Secondary Data Analysis of Nutritional Status of Canadians using the Canadian Community Health Survey and the Canadian Health Measures Survey

by

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ABSTRACT

Data from the Canadian Community Health Survey, 2.2 (N=32,776) and 3 cycles of the Canadian Health Measures Survey (N=15,754) were used for this thesis. The general objectives of this thesis were to: 1) explore the impact of soy and pulse food consumption as a healthy food source in the Canadian diet, 2) examine the post-fortification folate intake and red blood cell folate status of the average Canadian and 3) explore new methodological developments in estimating nutrient intakes based on food frequency data and correlations of nutrient intakes with blood nutrient levels. Results indicated that soy consumption was associated with an improvement in dietary quality of Canadians; however, neither pulse *or* soy consumption did not appear to relate to the nutrient profile of Manitobans 2-18 years but instead shed light on their overall poor eating habits. Secondary analysis highlighted potential groups who may be at risk for adverse health effects linked with their habitual folic acid intake. In particular 18% of respondents who reported consuming vitamin/mineral supplements containing folic acid were above the Upper Intake Level, and 25% had elevated blood folate levels, potentially increasing their risk for certain health problems associated with overconsumption. Using regression model parameters and "fitted" portion sizes were found to be reasonable approaches for estimating nutrient intakes from food frequency questionnaires, if 24 hr recall data is available. This thesis allows insight into nutritional short-comings of Canadians, demonstrates the potential amelioration of certain nutrient intakes such as protein and fibre by the inclusion of key foods in the diet (soy and pulse) and sheds light on potential adverse effects associated with fortification policy as well as aids in the development of new approaches in utilizing food frequency data to estimate nutrient intakes. Outcomes from this study may allow for the possibility of future

recommendations to be made in regards to nutrient intake, supplement use and public health and nutrition policy.

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PUBLISHED MATERIALS

A component of Chapter 2 has been previously published:

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Chapter 6 is in publishable format and will be submitted to the *British Journal of Nutrition*

Additionally, co-authors permission requests can be viewed in the Appendix of this dissertation.

LIST OF ABBREVIATIONS

AI	Adequate Intake
AICR	American Institute for Cancer Research
AMPM	Automated Multiple Pass Method
ATC	Anatomic Therapeutic Chemical
BMI	Body Mass Index
CCHS	Canadian Community Health Survey
CCHS 2.2	Canadian Community Health Survey, Cycle 2.2 (2004)
CFG	Canada's Food Guide
CHD	Coronary Heart Disease
CHMS	Canadian Health Measures Survey
CI	95% Confidence Interval
CIHI	Canadian Institute for Health Information
CNF	Canadian Nutrient File
CVD	Cardiovascular Disease
d	Day
DFE	Dietary Folate Equivalent(s)
DNA	Deoxyribonucleic acid
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
FAO	Food and Agricultural Organization
g	Gram
GI	Glycemic Index
HDL	High-density lipoprotein
HIV	Human Immunodeficiency Virus
hr	Hour
kcal	Kilocalorie
kg	Kilogram
LDL	Low-density lipoprotein
m	Metre (s)
µg	Microgram(s)
mg	Milligram(s)
min	Minute
mL	Millilitre
mm	Millimetre
MUFA	Monounsaturated Fatty Acid
NCNS	Nutrition Canada National Survey
NHANES	National Health and Nutrition Examination Survey
NHEFS	National Health and Nutrition Examination Survey Epidemiologic Follow-up Study
NS	Not Statistically Significant
OR	Odds Ratio (from a logistic regression analysis)
PA	Physical Activity Coefficient
PSU	Primary Sampling Unit

PUFA	Polyunsaturated Fatty Acid
RBC	Red blood cell
RDA	Recommended Dietary Allowance
SPSS®	Statistical Analysis Software
SD	Standard Deviation
SDF	Soluble Dietary Fibre
TDF	Total Dietary Fibre
UL	Tolerable Upper Intake Levels
US	United States
USDA	United States Department of Agriculture
WHO	World Health Organization
wt	Weight

CHAPTER 1.0: Introduction

1.1 Introduction to Secondary Analysis

Secondary analysis has been defined by Boslaugh in the broadest sense as "analysis of data collected by somebody else" (Boslaugh 2007). Other definitions put forth include the analysis of data that seeks to answer a research question other than the one for which the data were initially collected, or in which different teams of researchers are involved in the planning and collection of the data than those analyzing it (Church 2002, Koziol, Arthur 2011). There are many benefits of using a pre-existing data set for research purposes; namely they are cost and time effective, easily accessible and unobtrusive and provide access to potentially thousands of data records without comprising the actual collection time. However, caution must be exercised when using previously compiled data, namely making sure that the data is relevant to the research question, that the information regarding design and collection is available and sufficient and that the data source is dependable (Koziol, Arthur 2011).

Secondary data may come from many sources, such as school records, previously published reports and large government funded datasets, the latter of which were used for this thesis project. Secondary analysis of data has been used globally to analyze various aspects of population health (Matsushita et al. 2004, Deschênes, Burns & Schmitz 2015, Wang et al. 2016), including dietary intakes and nutrition patterns and their health related outcomes of various populations (Mitchell et al. 2009, Mudryj et al. 2012, Brisbois et al. 2014, Barr, DiFrancesco & Fulgoni 2016). Health Canada and Statistics Canada have conducted a few nationally representative surveys that sought to add to