: white Europeans)

2.3 Laboratory Assays

Pregnant women provided a fasting blood sample during their second trimester (24-28 weeks of gestation). The biochemical indicator data was only available in a subset of START as it has not been analysed in FAMILY or CHILD as of yet. RBC folate and plasma MMA were available for 233 SA participants whereas serum vitamin B12 and plasma Hcy were available for 236 participants. Based on the initial CIHR-ICMR grant, these women were the first 250 mothers recruited in the START study from Canada and were initially used for comparison with START India on common measures proposed in the grant. Vitamin B12 was extracted from frozen serum aliquots, and folate was extracted from frozen whole blood aliquots. Folate and vitamin B12 were quantified with the Roche E-170 analyser, based on the competition principle in which an enzyme donor and a folate or vitamin B12 conjugate compete for a binding site (i.e. folate binding protein for folate and intrinsic factor for vitamin B12). Hcy and MMA were extracted from frozen EDTA plasma aliquots and were quantified using the gas chromatography-mass spectrometry analytical method (START Study Protocol, 2016).

There is no established cut off ranges to define folate and vitamin B12 status in pregnancy for any of the mentioned biomarkers. As such, pregnant women were classified using non-pregnant adult cut-offs (Table 2.1 (a): Direct Biomarkers and Table 2.1 (b): Functional Biomarkers). Moreover, overt vitamin B12 deficiency was defined as a combination of serum vitamin B12 concentrations <148 pmol/l and plasma MMA concentrations >370 nmol/l. Similarly, overt folate deficiency was defined as a

combination of RBC folate concentrations <320 nmol/l and plasma Hcy concentrations >15 μM.

Table 2.1: The cut-off ranges for direct and functional biomarkers of folate and vitamin B12 status

a) **The cut-off ranges for deficient, adequate and high concentrations of the direct biomarkers (RBC folate and serum B12) of folate and vitamin B12 status**

Biochemical Indicator	Deficient	Adequate	High
RBC Folate	<320 nmol/L	320 – 1090 nmol/L	>1090 nmol/L
Serum vitamin B12	<148 pmol/L	148 – 220 pmol/L	>220 pmol/L

b) **The cut-off ranges for low, moderate and high concentrations of the functional biomarkers (plasma Hcy and plasma MMA) of folate and vitamin B12 status**

Biochemical Indicator	Low	Moderate	High
Plasma Hcy	<5 μM	5 – 15 μM	>15 μM
Plasma MMA	<210 nmol/L	210 – 370 nmol/L	>370 nmol/L

2.4 Statistical Considerations

2.4.1 Dietary Assessment & Estimations

Participants in both START and FAMILY completed a validated semi-quantitative FFQ adapted from the Study of Health and Risk in Ethnic Groups (SHARE) to capture usual dietary intake. Additional questions inquired about their use of dietary

supplements. Participants in the CHILD cohort used an FFQ adapted from the Fred Hutchinson Cancer Center tool. We used the data from these FFQs to estimate supplemental intake for each individual based on the reported amount consumed, the frequency of consumption and the average dose from common products (Table 2.2). For example, if a participant indicated on the FFQ that they took a folic acid supplement 1-3 times a week, then the daily intake of folic acid from the supplement would be calculated by multiplying the dose (i.e. 400 μg) by the average frequency (i.e. 2/7 days). This calculation resulted in an average intake of 114.29 μg of folic acid from a folic acid supplement per day. The mean folic acid and vitamin B12 intake from supplements per participant were calculated by using the sum of the average value of intake from all supplement sources including prenatal multivitamins.

Table 2.2: The average vitamin B12 and folic acid amounts assigned to each supplemental source

FFQ Item	Vitamin B12 (µg)	Folic Acid (µg)
Multivitamin	4.50	200.00
Multivitamin + Iron	5.00	266.67
Multivitamin + Minerals	31.99	417.60
B-Complex	26.05	337.18
Folic Acid	.	400.00
Vitamin B12	25.00	.

Micronutrient values for each supplemental source (1 tablet) was assigned based on a calculated average of common brands:
Multivitamin values were taken from Life Multivitamins only and Exact multivitamin 11 + beta carotene
Multivitamin + iron values were taken from Life multivitamins and iron only, One-A-Day advance fem (with iron) and Exact multivitamins + iron for women
Multivitamins + minerals values were taken from Life spectrum forte 29, Life gold over 50, Paramettes adult, GNC mega gold, Centrum, Centrum forte high potency, Centrum select 50+, Centrum protegra complete, One-A-Day advance adults 23 50+, One-A-Day advance adults 24, Com. Choice omniplex with beta carotene, Kirkland (5 tabs), Swiss One, Multiple Choice ultimate one 50+ men, Avon life woman's formula II, Swiss One 80 hi-potency, Swiss Super Swiss One 50, Paramettes 50+, Jamieson vita vim, Jamieson mega vita vim, Jamieson super vita vim, Jamieson vita vim with beta carotene, Pharma Plus 18 essential, Pharma Plus 12 essential with iron and calcium, Pharma Plus 29 essential high potency, Pharma Plus 29 high potency 50+, NatureMade mature bal. 50+, Life Spectrum 18 essential, Life nat source spectrum 24 and beta carotene, One-A-Day advance women's form 11 vit +, Life adult, Life adult 50+, Multiple Choice Ultimate one adult, Multiple Choice ultimate one, Multiple Choice Ultimate one 50+ women, Multiple Choice Ultimate one active men, Multiple Choice Ultimate one active women
B-complex values were taken from Beminal B complex with vitamin C forte, B50 complex, Surbex 500, BComplex 75, Redoxon B complex Stress formula & C, One-A-Day advance hi potency, b complex and C, Zinc polyvitamins B, C, E, Natural factors b-50, Quest superstress with B & C, Albee C-800 plus iron, Albee c-550, Albee-C-800, Stress Tabs high potency B complex C+E, Pharma+B stress c600 +E, ZN, CU, Pharma + B anti-stress c600 B+C, Pharma +B anti stress c600 + FE (B+C+E), Pharma Plus Bplex 50, Wampole high potency B + C500, Wampole stress high potency B+C500 with iron, Wampole stress high potency B+C with zinc, Swiss B Compound with C500, Swiss B Compound with C1000mg, Swiss B 100 Compound, Swiss high potency B50, Jamieson B complex 100, Jamieson B complex 50, Jamieson B complex with C250mg, NatureMade B complex with C100+E, NatureMade B complex with C300, Nature Made B complex 50, NatureMade stress B complex with C,E,ZN, CU, NatureMade B complex with C, E and iron, Stress Tabs hi potency B complex, Zn, Cu, Stress Tabs hi potency b complex + iron, Stress Tabs B complex plus, Exact B complex with C,EZN CU, Exact B complex with C,E,iron, Exact B complex with C,E and Exact balance B50 complex
Folic acid and vitamin B12 were reported based on the value given in the FFQ. Folic Acid = 400µg and vitamin B12 = 25 µg

Folate and vitamin B12 intake were examined in three forms: dietary, supplemental and total. Dietary folate and vitamin B12 were estimated based on the FFQ and considers natural and fortified sources. Supplemental folate was calculated using all sources of folic acid such as prenatal multivitamins, B-complex and folic acid. Supplemental vitamin B12 was calculated using all sources of vitamin B12 such as prenatal multivitamins, B-complex and vitamin B12. Lastly, total folate or total vitamin B12 were the sum of dietary and supplemental intakes, expressed as µg.

2.4.2 Methods: Sampling Strategy

For this thesis, the inclusion criteria were individuals of SA or WE ethnicity based on self-report. SA women were individuals originating from the subcontinent of India, Bangladesh, Pakistan and Sri Lanka. However, it is important to note that we did not have control over the selection and recruitment of participants, and we used existing data to conduct a secondary analysis. The exclusion criteria for this thesis were women who reported an implausible energy intake of <500 kcal and >6500 kcal per day and women with missing variable values (Figure 2.2a and 2.2b).

2.4.3 Methods: Power Analysis

To check whether our design in objectives 1-3 had enough power to detect an effect, if one truly existed, we conducted multiple linear regression power analyses using the software program G*Power prior to beginning the analysis (Faul et al, 2009). Power (1 - β) was set at 0.80, α = 0.05 and the calculation was two-tailed. The recommended effect sizes used for this assessment were as follows: small ($f^2 = 0.02$), medium ($f^2 = 0.15$), and large ($f^2 = 0.35$) (see Cohen 1977). This showed us that our sample sizes were adequate to reach statistical significance at the 0.05 level with effect sizes in the small to medium range depending on the analysis. Appendix C depicts the power, effect size and sample size calculation for each multiple linear regression. Thus, it is unlikely that our findings can be attributed to a limited sample size and as a result can confirm that our non-significant results were not due to a lack of statistical power.

a)

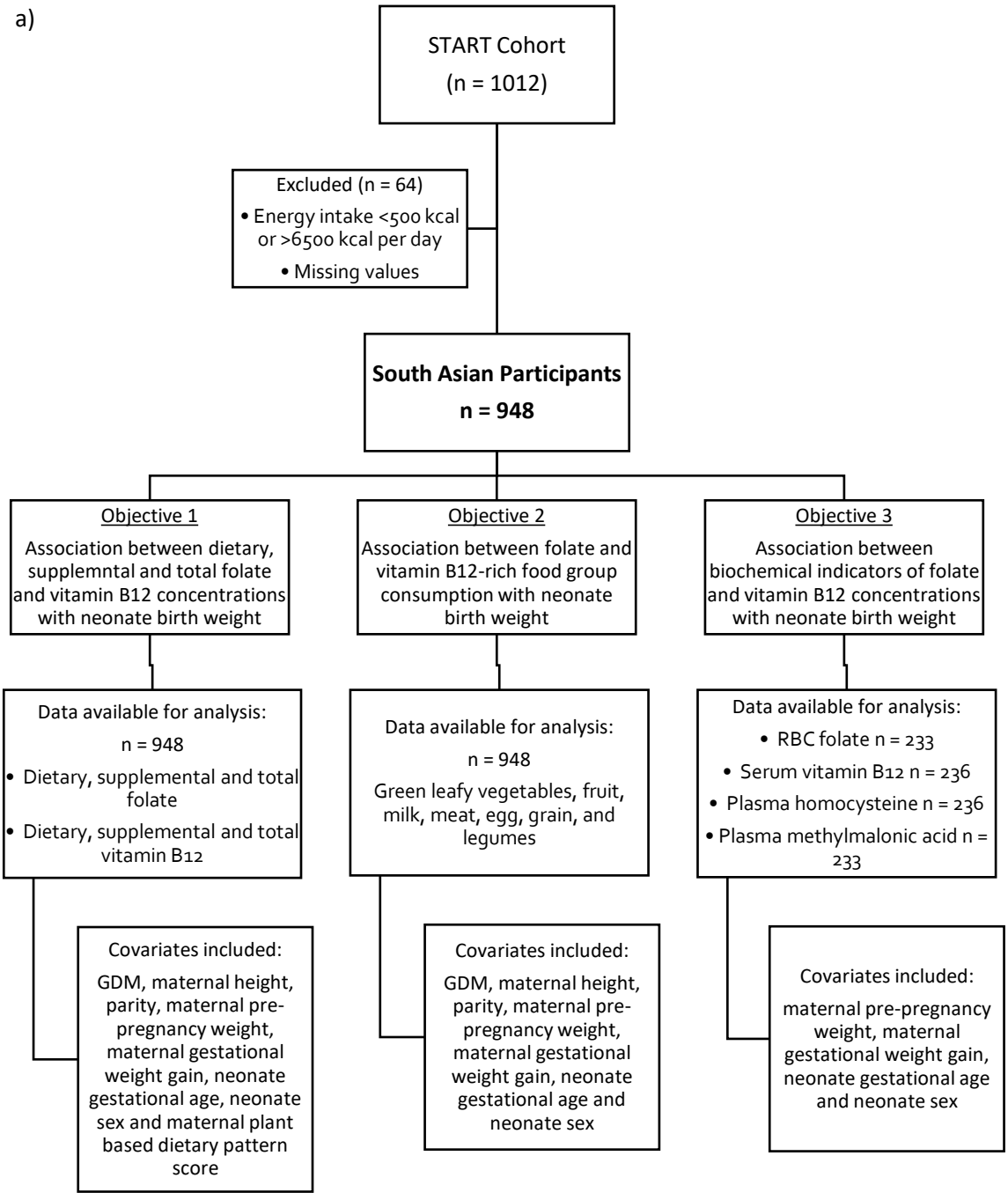

Figure 2.2a: A schematic flow diagram representing the participants from START used for analytical purposes

b)

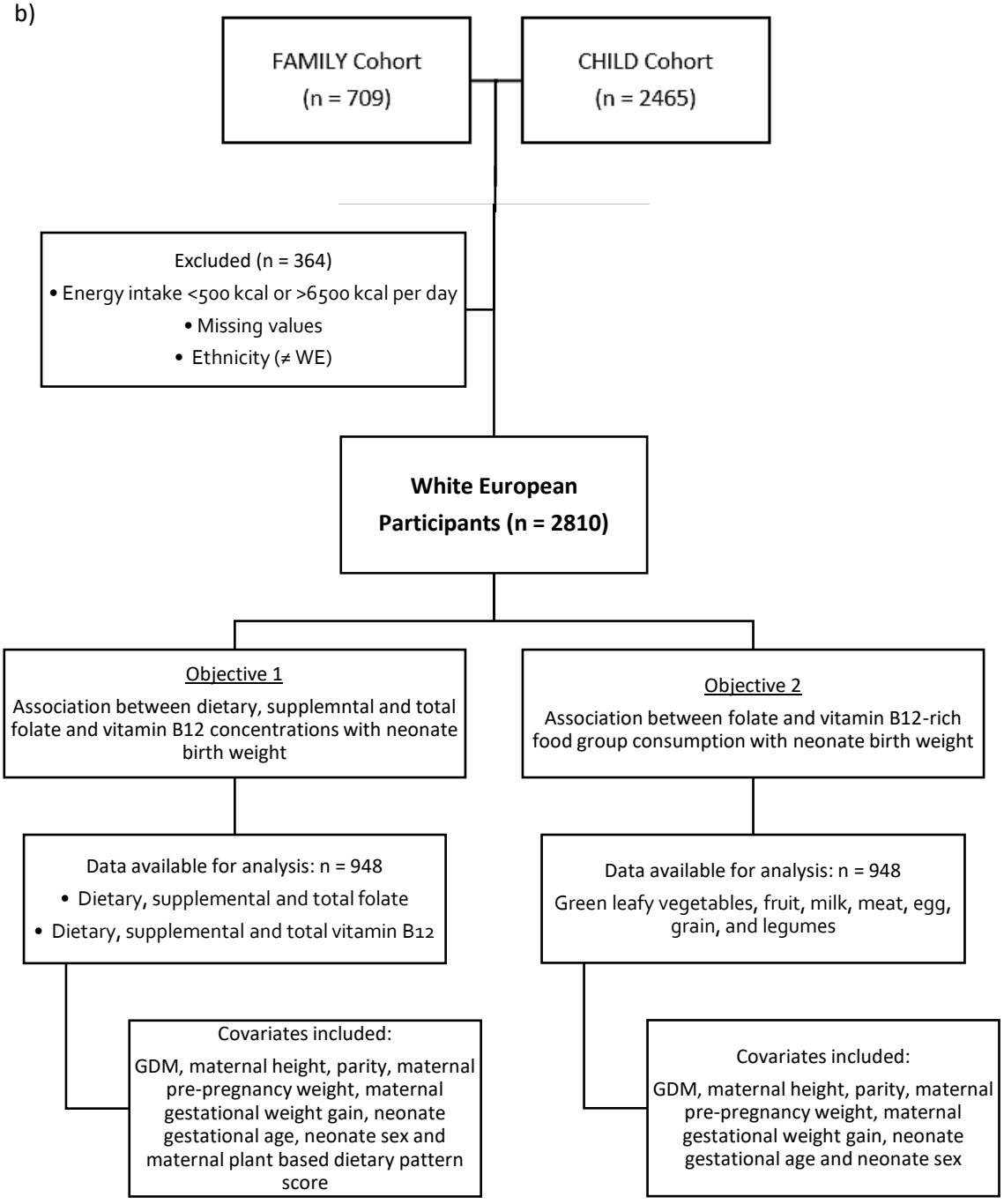

Figure 2.2b: A schematic flow diagram representing the participants from FAMILY and CHILD used for analytical purposes

2.4.4 Methods: Statistical Analysis

Variables were tested for normality based on a visual interpretation of histograms (for example, Figure 2.3 and Figure 2.4), as well as the Shapiro-Wilk test. Skewed variables were transformed to normality using either the natural log (ln) or square root transformation, in order to satisfy the assumptions of a regression. To maintain a zero-value assigned to a variable, the square root transformation was used, otherwise the natural log transformation was applied. Multiple linear regression using the forward stepwise method was conducted to assess the association between the main effects and covariates on neonate birth weight. The covariates, ethnicity and the maternal dietary patterns were included in a combined model. In this model, ethnicity was significantly associated with infant birth weight (Table 3.12). Therefore, a stratified analysis was completed in which multiple linear regression models, stratified by ethnicity (WE and SA) were adjusted for the covariates. Continuous data are presented as means ± SDs and categorical variables are presented as percentages. Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA).

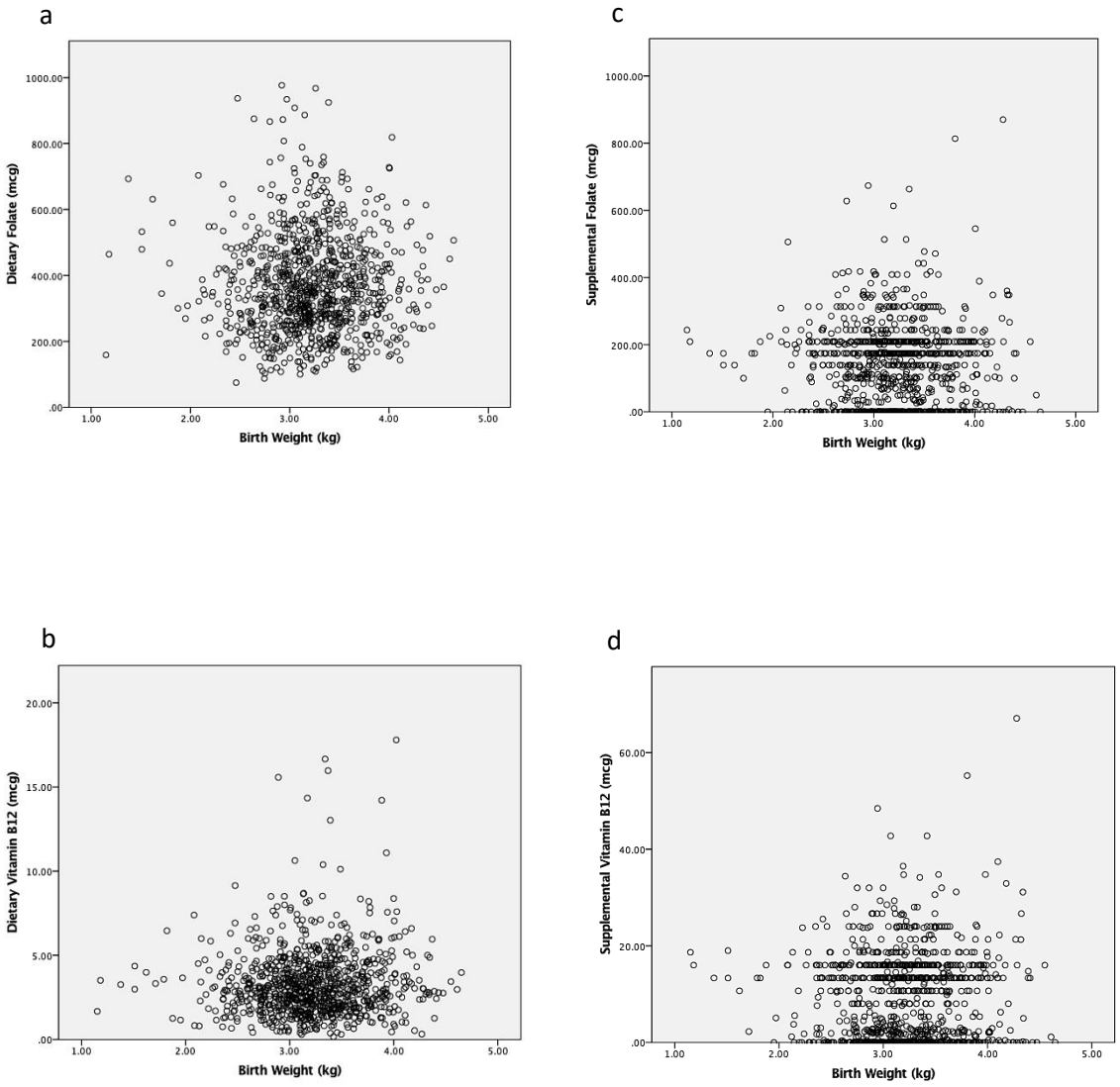

Figure 2.3: Scatterplots of SA participants depicting a) dietary folate (y-axis) and neonate birthweight (x-axis); b) dietary vitamin B12 (y-axis) and neonate birthweight (x-axis); c) supplemental folate (y-axis) and neonate birthweight (x-axis); d) supplemental vitamin B12 (y-

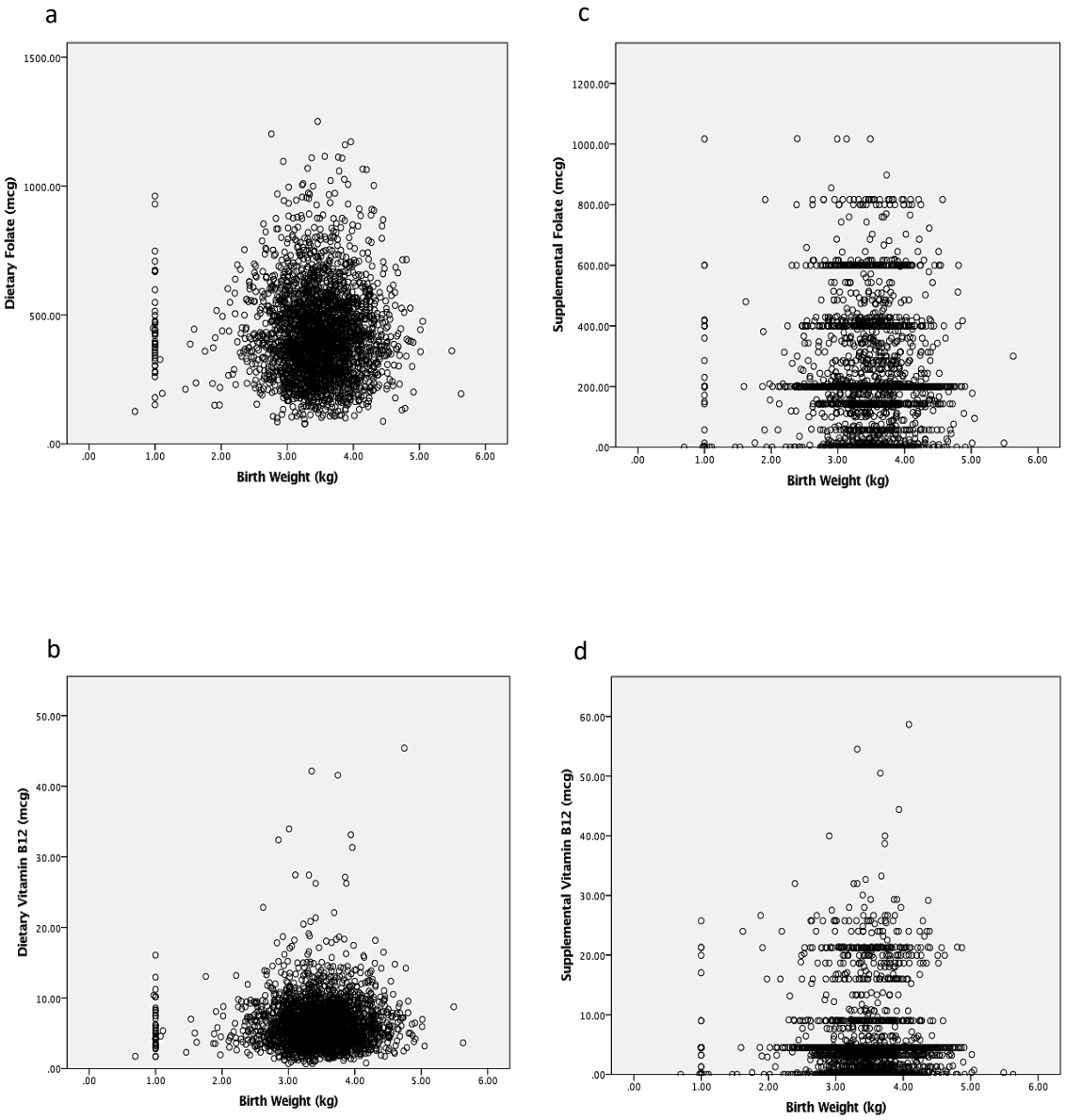

Figure 2.4: Scatterplots of WE participants depicting a) dietary folate (y-axis) and neonate birthweight (x-axis); b) dietary vitamin B12 (y-axis) and neonate birthweight (x-axis); c) supplemental folate (y-axis) and neonate birthweight (x-axis); d) supplemental vitamin B12

2.4.4a Objective 1

Objective 1: To assess the associations between maternal dietary, supplemental and total folate and vitamin B12 concentrations and neonate birth weight in SA and WE populations, we fit a multiple linear regression model. We regressed the dependent variable (Y, birth weight) against the main effect of :1) dietary folate; 2) supplemental folate; 3) total folate; 4) dietary vitamin B12; 5) supplemental vitamin B12; and 6) total vitamin B12, each adjusted for covariates as appropriate. For example

$$E(\bar{Y}) = \alpha + \beta_1 dietaryfolate + \beta_i Y_{j\ldots} + \varepsilon_{i,j}$$

2.4.4b Objective 2

Objective 2: To assess the association of folate and vitamin B12-rich food group (i.e. green leafy vegetables, fruits and milk) consumption with neonate birth weight in SA and WE populations, we fit a multiple linear regression model. We regressed the dependent variable (Y, birth weight) against the main effect of: 1) green leafy vegetables; 2) fruits; 3) legumes; 4) grains; 5) milk; 6) meats; and 7) egg, each adjusted for covariates as appropriate. For example

$$E(\bar{Y}) = \alpha + \beta_1 greenleafyvegetables + \beta_i Y_{j\ldots} + \varepsilon_{i,j}$$

2.4.4c Objective 3

Objective 3: To assess the association between maternal biochemical indicators of folate and vitamin B12 and neonate birth weight in the SA population, we fit a multiple linear regression model. We regressed the dependent variable (Y, birth weight) against the main effect of 1) RBC folate; 2) serum vitamin B12; 3) plasma Hcy; and 4) plasma MMA, each adjusted for covariates as appropriate. For example

$$E(\overline{Y}) = \alpha + \beta_1 RBCfolate + \beta_i Y_{j\ldots} + \varepsilon_{i,j}$$

Pearson correlation coefficient tests were done to examine the correlation between the direct and functional biomarkers of folate and vitamin B12 status to ensure the biologically expected association held true.

A cross tabulation analysis was conducted to describe the relationship between the two categorical variables: direct and functional biomarkers of folate and vitamin B12 status. The cross tabulation analysis was used to examine the number of individuals in each category (i.e. low in plasma vitamin B12 yet high in MMA signifying overt vitamin B12 deficiency) 1) serum vitamin B12 (deficient, adequate or high) and plasma MMA (low, moderate or high); 2) RBC folate (deficient, adequate or high) and serum vitamin B12 (deficient, adequate or high); 3) RBC folate (deficient, adequate or high) and plasma Hcy (low, moderate or high); and 4) serum vitamin B12 (deficient, adequate or high) and plasma Hcy (low, moderate or high).

Independent sample t-tests were used to compare dietary and supplemental intakes of folate and vitamin B12 in participants categorized as deficient, adequate or high RBC folate or serum vitamin B12.

2.4.5 Covariates

We selected a set of potential covariates for the multivariable model after a thorough literature search to identify variables associated with neonate birth weight. Collinearity diagnostics were conducted to test for multicollinearity between selected potential covariates. We dealt with multicollinearity by removing highly correlated variables based on the exclusion criteria of a tolerance value <0.10 and a variance

inflation factor >3. As a result, maternal pre-pregnancy body mass index and maternal final weight were removed from the model. The remaining covariates included maternal age, maternal pre-pregnancy weight, maternal gestational weight gain, maternal height, maternal GDM status, maternal smoking, parity, maternal total energy, neonate sex, neonate gestational age and maternal dietary pattern scores (see de Souza et al., 2017). The list of remaining candidate covariates then each separately entered into a simple regression model, sequentially. A parsimonious model, stratified by ethnicity, was fit after subjecting these covariates to a forward stepwise regression procedure, with neonate birth weight as the dependent variable. Standardized regression coefficients, β, denoting the increase in the dependant variable for a one unit increase in the independent variable are presented with accompanying R^2 values denoting the variation in the outcome variable explained by the variables included in the regression model. Variables in the multiple linear regression model with a $p< 0.05$ were considered to be significant.

Chapter 3: Results

3.1 Maternal & Neonatal Characteristics

3.1.1 Maternal & Neonate Anthropometric Characteristics & Health Behaviours

Table 3.1 summarizes the anthropometric characteristics of the mothers and neonates as well as the maternal behaviours. Overall, there are 948 SA and 2810 WE participants included in this study. WE have a higher maternal pre-pregnancy weight, gestational weight gain, pre-pregnancy BMI, final weight and height than SA women, even though participants are of a similar age. SA women have a higher prevalence of GDM (36.30% vs 7.58%) and social disadvantage (1.70 vs 0.49) while WE have a higher prevalence of smoking during pregnancy (11.18% vs 0.20%). Furthermore, neonatal birth weight is higher in WE (3.45 kg) than in SA (3.22 kg).

Table 3.1 : Maternal and neonatal anthropometric characteristics and health behaviours in South Asian and White European pregnant women

Variable	South Asian	White European
Sample size (n)	948	2810
Maternal age (years)	30.98 ± 3.94	32.14 ± 4.76
Maternal pre-pregnancy weight (kg)	62.52 ± 12.00	69.16 ± 14.31
Maternal gestational weight gain (kg)	14.31 ± 7.81	15.27 ± 5.96
Maternal pre-pregnancy BMI (kg/m^2)	23.79 ± 4.44	25.21 ± 5.11
Maternal final pregnancy weight (kg)	76.52 ± 13.00	84.20 ± 15.37
Maternal height (cm)	162.12 ± 6.22	165.65 ± 6.55
Maternal GDM (%)	36.30	7.58
Smoking during pregnancy (%)		
Never smoked	99.60	68.47
Quit before pregnancy	0.20	19.15
Quit during pregnancy	0.20	7.05
Currently smoking	.	4.13
Social Disadvantage Index (1-5)*	1.70 ± 1.42	0.49 ± 1.00
By Birth Cohort		FAMILY: 0.59 ± 1.15 CHILD: 0.46 ± 0.96
Primiparous (%)	40.80	48.93
Neonate sex (% male)	49.50	51.89
Neonate gestational age (weeks)	39.14 ± 1.50	39.52 ± 1.48
Full Term Neonate (%)	57.8	53.27
Neonate birth weight (kg)	3.22 ± 0.49	3.45 ± 0.58

Continuous variables are presented as means ± SD and categorical variables are presented as percentages.
Untransformed values are presented
Full term neonate is defined as 39 weeks to 40 weeks and 6 days of gestation
*Social Disadvantage Index (0 = no; 1 = low; 2/3 = moderate; 4/5 = high)
Abbreviations (BMI: body mass index; GDM: gestational diabetes mellitus)

3.1.2 Maternal Dietary Characteristics

WE reported a higher average daily caloric intake of 1938 kcal compared to 1680 kcal consumed by SA. SA pregnant women consumed more daily servings of milk, egg, grains and legumes, whereas WE consumed more daily servings of green leafy vegetables, fruits and meat (Table 3.2). As previously reported by Zulyniak et al (2017),

SA have a higher plant-based dietary score (1.41 vs -0.34) and WE have a higher Western dietary score (0.17 vs -0.55).

Table 3.2 : Maternal dietary characteristics of South Asian and White European pregnant women

Variable	South Asian	White European
Sample size (n)	948	2810
Total Energy (calories)	1796.60 ± 657.22	2048.15 ± 682.57
Green Leafy Vegetables (average daily servings)	0.55 ± 2.04	0.61 ± 0.59
Milk (average daily servings)	4.52 ± 2.55	4.04 ± 2.39
Fruits (average daily servings)	2.65 ± 1.97	3.02 ± 1.96
Meat (average daily servings)	0.44 ± 0.59	1.20 ± 0.70
Egg (average daily servings)	0.39 ± 0.57	0.24 ± 0.27
Grains (average daily servings)	4.05 ± 2.34	2.91 ± 1.44
Legumes (average daily servings)	0.76 ± 0.62	0.21 ± 0.24
Plant-Based Dietary Score	1.41 ± 1.10	-0.34 ± 0.56
Western Dietary Score	-0.55 ± 0.62	0.17 ± 0.96
Health Conscious Dietary Score	-0.42 ± 0.80	-0.004 ± 0.85

Continuous variables are presented as means ± SD.
Untransformed values are presented
Dietary scores presented are created by Zulyniak et al (2017) using PCA

Table 3.3 presents the average dietary, supplemental and total folate and vitamin B12 in SA and WE women as well as the prevalence of supplement use. Average dietary and supplemental folate and dietary vitamin B12 intakes are higher in WE, however supplemental vitamin B12 intake is higher in SA. The total folate and vitamin B12 intakes are determined by estimating the folate and vitamin B12 contribution of all food items and supplements. WE had a higher total folate concentration than SA (663.78 mcg vs 517.59 mcg, respectively; $p < 0.01$), however, total vitamin B12 concentrations were

higher in SA (12.81 mcg vs 11.99 mcg; p = 0.01). 72.57% of SA and 88.4% of WE participants report using multivitamin supplements during pregnancy. WE (29.5%) report a higher use of folic acid supplements than SA (9.3%). Vitamin B-complex, which includes vitamin B12 is used by 5.9% of the WE population and rarely (0.6%) by SA. Vitamin B12 supplement use is more prevalent in SA women than WE women enrolled in FAMILY. CHILD participants were not included in the vitamin B12 supplement analysis because vitamin B12 supplement use was not captured by the CHILD FFQ. Figure 3.1 depicts the estimated number of participants consuming below and above the RDA for total folate and vitamin B12 in (a) SA and (b) WE. In both populations, a high proportion of women are above the RDA for folate and vitamin B12.

Table 3.3: Maternal dietary, supplemental and total folate and vitamin B12 characteristics of South Asian and White European pregnant women

Variable	South Asian	White European
Sample size (n)	948	2810
Average Folate - Diet (mcg)	376.37 ± 180.05	425.30 ± 169.74
Average Folate - Supplements (mcg)	141.22 ± 119.10	238.57 ± 175.69
Average Vitamin B12 - Diet (mcg)	3.34 ± 2.15	6.35 ± 3.46
Average Vitamin B12 - Supplement (mcg)	9.47 ± 9.05	5.63 ± 6.06
Multivitamin (%)	72.47	88.40
B-complex (%)	0.63	5.94
Folic Acid (%)	9.28	29.54
Vitamin B12 (%)	10.34	2.88[T]
Average Total Folate (mcg)	517.59 ± 216.62	663.87 ± 256.23
Average Total Vitamin B12 (mcg)	12.81 ± 9.31	11.99 ± 6.97

Categorical variables are presented as percentages
Continuous variables are presented as means ± SD
Untransformed values are presented
ᴛ Denotes vitamin B12 supplementation was not specifically captured by the CHILD FFQ therefore, the mean intake is of the FAMILY cohort only
The multivitamin category includes general multivitamins and prenatal multivitamins

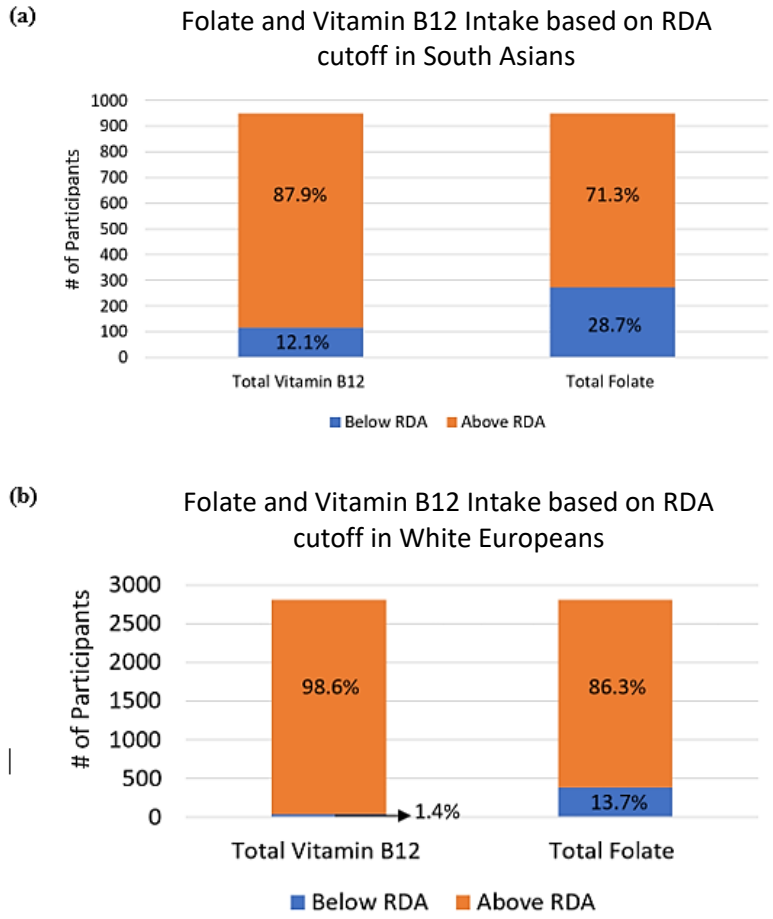

Figure 3.1: Total folate and vitamin B12 intakes based on RDA cut-offs in (a) South Asians and (b) White Europeans

3.1.3 Maternal Biochemical Characteristics in South Asians

The mean RBC folate, serum vitamin B12, plasma Hcy and plasma MMA concentrations of participants are as shown in Table 3.4. The direct indicators, RBC folate and serum vitamin B12 have a mean concentration of 492.90 nmol/L and 223.86 pmol/L, respectively. The functional indicators, plasma Hcy and plasma MMA have a mean concentration of 6.21 μM and 465.84 nmol/L, respectively.

Table 3.4: Biochemical characteristics of a subgroup of the START study population

Variable	START	N
RBC Folate (nmol/L)	492.90 ± 154.30	233
Serum Vitamin B12 (pmol/L)	223.86 ± 104.65	236
Plasma Methylmalonic Acid (nmol/L)	465.84 ± 227.80	233
Plasma Homocysteine (μM)	6.21 ± 1.94	236

Continuous variables are presented as means ± SD
Untransformed values are presented
Biochemical indicators are extracted from fasting maternal blood samples during the second trimester

3.2 Maternal Dietary Intake

During the baseline visit, participants were administered an FFQ to estimate their frequency of food consumption, which was used to estimate folate and vitamin B12 intakes. The FFQ shows an average caloric intake of 1682.27 kcal in SA and 1937.78 kcal in WE (Table 3.2).

3.2.1 Maternal Frequency of Food Consumption & Folate & Vitamin B12 Content

Table 3.5 presents the top 10 consumed foods by SA in the START cohort. SA primarily consumed lentil/dal curry, roti/chapati, garlic, ginger, dark leafy vegetables, onions, apple/pear, chilies, chickpea curry and cucumber, in descending order. The top 10 food items were consumed by over 90% of the SA population. Of these food items, lentils/dal curry (105.61 mcg), dark leafy vegetables (70.01 mcg) and chickpeas curry (72.23 mcg) contain high amounts of dietary folate, indicated in Appendix D (a). However, Appendix D (b) shows that the top consumed food items from the SA FFQ are not included in the top 10 vitamin B12-rich food items.

Table 3.5: Top 10 consumed foods by South Asians in the START cohort, in descending order

FFQ Food Item	% (n=948)	Folate (mcg)	Vitamin B12 (mcg)	Serving Size
Lentil/Dal Curry	96.84 (918)	105.61	0.00	Medium (½ cup)
Roti, Chapati	94.41 (895)	22.71	0.01	1, 6" diameter
Fresh Garlic	94.09 (892)	0.05	0.00	½ tsp
Ginger	93.25 (884)	0.11	0.00	½ tsp
Dark Leafy Vegetables	92.41 (876)	70.01	0.01	½ cup or 125 ml
Onions	91.88 (871)	13.87	0.00	½ cup or 125 ml
Apple, Pear	91.56 (868)	3.34	0.00	1 medium
Chilies Chickpeas Curry	91.14 (864)	5.27 72.23	0.00 0.00	1 small Medium (½ cup)
Cucumber	90.08 (854)	7.00	0.00	½ cup or 125 ml
Banana	89.24 (846)	21.97	0.00	1 medium
Based on the START FFQ				

Table 3.6 presents the top 10 consumed foods by WE in the FAMILY cohort. WE from FAMILY primarily consumed potatoes, lettuce, chicken/turkey, crisp snacks, carrots, cookies, apple/pear, chocolate, French fries, corn, berries, ice cream, rice, grapes and other vegetables, in descending order. The top 10 food items were consumed by over 95% of the WE population. Of these food items, lettuce (49.25 mcg) and corn (46.21 mcg) contain high amounts of dietary folate, as seen in Appendix E (a). However, as depicted by Appendix E (b), the top consumed food items from the WE FAMILY FFQ are not included in the top 10 vitamin B12-rich food items.

Table 3.6: Top 10 consumed foods by White Europeans in the FAMILY cohort, in descending order

FFQ Food Item	% (n=624)	Folate (mcg)	Vitamin B12 (mcg)	Serving Size
Potatoes (Boiled, or Mashed Baked)	98.88 (617)	8.07	0.03	1 Medium or ½ cup
Lettuce Chicken, Turkey (Roasted or Oven-Baked)	98.08 (612)	49.25 8.22	0.00 0.29	1 cup or 250 ml Medium
Crisp Snacks (Popcorn, Potato Chips)	97.92 (611)	2.61	0.00	½ cup or 125 ml
Carrots	97.76 (610)	9.95	0.00	1 Medium or ½ cup
Cookie	97.60 (609)	1.95	0.01	1 Cookie
French Fries or Fried Potatoes Apple, Pear Chocolate	97.44 (608)	16.39 4.18 8.65	0.02 0.00 0.09	1 cup or small McDonald's 1 Medium 1 Small Bar, 45g or 5 Chocolates
Corn Berries Ice Cream	97.28 (607)	46.21 16.66 2.24	0.00 0.00 0.30	1 Cob or ½ cup ½ cup or 125 ml ½ cup or 125 ml
Rice (Boiled)	95.99 (599)	8.97	0.00	½ cup or 125 ml
Grapes	95.35 (595)	2.90	0.00	½ cup or 125 ml
Other Vegetables (Celery, Mushroom, Artichokes)	95.03 (593)	29.74	0.00	½ cup or 125 ml
Based on the FAMILY FFQ				

3.2.2 Major Contributors of Folate & Vitamin B12 in the Maternal Diet

The top 10 contributors of folate in the SA diet were roti/chapati, lentil/dal curry, lettuce, onions, tomato, paratha made with oil, 2% milk, plain regular fat yogurt, tea and dark leafy vegetables, in descending order. The overall contribution of these 10 foods was approximately 46.26% of all folate consumed by the SA women. The contribution of each

food item to the total folate intake is presented in Table 3.7. Foods created with folic acid fortified flour contributed substantially to daily folate intake. For instance, roti/chapati and paratha, which are types of Indian bread, are made from folic acid fortified wheat flour and together contributed 13.20% of the participants daily folate status.

Table 3.7: Top 10 foods contributing to folate in the South Asian population, in descending order (n = 948)

FFQ Food Item	% of Contribution	Folate Contribution (mcg)	Serving Size
Roti, Chapati	9.59	34,226.16	1, 6” diameter
Lentil/Dal Curry	8.25	29,442.40	Medium ((½ cup)
Lettuce	5.21	18,597.93	1 cup or 250 ml
Onions	3.75	13,391.13	½ cup or 125 ml
Tomato	3.61	12,889.26	1 Medium
Paratha made with Oil	3.61	12,862.66	1, 6” diameter
2% Milk	3.19	11,389.24	1 cup or 250 ml
Yogurt, Curd, Plain, Regular Fat	3.14	11,188.53	¾ cup or 175 ml
Tea, Regular	3.07	10,970.63	1 cup or 250 ml
Dark Leafy Vegetables	2.83	10,107.56	½ cup or 125 ml
TOTAL	**46.26**	**165,065.50**	
Based on the START FFQ			

The top 10 contributors of vitamin B12 in the SA diet were 2% milk, plain regular fat yogurt, whole milk, liver, yogurt drink, fish, egg, buttermilk low fat yogurt, fruit yogurt and raita with vegetables, in descending order. The overall contribution of these 10 foods was approximately 77.54% of all vitamin B12 consumed by SA women. The contribution of each food item to the total vitamin B12 intake is presented in Table 3.8.

Primarily milk-based food items were contributing to the vitamin B12 intake (63.66%) in the SA population as well as fish and egg.

Table 3.8: Top 10 foods contributing to vitamin B12 in the South Asian population, in descending order (n = 948)

FFQ Food Item	% of Contribution	Vitamin B12 Contribution (mcg)	Serving Size
2% Milk	25.70	813.00	1 cup or 250 ml
Yogurt, Curd, Plain, Regular Fat	16.99	537.39	¾ cup or 175 ml
Whole Milk (Homo)	9.12	288.54	1 cup or 250 ml
Liver	7.34	232.13	Medium
Yogurt Drink	4.03	127.52	1 cup or 250 ml
Fish, Machli (Steamed or Baked)	3.32	105.15	Medium
Egg (Fried, Scrambled, Curry)	3.23	102.13	1 Egg
Yogurt, Buttermilk, Plain, Low Fat	2.98	94.25	¾ cup or 175 ml
Yogurt, Fruit Flavoured	2.43	76.83	¾ cup or 175 ml
Raita, with Vegetables	2.41	76.33	½ cup or 125 ml
TOTAL	**77.54**	**2,453.28**	
Based on the START FFQ			

The top 10 contributors of folate intake in the WE diet were lettuce, orange/grapefruit juice, 100% whole wheat bread, 1% milk, banana, vegetables (celery, mushroom, artichokes), citrus fruits, skim milk, 2% milk and broccoli, in descending order. The overall contribution of these 10 foods was approximately 36.66% of all folate consumed by the women in the cohort. The contribution of each food item to the total folate intake is presented in Table 3.9. Primarily fruit, vegetable and milk-based food items were contributing to the folate intake (33.27%) in the WE FAMILY population.

Table 3.9: Top 10 foods contributing to folate in the White European FAMILY population, in descending order (n = 624)

FFQ Food Item	% of Contribution	Folate Contribution (mcg)	Serving Size
Lettuce	7.65	14,505.34	1 cup or 250 ml
Orange, Grapefruit Juice	6.55	12,415.63	¾ cup or 175 ml
Whole Wheat Bread 100%	3.41	6,461.70	1 Slice
1% Milk	3.22	6,101.89	1 cup or 250 ml
Banana	3.15	5,968.38	1 Medium
Vegetables (Celery, Mushroom, Artichokes)	2.98	5,651.79	1/2 cup or 125 ml
Citrus Fruits	2.79	5,294.09	1 Orange or 2 Clementine or ½ Grapefruit
Skim Milk	2.40	4,547.07	1 cup or 250 ml
2% Milk	2.27	4,300.80	1 cup or 250 ml
Broccoli	2.26	4,278.43	½ cup or 125 ml
TOTAL	**36.66**	**69525.11**	
Based on the FAMILY FFQ			

The top 10 contributors of vitamin B12 in the WE FAMILY diet were 1% milk, skim milk, 2% milk, ground beef, fish, chocolate milk, fruit low fat yogurt, regular fat cheese, steak and regular fat fruit yogurt, in descending order. The overall contribution of these 10 foods was approximately 61.48% of all vitamin B12 consumed by the women in the cohort. The contribution of each food item to the total vitamin B12 intake is presented in Table 3.10. Primarily milk-based food items were contributing to the vitamin B12 intake (47.52%) in the WE population.

Table 3.10: Top 10 foods contributing to vitamin B12 in the White European FAMILY population, in descending order (n = 624)

FFQ Food Item	% of Contribution	Vitamin B12 Contribution (mcg)	Serving Size
1% Milk	13.74	449.14	1 cup or 250 ml
Skim Milk	10.11	330.50	1 cup or 250 ml
2% Milk	9.39	307.00	1 cup or 250 ml
Ground Beef	6.27	204.91	3" patty or 90g
Fish (Steamed, Baked)	4.24	138.63	Medium
Chocolate Milk, Hot Chocolate	4.20	137.44	1 cup or 250 ml
Yogurt, Fruit, Low Fat	3.87	126.68	¾ cup or 175 ml
Cheese, Regular Fat	3.53	115.45	1 Slice or 30g
Steak	3.44	112.52	Medium
Yogurt, Fruit, Regular Fat	2.68	87.78	¾ cup or 175 ml
TOTAL	**61.48**	**2,010.06**	
Based on the FAMILY FFQ			

Unfortunately, the folate and vitamin B12 breakdown for the CHILD FFQ was not made available, therefore the top 10 consumed foods was the only analysis completed. Based on these top 10 food items consumed by WE from the CHILD cohort, the ones most likely to be high in folate were granola/cereal bars, green salad, berries, breads/rolls and apples/applesauce/pears, in accordance with the available data from START and FAMILY (Table 3.11). Moreover, the food most likely to be high in vitamin B12 was whole eggs. Primarily fruit, vegetable and grain-based food items were contributing to the diet of WE in the CHILD population.

Table 3.11: Top 10 foods consumed by White Europeans in the CHILD cohort

FFQ Food Item	% (n=2186)	Serving Size
Granola Bars and Cereal Bars	99.45 (2174)	1 bar
Green Salad (Lettuce)	97.85 (2139)	1 cup
Berries (Strawberries and Blueberries)	96.66 (2113)	½ cup
Carrots	96.34 (2106)	½ cup
Breads and Rolls (as part of a sandwich)	96.07 (2100)	2 slices or 1 large roll
Apples, Applesauce and Pears	95.88 (2096)	1 medium or ½ cup
Other Vegetables (Bean Sprouts, Celery, Cucumber, Bamboo Shoots, Water Chestnuts, Mushrooms, Turnips, Lotus Root)	95.79 (2094)	½ cup
Potatoes (Boiled, Baked or Mashed)	94.56 (2067)	I medium or ¾ cup
Whole Eggs Pizza	94.01 (2055)	2 eggs 2 slices
Salad Dressing (All Types)	93.60 (2046)	2 tablespoons
Based on the CHILD FFQ		

3.3 Ethnicity – Combined Model

Covariates, dietary pattern scores and ethnicity were forced into the model. In the final multivariable model, only maternal height ($\beta < 0.01$; $p = 0.03$), maternal pre-pregnancy weight ($\beta = 0.51$; $p < 0.01$), maternal gestational weight gain ($\beta = 0.10$; $p < 0.01$), neonate gestational age ($\beta = 4.01$; $p < 0.01$) and parity ($\beta = 0.03$; $p = 0.01$) were retained as significant contributors of the model after a forward selection procedure. The final multivariable model for ethnicity is presented in Table 3.12. Maternal ethnicity was significantly associated with neonate birth weight after adjustment for covariates in the entire study population ($\beta = -014$; $p < 0.01$).

Table 3.12: Multiple linear regression model of the association between ethnicity and neonate birth weight (n = 2740)

Variable Outcome = Birth weight (kg)	β	P-Value	R^2
Maternal Height (cm)	<0.01	<0.03	0.19
Parity	0.03	0.01	
Pre-Pregnancy Weight (per kg)*	0.51	<0.01	
Gestational Weight Gain (per kg)**	0.10	<0.01	
Gestational Age (per week)*	4.01	<0.01	
Ethnicity (1 = SA)	-0.14	<0.01	
* Denotes log transformed variables ** Denotes square root transformed variables Ethnicity (0 = WE; 1 = SA)			

3.4 Dietary, Supplemental & Total Folate & Vitamin B12 – Stratified Analysis

3.4.1 Maternal Association of Dietary, Supplemental & Total Folate & Vitamin B12 with Neonate Birth Weight in South Asians

Covariates, dietary pattern scores and the independent variable (dietary, supplemental and total folate or vitamin B12) were forced into the model. In the final multivariable model, only GDM status (β = 0.07; p = 0.02), maternal height (β <0.01; p = 0.01), parity (β = 0.04; p = 0.02), neonate sex (β = -0.09; p <0.01), maternal pre-pregnancy weight (β = 0.47; p <0.01), maternal gestational weight gain (β = 0.07; p <0.01), and neonate gestational age (β = 6.38; p <0.01) were retained as significant contributors of the model after a forward selection procedure (Table 3.13). Furthermore, the plant-based dietary score (β = 0.03; p <0.01) was shown to be significantly associated with neonate birth weight in the SA population, as expected. The final multivariable models for dietary, supplemental and total folate and vitamin B12 are presented in Table 3.14. Maternal dietary, supplemental and total folate and vitamin B12 status were not

significantly associated with neonate birth weight after adjustment for covariates and maternal dietary patterns in the SA population.

Table 3.13: Covariates that were significantly associated with neonate birthweight in South Asians (n = 903)

Variable **Outcome = Birth weight (kg)**	**β**	**P-Value**	**R^2**
Constant	-23.46	<0.01	0.34
GDM (yes vs. no) The mother reports having GDM or using insulin during pregnancy, it is specified on her birth chart, or her OGTT came out positive, using Born in Bradford thresholds	0.07	0.02	
Maternal Height (cm)	<0.01	0.01	
Parity	0.04	0.02	
Neonate Sex (female vs. male)	-0.09	<0.01	
Pre-Pregnancy Weight (per kg)*	0.47	<0.01	
Gestational Weight Gain (per kg)**	0.07	<0.01	
Gestational Age (per week)*	6.38	<0.01	

* Denotes log transformed variables
** Denotes square root transformed variables
GDM (0=no; 1=yes)
Sex (0=male; 1=female)

Table 3.14: Unadjusted and adjusted multiple linear regression models in South Asian mothers for dietary, supplemental and total folate and vitamin B12

Independent Variable Outcome = Birth weight (kg)	Adjustment	β	R^2	P-Value	N
Dietary Folate*	Unadjusted Adjusted	0.02 .	<0.01 0.34	0.54 .	948 903
Dietary vitamin B12*	Unadjusted Adjusted	0.03 .	<0.01 0.34	0.34 .	948 903
Supplemental Folate**	Unadjusted Adjusted	< -0.01 .	<0.01 0.34	0.23 .	948 903
Supplemental vitamin B12**	Unadjusted Adjusted	< -0.01 .	<0.01 0.34	0.83 .	948 903
Total Folate*	Unadjusted Adjusted	-0.02 .	<0.01 0.34	0.66 .	948 903
Total vitamin B12*	Unadjusted Adjusted	< -0.01 .	<0.01 0.34	0.86 .	948 903

* Denotes log transformed variables
** Denotes square root transformed variables
Unadjusted refers to a simple linear regression model
Adjusted refers to a multiple linear regression model with inclusion of covariates (GDM, maternal height, parity, maternal pre-pregnancy weight, maternal gestational weight gain, neonate gestational age, neonate sex and plant based dietary pattern score)
"." Denotes non-significant association and was therefore removed from the stepwise forward regression analysis

3.4.2 Maternal Association of Dietary, Supplemental & Total Folate & Vitamin B12 with Neonate Birth Weight in White Europeans

Covariates, dietary pattern scores and the independent variable (dietary, supplemental and total folate or vitamin B12) were forced into the model. In the final multivariable model, only maternal pre-pregnancy weight ($\beta = 0.47$; $p < 0.01$), maternal gestational weight gain ($\beta = 0.07$; $p < 0.01$), and neonate gestational age ($\beta = 6.38$; $p < 0.01$) were retained as significant contributors of the model after a forward selection procedure (Table 3.15). The final multivariable models for dietary, supplemental and total folate and vitamin B12 are presented in Table 3.16. Maternal dietary, supplemental and

total folate and vitamin B12 status were not significantly associated with neonate birth weight after adjustment for covariates and maternal dietary patterns in the WE population.

Table 3.15: Covariates that were significantly associated with neonate birthweight in White Europeans (n = 1844)

Variable Outcome = Birth weight (kg)	β	P-Value	R^2
Pre-Pregnancy Weight (kg)*	0.51	<0.01	0.10
Gestational Weight Gain (kg)**	0.12	<0.01	
Gestational Age (weeks)*	2.90	<0.01	

* Denotes log transformed variables
** Denotes square root transformed variables

Table 3.16: Unadjusted and adjusted multiple linear regression models in White European mothers for dietary, supplemental and total folate and vitamin B12

Independent Variable Outcome = Birth weight (kg)	Adjustment	β	R^2	P-Value	N
Dietary Folate*	Unadjusted Adjusted	-0.01 .	<0.01 0.10	0.73 .	2810 1844
Dietary vitamin B12*	Unadjusted Adjusted	0.05 .	<0.01 0.10	0.03 .	2810 1844
Supplemental Folate**	Unadjusted Adjusted	< 0.01 .	<0.01 0.07	0.74 .	2595 1675
Supplemental vitamin B12**	Unadjusted Adjusted	< 0.01 .	<0.01 0.07	0.53 .	2500 1609
Total Folate*	Unadjusted Adjusted	-0.01 .	<0.01 0.10	0.84 .	2810 1844
Total vitamin B12*	Unadjusted Adjusted	0.03 .	<0.01 0.10	0.14 .	2810 1844

* Denotes log transformed variables
** Denotes square root transformed variables
Unadjusted refers to a simple linear regression model
Adjusted refers to a multiple linear regression model with inclusion of covariates (maternal pre-pregnancy weight, maternal gestational weight gain, neonate gestational age)
"." Denotes non-significant association and was therefore removed from the stepwise forward regression analysis

3.5 Folate and Vitamin B12-Rich Foods

The folate and vitamin B12-rich food groups were derived and summed from the FFQ, so that each participant was assigned an average daily intake (expressed as servings per day). The folate-rich food groups included green leafy vegetables, fruit, milk, grain and legumes food items. The vitamin B12-rich food groups included milk, egg and meat food items.

3.5.1 Association of Maternal Consumption of Folate & Vitamin B12-Rich Foods with Neonate Birth Weight in South Asians

In the adjusted regression models, the covariates, dietary pattern scores and the independent variable of interest were included. In the final multivariable model, only GDM status ($\beta = 0.07$; $p = 0.02$), maternal height ($\beta < 0.01$; $p = 0.01$), parity ($\beta = 0.04$; $p = 0.02$), neonate sex ($\beta = -0.09$; $p < 0.01$), maternal pre-pregnancy weight ($\beta = 0.49$; $p < 0.01$), maternal gestational weight gain ($\beta = 0.07$; $p < 0.01$), and neonate gestational age ($\beta = 6.33$; $p < 0.01$) were retained as significant contributors of the model after a forward selection procedure. Table 3.17 summarizes the unadjusted (simple linear regression) and adjusted (multiple linear regression) models for each independent variable of interest. The green leafy vegetables, fruit, meat, egg, grains and legumes food groups were not significantly associated with neonate birth weight after adjustment for covariates. However, the milk food group ($\beta=0.06$; $p=0.01$) was significantly associated with neonate birth weight in the SA population, after adjustment for covariates.

Table 3.17: Unadjusted and adjusted multiple linear regression models in South Asian mothers

Independent Variable** Outcome = Birth weight (kg)	Adjustment	β	R^2	P-Value	N
Green Leafy Vegetables	Unadjusted Adjusted	<0.01 .	<0.01 0.34	0.89 .	948 903
Fruit	Unadjusted Adjusted	0.02 .	<0.01 0.34	0.45 .	948 903
Milk	Unadjusted Adjusted	0.06 0.06	0.01 0.34	0.03 0.01	948 903
Meat	Unadjusted Adjusted	-0.03 .	<0.01 0.34	0.34 .	948 903
Egg	Unadjusted Adjusted	-0.04 .	<0.01 0.34	0.33 .	948 903
Grain	Unadjusted Adjusted	0.02 .	<0.01 0.34	0.63 .	948 903
Legumes	Unadjusted Adjusted	-0.06 .	<0.01 0.34	0.22 .	948 903

** Denotes square root transformed variables
Unadjusted refers to a simple linear regression model
Adjusted refers to a multiple linear regression model with inclusion of covariates (GDM, maternal height, parity, maternal pre-pregnancy weight, maternal gestational weight gain, neonate gestational age and neonate sex)
"." Denotes non-significant association and was therefore removed from the stepwise forward regression analysis

3.5.2 Association of Maternal Consumption of Folate & Vitamin B12-Rich Foods with Neonate Birth Weight in White Europeans

In the adjusted regression models, the covariates (Table 3.15), dietary pattern scores and the independent variable of interest were included. In the final multivariable model, maternal pre-pregnancy weight ($\beta = 0.51$; $p < 0.01$), maternal gestational weight gain ($\beta = 0.12$; $p < 0.01$), and neonate gestational age ($\beta = 2.91$; $p < 0.01$) were retained as significant contributors of the model after a forward selection procedure. Table 3.18 summarizes the unadjusted (simple linear regression) and adjusted (multiple linear regression) models for each independent variable of interest. The green leafy vegetables,

fruit, meat, milk, grains and legumes food groups were not significantly associated with neonate birth weight after adjustment for covariates. However, the egg food group (β=-0.19; p<0.01) was significantly associated with neonate birth weight after adjustment for covariates in the WE population.

Table 3.18: Unadjusted and adjusted multiple linear regression models in White European mothers

Independent Variable** Outcome = Birth weight (kg)	Adjustment	β	R^2	P-Value	N
Green Leafy Vegetables	Unadjusted Adjusted	0.03 .	<0.01 0.10	0.42 .	2810 1844
Fruit	Unadjusted Adjusted	-0.02 .	<0.01 0.10	0.49 .	2809 1844
Milk	Unadjusted Adjusted	0.05 .	<0.01 0.10	0.01 .	2809 1844
Meat	Unadjusted Adjusted	0.03 .	<0.01 0.10	0.41 .	2810 1844
Egg	Unadjusted Adjusted	-0.08 -0.19	<0.01 0.10	0.08 <0.01	2810 1844
Grain	Unadjusted Adjusted	0.06 .	<0.01 0.10	0.02 .	2810 1844
Legumes	Unadjusted Adjusted	0.05 .	<0.01 0.10	0.23 .	2810 1844

** Denotes square root transformed variables
Unadjusted refers to a simple linear regression model
Adjusted refers to a multiple linear regression model with inclusion of covariates (maternal pre-pregnancy weight, maternal gestational weight gain, neonate gestational age)
"." Denotes non-significant association and was therefore removed from the stepwise forward regression analysis

3.6 Biochemical Indicators

The biochemical indicators of folate and vitamin B12 status were measured to get a better understanding of the participants micronutrient status. RBC folate and serum

vitamin B12 were used as direct biomarkers of folate and vitamin B12 status. Plasma Hcy was used as a functional biomarker of folate and vitamin B12 status. Plasma MMA was used as a functional biomarker of vitamin B12 status. The biochemical indicators of folate and vitamin B12 status were only available in a select group of SA women from START. As expected, there was an inverse association between RBC folate and plasma Hcy ($\beta = -0.22$; $p < 0.01$; $n = 233$), serum vitamin B12 and plasma Hcy ($\beta = -0.22$; $p < 0.01$; $n = 236$), and serum vitamin B12 and plasma MMA ($\beta = -0.29$; $p < 0.01$; $n = 233$). There was a positive association between RBC folate and serum vitamin B12 ($\beta = 0.23$; $p < 0.01$; $n = 233$).

3.6.1 Folate & Vitamin B12 Deficiency

Based on the biochemical indicators, none of the participants had a high RBC folate concentration, above the adequate reference concentration of 320-1090 nmol/L, whereas, 32 (13.73%) participants had a folate status <320 nmol/L, which was defined as folate deficiency. The vitamin B12 concentration in 90 (38.14%) participants was >220 pmol/L, which was higher than the adequate reference range of 148-220 pmol/L and 42 (17.80%) participants were deficient in vitamin B12 (<148 pmol/L) (Table 3.19). Food frequency analysis of the participants diet shows that their overall consumption of folate and vitamin B12 was good considering the mean biochemical variables were within the adequate reference range for most participants. Participants classified as either low vitamin B12, high vitamin B12 or low folate did not significantly differ in their dietary intakes relative to those classified within the adequate range of intake ($p = 0.07$, $p = 0.44$ and $p = 0.51$, respectively). However, participants classified as low vitamin B12 or low

folate did significantly differ in their supplemental use relative to those classified within the adequate range of intake ($p = 0.02$ and $p = 0.04$, respectively); but this was not the case for high vitamin B12 supplement users ($p = 0.11$).

Table 3.20 (a-d) demonstrates the results of a crosstabulation analysis between the biochemical indicators of folate and vitamin B12 status. Plasma MMA concentration was high (>370 nmol/L) in 142 (60.94%) participants, which was also indicative of vitamin B12 deficiency. However, participants did not have a high homocysteine status (>15 μM), which was a marker for both folate and vitamin B12 deficiency. 11 (4.7%) of participants were deficient in both RBC folate and serum vitamin B12. Based on independent sample t-tests, these individuals had higher plasma Hcy and MMA concentrations on average compared to the rest of the subgroup. Moreover, 31 (13.3%) women were identified as being overtly deficient in vitamin B12 (low vitamin B12 [<148 pmol/l] and high MMA [>370 nmol/l]). In contrast, none of the participants were identified as being overtly deficient in folate (low folate [<320 nmol/l] and high Hcy [>15 μM]).

Table 3.19: Prevalence of South Asian pregnant women classified based on biochemical indicator cut-off ranges

Biochemical Indicators	Cut-off Ranges	Status	N	Total
RBC Folate (nmol/L)	<320 320-1090 >1090	Deficient Adequate High	32 201 0	233
Serum Vitamin B12 (pmol/L)*	<148 148-220 >220	Deficient Adequate High	42 104 90	236
Plasma Homocysteine (μM)*	<5 5-15 >15	Low Moderate High	67 169 0	236
Plasma Methylmalonic Acid (nmol/L)*	<210 210-370 >370	Low Moderate High	18 73 142	233
* Denotes log transformed variables Determined based on non-pregnant adult cut-off ranges				

Table 3.20: Crosstabulation results of South Asian pregnant women classified based on biochemical indicator cut-off ranges for folate and vitamin B12 status

(a) Crosstabulation between serum vitamin B12 and plasma MMA

		Plasma MMA Status			
Serum Vitamin B12 Status n = 233		*Low 7.73%*	*Moderate 31.33%*	*High 60.94%*	**Total**
	Deficient 18.03%	1 (0.43%)	10 (4.29%)	31 (13.30%)	42
	Adequate 43.78%	6 (2.58%)	27 (11.59%)	69 (29.61%)	102
	High 38.19%	11 (4.72%)	36 (15.45%)	42 (18.03%)	89
	Total	18	73	142	

(b) Crosstabulation between RBC folate and serum vitamin B12

		Serum Vitamin B12 Status			
RBC Folate Status n = 233		*Deficient 18.03%*	*Adequate 44.21%*	*High 37.77%*	**Total**
	Deficient 13.73%	11 (4.72%)	18 (7.73%)	3 (1.29%)	32
	Adequate 86.27%	31 (13.30%)	85 (36.48%)	85 (36.48%)	201
	High 0%	0 (0%)	0 (0%)	0 (0%)	0
	Total	42	103	88	

(c) Crosstabulation between RBC Folate and plasma Hcy

		Plasma Hcy Status			
RBC Folate Status n = 233		*Low 28.76%*	*Moderate 71.24%*	*High*	**Total**
	Deficient 13.73%	5 (2.15%)	27 (11.59%)	0 (0%)	32
	Adequate 86.27%	62 (26.61%)	139 (59.66%)	0 (0%)	201
	High 0%	0 (0%)	0 (0%)	0 (0%)	0
	Total	67	166	0	

(d) Crosstabulation between serum vitamin B12 and plasma Hcy

	Serum Vitamin B12 Status				
Plasma Hcy Status n = 236		*Deficient 17.80%*	*Adequate 44.07%*	*High 38.14%*	**Total**
	Low 28.39%	9 (3.81%)	26 (11.02%)	32 (13.56%)	67
	Moderate 71.61%	33 (13.98%)	78 (33.05%)	58 (24.58%)	169
	High (0%)	0 (0%)	0 (0%)	0 (0%)	0
	Total	42	104	90	

3.6.2 Association of Maternal Biochemical Indicators with Neonate Birth Weight

The association between maternal folate and vitamin B12 biochemical indicators (RBC folate, serum vitamin B12, plasma Hcy and plasma MMA) status with neonate birth weight was determined by a multiple linear regression model. The covariates (Table 3.13), dietary pattern scores and the biochemical variable were included in the model. In the final multivariable model, maternal pre-pregnancy weight ($\beta = 0.92$; $p<0.01$), maternal gestational weight gain ($\beta = 0.07$; $p=0.01$), neonate gestational age ($\beta = 8.25$; $p<0.01$) and neonate sex ($\beta = -0.17$; $p<0.01$) were retained as significant contributors of the model after a forward selection procedure. The unadjusted (simple linear regression) and adjusted (multiple linear regression) models for each biochemical variable of interest are summarized in Table 3.21. There was a significant negative association between serum vitamin B12 concentrations and neonate birth weight after adjustment for covariates ($\beta=-0.16$; $p=0.03$).

Table 3.21: Unadjusted and adjusted multiple linear regression models in South Asian mothers only for biochemical indicators of folate and vitamin B12 status

Independent Variable Outcome = Birth weight (kg)	Adjustment	β	R^2	P-Value	N
RBC Folate	Unadjusted Adjusted	0.02 .	<0.01 0.51	0.54 .	233 218
Serum vitamin B12*	Unadjusted Adjusted	0.03 -0.16	< 0.01 0.52	0.34 0.03	236 221
Plasma Hcy*	Unadjusted Adjusted	< -0.01 .	0.49 0.51	0.49 .	236 221
Plasma MMA*	Unadjusted Adjusted	< -0.01 .	<0.01 0.51	0.83 .	233 219

* Denotes log transformed variables
Unadjusted refers to a simple linear regression model
Adjusted refers to a multiple linear regression model with inclusion of covariates (maternal pre-pregnancy weight, maternal gestational weight gain, neonate gestational age and neonate sex)
"." Denotes non-significant association and was therefore removed from the stepwise forward regression analysis

3.7 Summary of Results

- Maternal folate and vitamin B12 levels (dietary, supplemental and total) were not associated with neonate birth weight in SA and WE women
- The maternal plant-based dietary score was associated with higher neonate birth weight in SA women ($\beta = 0.03$; $p < 0.01$)
- Higher consumption of milk-products (servings/day) by SA women was associated with higher neonate birth weight ($\beta=0.06$; $p=0.01$)
- Higher consumption of egg products (servings/day) by WE women was associated with lower neonate birth weight ($\beta=-0.19$; $p<0.01$)
- Maternal serum vitamin B12 status was inversely associated with neonate birth weight in the SA subgroup ($\beta=-0.16$; $p=0.03$)

Chapter 4: Discussion, Future Directions and Conclusion

4.1 Discussion

In this cross-sectional analysis of a prospective cohort study of 3758 SA and WE women, we studied maternal folate and vitamin B12 status in relation to neonatal birth weight. We also examined circulating folate and vitamin B12 concentrations during the second trimester in a subgroup ($n \approx 236$) of SA. We did not find any statistically significant relationships between maternal folate (dietary, supplemental, or total) or vitamin B12 (dietary, supplemental or total) intake and neonate birth weight in either SA or WE participants assessed during the second trimester of pregnancy. The nature of the participants studied may be the reason a significant association was not identified. These participants were residing in a high resource country and therefore differ from the participants studied in India. The populations studied in India were an unusual sample as they were based in highly poor environments (i.e. slum-dwelling or malnourished individuals) and this may explain why an association between higher intakes of folate or vitamin B12 with neonate birth weight was found. Furthermore, statistically, the range of folate and vitamin B12 within one population is limited as well as the range of neonate birth weight in healthy women. These Canadian women (both WE and SA) are considered to be healthy in terms of folate and vitamin B12 given the mandatory food fortification and awareness surrounding supplement usage during pregnancy. Therefore, the ceiling effect occurs in which the independent variable (i.e. dietary or supplemental

folate and vitamin B12) no longer has an effect on the dependant variable (i.e. neonate birth weight), as a saturation point has been reached (Baker, 2004).

In the cross-sectional SHARE study that enrolled 818 SA, Chinese and WE Canadians between December 1996 and August 1998, 342 SA and 326 WE participants from 3 Canadian centers (Hamilton, Ontario; Toronto, Ontario and Edmonton, Alberta) (Kelemen et al, 2003) completed validated semi-quantitative FFQs. Average total folate intake was higher in START than SHARE SA (476.37 mcg and 427 mcg, respectively), and in FAMILY than in SHARE WE (613.08 mcg vs 438 mcg). Average total vitamin B12 intake was similar between the SHARE (10.4 mcg) and START SA populations (9.02 mcg); but average total vitamin B12 intake was higher in the SHARE WE study population compared to FAMILY (17.1 mcg vs 10.38 mcg) (Table 4.1; from Kelemen et al., 2003). The SHARE study collected samples of serum folate whereas the START study collected RBC folate levels, therefore a direct comparison of folate status could not be made. Mean serum vitamin B12 in START (208.05 pmol/L) was lower than in the SA (237 pmol/L) and WE (279 pmol/L) populations of SHARE (Kelemen et al, 2003). Furthermore, plasma Hcy was lower in START (5.90 μmol/L) compared to SA (10.5 μmol/L) and WE (9.68 μmol/L) in SHARE.

Table 4.1: Mean Biochemical Indicator Concentrations in SHARE and START populations

Nutrient[a]	South Asians (SHARE N=283)	White Europeans (SHARE N=260)	South Asians (START N=236)
Serum Folate (μmol/L)	18.9	21.7	.
RBC Folate (nmol/L)	.	.	492.90*
Serum Vitamin B12 (pmol/L)	237	279	208.05
Plasma Homocysteine (μmol/L)	10.5	9.68	5.90

[a] Denotes geometric mean
* N = 233 participants
SHARE data (Kelemen et al, 2004)

In SA, higher maternal cow's milk consumption during the second trimester of pregnancy was significantly associated with neonate birth weight. Overall, maternal consumption of green leafy vegetables, fruit, meat, egg, grain and legume foods were not independently associated with neonate birth weight. In WE, higher maternal egg consumption during the second trimester of pregnancy was inversely associated with neonate birth weight. Overall, maternal consumption of green leafy vegetables, fruit, milk, meat, grain and legume foods were not independently associated with neonate birth weight. Similar to our study, the Pune Maternal Nutrition Study also found that increased milk consumption at 18 weeks of gestation was related to birth size (Rao et al, 2001). A cross-sectional study from Calgary, Canada of 279 pregnant women (1997-1999), found that for each additional cup of milk consumed per day, birth weight increased by 41g (Mannion et al, 2006). Papanikolaou & Fulgoni (2018) found that egg consumption was associated with lower intakes of dietary folate, iron, magnesium and niacin relative to non-consumers of eggs. Thus, it may not be the egg itself that is lowering neonate birth weight but the associated limited intake of other nutrients such as folate or iron.

Moreover, in a systematic review of observational studies (Murphy et al, 2014), most cohort studies (Mikkelsen et al, 2006; Mitchell et al, 2004; McCowan et al, 2010; Petridou et al, 1998; Ricci et al, 2010; Balazs et al, 2014) from highly developed areas failed to demonstrate any association between consuming fruits or vegetables during pregnancy and neonate birth weight. However, fruits and vegetable intake were positively associated with neonate birth weight in a lower developed area. In a population of rural, undernourished women in India assessed at 28 weeks of gestation, each additional serving of green leafy vegetables was associated with an increase in birth weight of 19.4g (95% CI 8-30g; $p<0.001$) (Rao et al, 2001). Similarly, the Pune Maternal Nutrition Study conducted in India, found that birth size was associated with both green leafy vegetable and fruit intake at 28 weeks' gestation (Rao et al, 2001). Therefore, dietary intake of foods may have a stronger effect on neonatal birth weight in a less developed area (i.e. India) rather than foods consumed in a highly developed area (i.e. Canada). Although the available evidence is limited, the association of increased fruit and vegetable consumption with birth weight is stronger in cohorts from resource-poor settings than from highly developed areas (Murphy et al, 2014).

This study found a high prevalence of adequate RBC folate status among pregnant SA women--86.3% of our SA subsample fell within the adequate range (320-1090 nmol/L); and 44% of SA women in the subsample had adequate serum vitamin B12 (148-220 pmol/L), and 38.1% had high (>220 pmol/L) serum vitamin B12. This suggests that our population was generally replete with folate and vitamin B12 during the second trimester, but we did observe deficiencies in folate (13.7%) and vitamin B12 (17.8%) in a

proportion of the population. In a retrospective cohort study of 748 healthy SA (n = 371) and WE (n = 377) women from Vancouver Canada, the prevalence of vitamin B12 deficiency (serum vitamin B12 <148 pmol/L) in SA women was 36% in the second trimester. Our study reported a lower (17.8%) prevalence of vitamin B12 deficiency in SA women (n = 236). Likewise, in a Canadian cross-sectional study conducted in Vancouver of 340 pregnant women, 18% of women were classified as vitamin B12 deficient (Jeruszka-Bielak et al, 2017). Nevertheless, these Canadian studies reported a prevalence of vitamin B12 deficiency that was lower than the findings in pregnant women living in India. Studies from India reported a substantially higher prevalence (≈50-70%) of vitamin B12 deficiency ([serum vitamin B12] <150 pmol/L) during early pregnancy (Duggan et al, 2014; Yajnik et al, 2008). In addition, studies from India reported 70-95% of pregnant women had MMA concentrations >260 nmol/L (Duggan et al, 2014; Yajnik et al, 2008; Schroder et al, 2017), substantially higher than the 60.9% prevalence of high MMA (>370 nmol/L) observed in the present study.

A weak inverse association of maternal serum vitamin B12 concentration with neonate birth weight was found, after controlling for maternal pre-pregnancy weight, maternal gestational weight gain, neonate gestational age and neonate sex. This model explained 52% (R^2) of the variation in birth weight, and addition of serum vitamin B12 to the model explained an additional 18% of variance above established covariates in our cohort (R^2 =34%). There was no association of maternal RBC folate, plasma Hcy or plasma MMA concentrations in the second trimester with birth weight. The lack of association between maternal folate concentrations and neonate birth weight in this study

agrees with a cross-sectional birth cohort study from Singapore, a rich and resourceful country similar to Canada, involving 999 mother-child dyads, at 26-28 weeks of gestation. They found that maternal concentrations of folate and vitamin B12 were not independently associated with birth weight. Similar to our study (β=-0.196; p=0.007), they reported an inverse association between maternal vitamin B12 and neonate birth weight (β=-1.2; p=0.93), however this association was not significant (Chen et al, 2014).

Importantly, folate and vitamin B12 may be proxies for poor nutritional status and it is possible that the previously reported studies from India were not specific to folate and vitamin B12 but rather was an indicator of malnutrition (Rogne et al, 2017). 13.7% of the women in the present study were deficient in folate based on the <320 nmol/L cut-off. However, folate status in Canadian pregnant women (Fayyaz et al, 2014; Plumptre et al, 2015), and reproductive-aged women (Colapinto et al, 2015) was reported to be substantially elevated, because >90% of these women use high-dose folic acid (1 mg/d) prenatal supplements (>90%) (Fayyaz et al, 2014; Plumptre et al, 2015). In our study, 73% of the SA and 88% of the WE cohort reported taking supplements during pregnancy. Plumptre et al (2015) reported that over 50% of women had an RBC folate concentration >1360 nmol/l. Similarly, Fayyaz et al (2014) reported a mean RBC folate of 2417 nmol/l, which is considered to be high. Moreover, our SA population (n = 233) did not have elevated folate (>1090 nmol/l). The normal range of folate and vitamin B12 in our participants likely explains the low Hcy concentrations observed.

The association between folate status and neonate birth weight in some studies may be due to different participant characteristics and folate ranges. Given the differences

in nourishment, the folate ranges are lower in studies from India than from high income countries like Canada. The association between folate and birth weight in the Canadian context is not evident among SA participants in this study possibly because their RBC folate and serum vitamin B12 may not have been low enough to influence neonate birth weight (as seen in Figure 4.1). As evident from the mean biochemical indicator values (Table 3.4), mean values are within the adequate reference range. Furthermore, the number of participants in the low folate (13.7%) and low vitamin B12 (17.8%) group are minimal, and no participant was in the high folate group. Moreover, in our study of 233 participants with data for correlation analyses, plasma Hcy concentrations were inversely associated with RBC folate and serum vitamin B12, which agrees with the investigations of Kelemen et al, 2003. Similarly, plasma MMA concentrations were significantly inversely associated with serum vitamin B12.

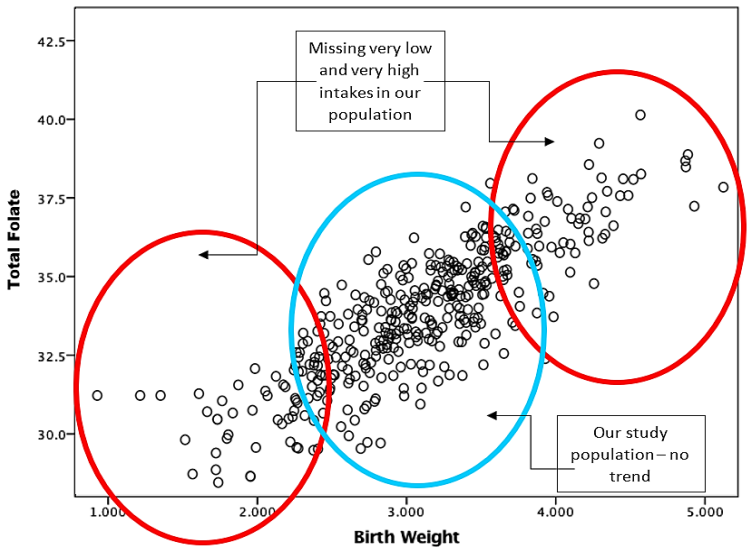

Figure 4.1: A scatterplot representing the trend of association between folate and neonate birth weight in our study population relative to studies in which an

4.5 Strengths and Limitations

Our study had some limitations. First, this thesis used internal secondary data as it was collected and designed by others based on the cohort's study requirements, goals and objectives. Participant selection and recruitment were pre-determined by other investigators and not by the student who conducted this analysis. Therefore, the participants used in this thesis do not represent a random selection of the population or of the study cohort.

Second, this analysis represents a cross-sectional analysis of a prospective cohort with measures of folate and vitamin B12 at a single time point, therefore the findings are not representative of the early or late stages of pregnancy. Samples from more than one time point of pregnancy would have facilitated exploration of differences in biomarker concentrations between the first, second and third trimesters of pregnancy. Further, folate and vitamin B12 concentrations fluctuate throughout gestation leading to a potential misclassification of vitamin status using non-pregnant cut-offs (Schroder et al, 2017). The fluctuation in folate and vitamin B12 concentrations during pregnancy are important physiological changes that need to be taken into consideration in future studies assessing the association between these two micronutrients and birth weight outcomes.

Third, maternal data on periconceptional multivitamin, folic acid or vitamin B12 supplement use were also not collected. Periconceptional measurements could have provided more insight into the timing of supplement use to improve neonate birth weight outcomes. In a prospective study of Canadian pregnant women (n = 368), >90% of whom were taking a periconceptional folic acid containing supplement, serum and RBC folate

concentration in early pregnancy as well as at delivery were in ranges considered to be high (Plumptre et al, 2015). Therefore, folic acid and vitamin B12 containing supplements may have a stronger and detectable effect on neonate birth weight if consumption begins periconceptionally and continues post-delivery.

Fourth, in the biochemical indicator analysis having WE biochemical samples would have provided more insight, as a comparison could have been done with the SA biochemical samples.

However, we remain confident in our findings because of the prospective data collection, a large sample size for the FFQ-based analyses, the availability of a wide range of variables and the use of validated and ethnic-specific FFQs. We measured circulating maternal biochemical indicators of folate and vitamin B12 status during pregnancy, which is a more robust indicator of maternal nutritional status than dietary or supplemental intake levels based on an FFQ assessment (Krishnaveni et al, 2014). Yetley et al (2011) recommended the use of at least one direct and one functional indicator. The combination of direct (RBC folate and serum vitamin B12) and functional (plasma Hcy and MMA) indicators of folate and vitamin B12 status and how they individually relate to birth weight was a strength of this study, as these provide a better reflection of maternal status. A general limitation of previous studies was the lack of complete information on total intake of folate and vitamin B12 from the diet and supplements, as well as maternal biochemical blood concentrations. We overcome this limitation through a comprehensive range of dietary, supplemental, and biochemical measurements during pregnancy in both the mother and neonate.

4.6 Future Directions

Future studies are necessary to replicate these results and to elucidate potential mechanisms that underlie these ethnic-specific associations. We have identified some associations but cannot define a causal relationship due to the observational nature of this study.

Firstly, due to the high intakes commonly seen in rich and resourceful countries such as Canada it is not ethnical or feasible to implement a randomized control trial to test for causality. Instead, future studies should aim to assess the causal association between maternal folate, vitamin B12 status and neonate birth weight through a Mendelian randomization study. In a Mendelian randomization study, genetic variants are used to investigate the causal association of a modifiable risk factor (in this case folate and vitamin B12) and health outcomes (i.e. neonate birth weight) in observational data. Mendelian randomization design is similar to randomized controlled trials as it investigates participants irrespective of their genotypes which may lead to differences in the outcome (Gupta, Sachdeva & Walia, 2019; Bennett & Holmes, 2017). By doing so, the causal relationship between maternal dietary, supplemental and biochemical folate and vitamin B12 concentrations with neonate birth weight can be identified.

Secondly, a study comparing the pre- and post-acculturation diet of recent and more longstanding SA immigrants is needed. Acculturation may affect general dietary and vegetarian patterns of migrant SA populations, as such this needs to be studied further, as well as a detailed analysis of dietary history and patterns before and after migrating, to truly encompass the effect of acculturation on this relationship (Gammon et

al, 2012). For example, a long-term (~ 5 years) prospective study which examines the effect of acculturation over time among SA women living in Canada. To measure acculturation, a survey capturing attitudes about the practice of SA traditions, dietary preferences, and health behaviours would be administered and respondents would be scored accordingly.

Thirdly, a large, well-powered and multi-ethnic observational study of SA and WE pregnant women would be beneficial. This observational study should include repeated measures of folate and vitamin B12 over time (i.e. trimesters) to further understand the presence of the association of maternal folate and vitamin B12 concentrations with neonate birth weight. Furthermore, the preconceptual folate and vitamin B12 intake should be collected to test the effect on neonate birth weight.

In addition to folate, vitamin B12, Hcy and MMA, assessment of maternal holotranscobalamin may also be more reflective of circulating vitamin B12 status. Holotranscobalamin is the proportion of circulating vitamin B12 that binds to haptocorrin for cellular uptake, which usually ranges from 10 to 30%. For this reason, holotranscobalamin is also referred to as active vitamin B12 (Finkelstein et al, 2015). There is therefore a direct relationship between vitamin B12 and holotranscobalamin concentrations. Studies that investigate other maternal B-vitamins, such as vitamin B6 and B2, due to their interdependent functions in one-carbon metabolism, are needed as well, in order to understand the true interplay between folate and vitamin B12 and neonate birth weight. Future work to build upon this research and to advance our

understanding about the association of maternal folate and vitamin B12 concentrations with neonate birth weight in the Canadian population is needed.

4.7 Conclusion

The overarching aim of this thesis was to describe the relationship between maternal folate and vitamin B12 concentrations and neonatal birth weight in SA and WE pregnant women. Through the work conducted for this thesis, we found that second trimester maternal dietary, supplemental and total folate and vitamin B12 intakes were not significantly associated with neonate birth weight in both SA and WE study populations. In SA, a higher maternal milk consumption during the second trimester of pregnancy was significantly associated with a higher neonate birth weight. Overall, maternal consumption of green leafy vegetables, fruit, meat, egg, grain and legume foods were not independently associated with neonate birth weight in SA. In WE, a higher maternal egg consumption during the second trimester of pregnancy was significantly inversely associated with neonate birth weight. Overall, maternal consumption of green leafy vegetables, fruit, milk, meat, grain and legume foods were not independently associated with neonate birth weight in WE. There was a significant negative association between serum vitamin B12 concentrations and neonate birth weight, whereas, RBC folate, plasma Hcy and plasma MMA concentrations were not associated with neonate birth weight in the SA sub-population. The absence of significant associations of dietary, supplemental, total and RBC folate and plasma vitamin B12 with neonate birth weight

might be explained by an adequate intake of folate and vitamin B12 by the population and thus a higher nutritional baseline status.

This study adds to the knowledge of vitamin B12 and folate status and birth weight in a large and vulnerable ethnic population in Canada. It also raises some questions that need to be investigated further such as a priori supplement use, folate and vitamin B12 concentrations across the trimesters and the influence of dietary acculturation. These findings complement current research on folate and vitamin B12 concentrations with birth weight in well-nourished populations and offers an insight into these associations in the Canadian context.

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Appendix A

a) Examples of High Source of Folate Foods

Food Item	Serving Size	Folate (µg)*
Edamame/Baby Soybeans (Cooked)	125 ml (½ cup)	106-255
Spinach (Cooked)	125 ml (½ cup)	121-139
Asparagus (Cooked)	4 Spears	128-141
Turnip Greens, Collards (Cooked)	125 ml (½ cup)	68-93
Lettuce	250 ml (1 cup)	65-80
Pasta, White (Enriched, Cooked)	125 ml (½ cup)	88-113
Peas – Chickpeas, Black-Eyed, Pigeon (Cooked)	175 ml (¾ cup)	138-263
Lentils (Cooked)	175 ml (¾ cup)	265
Beans	175 ml (¾ cup)	157-238
Liver	75 g (2½ oz)	122-518
Yeast Extract Spread – Vegemite, Marmite	30 ml (2 tbsp)	360
Papaya	½ fruit	56
Orange Juice	125 ml (½ cup)	25-39
Soy Burger/Vegetarian Meatloaf or Patty (Cooked)	75 g (2½ oz)	59
Soy Nuts	60 ml (¼ cup)	59

* According to the Dietitians of Canada

b) Examples of High Source of vitamin B12 Foods

Food Item	Serving Size	Vitamin B12 (µg)*
3.3%, 2% or 1% Milk	250 ml (1 cup)	1.2-1.4
Skim Milk	250 ml (1 cup)	1.3
Cottage Cheese	250 ml (1 cup)	1.1-1.5
Plain Yogurt (Regular, Low Fat)	175 g (¾ cup)	0.05
Greek Yogurt, Fruit Bottom (Regular, Low Fat)	175 g (¾ cup)	0.5
Soy, Almond, Oat or Rice Beverage (Fortified)	250 ml (1 cup)	1.0
Liver (Cooked)	75 g (2½ oz)	12.6-66.0
Kidney (Cooked)	75 g (2½ oz)	18.7-59.2
Chicken, Turkey or Duck (Cooked)	75 g (2½ oz)	0.2-0.3
Beef (Cooked)	75 g (2½ oz)	1.3-2.7
Pork (Cooked)	75 g (2½ oz)	0.5-0.9
Mackerel (Cooked)	75 g (2½ oz)	13.5-14.3
Tuna (Raw or Cooked)	75 g (2½ oz)	8.2-9.3
Salmon (Cooked)	75 g (2½ oz)	2.3-4.4
Soy Burger	75 g (2½ oz)	1.8
Egg (Cooked)	2 large	1.5-1.6

* According to the Dietitians of Canada

Appendix B

a) The FFQ food items included in the folate and vitamin B12-rich food categories in the START cohort

GLV	Fruit	Milk	Meat	Egg	Grain	Legumes
Dark leafy vegetables	Orange, grapefruit juice	Whole milk	Beef curry	Egg Anda	White bread	Peas or matar curry
Lettuce	Apple, pineapple, other juices	2% milk	Ground beef	Egg	Whole wheat bread 100%	Lentil/dal curry
	Apple, pear	1% milk	Other beef		Whole wheat bread 60%	Sambhar, rasam
	Citrus fruits	Skim milk	Pork curry		Bread rolls white	Chickpeas curry
	Banana	Homo milk tea/coffee	Other pork		Bread rolls whole wheat	Other dried beans curry
	Grapes	2% or 1% tea/coffee	Goat, lamb curry		Roti, chapati	Dhokla, idli
	Berries	Skim milk tea/coffee	Other goat or lamb		Naan, pita bread	Dosa
	Peach, plum, nectarine	Coffee cream tea/coffee	Hot dogs, sausages		Paratha oil	
	Cantaloupe	Half & half tea/coffee	Lunch meat		Paratha pure ghee	
	Watermelon, honeydew	Non-dairy creamer tea/coffee	Liver		Paratha vegetable ghee	
	Mango, papaya	Yogurt drink	Fried chicken		Puri/mathri	
	All other fruit	Cheese, cream cheese	Chicken curry		Bran/granola cereal	

	Canned fruit	Cheese part skim	Roast, tandoori chicken		Whole wheat cereals	
	Dried fruit	Yogurt curd	Fresh fish, machli		Sugar coated cereals	
		Yogurt buttermilk	Fish curry		No sugar cereals	
		Raita	Canned fish		Cooked cereals	
		Yogurt fruit	Deep fried fish		Crackers	
		Panir, ricotta cheese	Seafood, shrimp curry		Muffins	
		Butter/pure ghee on lentils			Rice boiled	
		Margarine on lentils			Fried rice	
		Butter on bread			Pizza no meat	
		Margarine on bread			Pizza with meat	
		Soup, creamed			Macaroni, spaghetti	
					Pasta with tomato sauce	
					Pasta with cream sauce	
					Pasta with cheese/meat	

b) The FFQ food items included in the folate and vitamin B12-rich food categories in the FAMILY cohort

GLV	Fruit	Milk	Meat	Egg	Grain	Legumes
Dark leafy vegetables	Orange, grapefruit juice	Whole milk	Meat stew	Egg boiled	White bread	Peas, lima beans
Lettuce	Apple, grape juices	2% milk	Ground beef	Egg	Whole wheat bread 100%	Dried beans or lentils
	Other juices	1% milk	Roast beef		Whole wheat bread 60%	
	Apple, pear	Skim milk	Steak		Bread rolls white	
	Citrus fruits	Homo milk tea/coffee	Pot roast		Bread rolls whole wheat	
	Banana	2% or 1% tea/coffee	Pork chop		Bran/granola cereal	
	Grapes	Skim milk tea/coffee	Baked ham		Whole wheat cereals	
	Berries	Coffee cream tea/coffee	Veal		Sugar coated cereals	
	Peach, plum, nectarine	Half & half tea/coffee	Lamb		No sugar cereals	
	Cantaloupe	Non-dairy creamer tea/coffee	Bacon		Cooked cereals	
	Watermelon, honeydew	Chocolate milk, hot chocolate	Hot dog, wieners		Crackers	
	Mango, papaya	Milkshake	Sausages		Bran or oat muffins	
	All other fruit	Yogurt drink	Luncheon ham, corned beef		Other muffins, fruit breads	

	Canned fruit	Cottage, ricotta cheese	Other luncheon meat		Rice boiled	
	Dried fruit	Cream cheese	Liver		Fried rice	
		Cheese regular fat	Fried chicken, chicken wings		Pizza no meat	
		Cheese part skim	Chicken or turkey		Pizza with meat	
		Sour cream, whipping cream	Fish steamed		Macaroni, spaghetti	
		Yogurt plain regular fat	Fish fried		Pasta with tomato sauce	
		Yogurt plain low fat	Canned fish		Pasta with cream sauce	
		Yogurt plain fruit regular fat	Seafood		Pasta with cheese/meat	
		Yogurt fruit low fat	Salted/dried meat or fish			
		Soup, creamed	Pickled meat or fish			
		Butter on vegetables				
		Margarine on vegetables				
		Butter on bread				
		Margarine on bread				
		Ice cream				

c) The FFQ food items included in the folate and vitamin B12-rich food categories in the CHILD cohort

GLV	Fruit	Milk	Meat	Egg	Grain	Legumes
Green salad	Apples, applesauce, pears	Milk on cereals	Bacon, breakfast sausage	Whole egg	Cold cereal	Idli and dosa
Cooked greens	Bananas	Butter, margarine, ghee on breads	Low fat hot dog and sausage	Egg whites, egg substitute	Cooked cereal	Bean soups
Raw leafy green vegetables	Peaches, nectarines, plums	Cream soups	Regular fat hot dog and sausage		Homemade pancakes, french toast, waffles	Green peas
	Apricots	Cottage, ricotta chees	Lunch meats		Store brought pancakes, French toast, waffles	Refried beans
	Dried fruit	Low fat cheese	All other lunch meats		Muffins, scones, croissant, biscuits	All other beans
	Oranges, grapefruit, tangerines	All other cheese	Canned tuna		Poptarts	
	Berries	Yogurt, lassi, raita	Beef, pork, ham		Breads and rolls part of sandwich	
	Cantaloupe, orange melon	Butter, margarine, sour cream on vegetables and rice	Game meat		Breads and rolls not part of sandwich	
	Watermelon, red melon	Cheese sauce, cream sauce	Ground meat		Roti, chapatti, tortilla, pita, naan	
	Lychees, rambutan, longan	Frozen dessert	Liver, organ meats		Cornbread and corn muffin	

	Papaya, mango, pineapple, other tropical fruits	Ice cream, milkshakes	Fried chicken		Soft pretzel	
	Any other fruit	Milk all types	Chicken and turkey		Low fat crackers	
	Orange, grapefruit juice	Latte, hot chocolate	Fried fish and shellfish		Regular crackers	
	Other 100% juice	Milk, cream, and creamer tea/coffee	Non-fried shellfish		Spaghetti with tomato or meat sauce	
			White fish		Lasagna, tortellini, ravioli	
			Dark fish		Pasta with oil, cheese or cream sauce	
			Meat stew		Asian-style noodles	
					Mantou	
					Baozi	
					Wontons, dumplings	
					Pizza	
					Ramen noodle soup	
					White rice, noodles and other grains	
					Whole grain, wild or brown rice	

Appendix C

Range of effect size and corresponding sample size for power = 0.80

Effect Size	Power	Sample Size	Calculation
Large (0.35)	0.8	26	Reference
Medium (0.15)	0.8	55	Reference
0.035	0.8	227	Biochemical – Folate Analysis
0.035	0.8	228	Biochemical – MMA Analysis
0.034	0.8	230	Biochemical – Vitamin B12 & Hcy Analysis
Small (0.02)	0.8	395	Reference
0.0084	0.8	932	Obj 1 & 2 - SA
0.0042	0.8	1888	Obj 1 & 2 - WE

Abbreviations – SA: South Asian; WE: White European; MMA: methylmalonic acid; Hcy: homocysteine; Obj: objective
Reference effect sizes are labelled in red

Appendix D

a) Top 10 folate-rich food items in the South Asian START cohort, in descending order

FFQ Food Item	% (n=961)	Folate (mcg)	Serving Size
Liver	9.9 (95)	465.97	Medium
Bhajia, Sev, Gathia	27.3 (262)	149.25	¼ cup or 60 ml
Lentil/Dal Curry	96.9 (931)	105.61	Medium (½ cup)
Dal Ki Pakori, Vada	40.1 (385)	105.51	½ cup or 125 ml
Vegetable Pakoras	60.5 (581)	84.83	Small (½ cup)
Paratha made with Oil	55.9 (537)	77.19	1, 6” diameter
Other Dried Beans Curry	80.9 (777)	72.55	Medium ((½ cup)
Chickpeas Curry	91.0 (874)	72.23	Medium ((½ cup)
Dark Leafy Vegetables	92.2 (886)	70.01	½ cup or 125 ml
Sambhar, Rasam	34.1 (328)	55.68	Medium (½ cup)
Based on the START FFQ			

b) Top 10 vitamin B12-rich food items in the South Asian START cohort, in descending order

FFQ Food Item	% (n=961)	Vitamin B12 (mcg)	Serving Size
Liver	9.9 (95)	77.1	Medium
Fresh Fish, Machli (Steamed or Baked)	30.2 (290)	3.89	Medium
Other Beef (Roast, Steak)	18.6 (179)	3.18	Medium
Fish Curry	18.4 (177)	1.76	Medium (½ cup)
Ground Beef (Mince, Hamburger, Keema, Kabob, Dry Kofta)	29.3 (282)	1.54	6" Kabob or 2 Kofta or 3" Patty
Other Goat, Lamb (Mince, Roast, Steak, Chop, Keema, Kabob, Dry Kofta, Raan)	23.5 (226)	1.46	6" Kabob or 2 Kofta or 3" Patty
Deep Fried Fish	27.9 (268)	1.09	Medium or 5 Fish Sticks
Skim Milk	1.80 (17)	0.98	1 cup or 250 ml
Yogurt, Buttermilk, Plain, Low Fat	21.9 (210)	0.98	¾ cup or 175 ml
Yogurt, Curd, Plain, Regular Fat	83.0 (798)	0.96	¾ cup or 175 ml
Based on the START FFQ			

Appendix E

a) Top 10 folate-rich food items in the White European FAMILY cohort, in descending order

FFQ Food Item	% (n=961)	Folate (mcg)	Serving Size
Liver	11.7 (74)	399.58	Medium
Dried Beans or Lentils	67.1 (424)	104.48	½ cup or 125 ml
Asparagus	75.8 (479)	67.32	4 Stalks
Avocado	41.6 (263)	66.62	½ Medium
Chili Con Carne	59.2 (374)	51.65	1 cup or 250 ml
Lettuce	98.1 (620)	49.25	1 cup or 250 ml
Corn	97.3 (615)	46.21	1 Cob or ½ cup
Brussel Sprouts	46.4 (293)	45.45	½ cup or 125 ml
Bean Sprouts, Alfalfa Sprouts	51.9 (328)	43.38	½ cup or 125 ml
Dark Leafy Vegetables	74.5 (471)	40.87	½ cup or 125 ml
Based on the FAMILY FFQ			

b) Top 10 vitamin B12-rich food items in the White European FAMILY cohort, in descending order

FFQ Food Item	% (n=961)	Vitamin B12 (mcg)	Serving Size
Liver	11.7 (74)	71.92	Medium
Salted/Dried Meat or Fish	11.7 (74)	8.77	Medium
Steak	87.8 (555)	3.42	Medium
Pot Roast	62.5 (395)	3.41	Medium
Lamb	26.0 (164)	3.32	Medium
Fish (Steamed, Baked)	71.4 (451)	3.31	Medium
Roast Beef	89.4 (565)	3.17	Medium
Seafood (Crab, Lobster, Shrimp)	64.4 (407)	3.00	Medium
Veal	29.1 (184)	1.99	Medium
Pickled Meat or Fish	3.3 (21)	1.85	Medium
Based on the FAMILY FFQ			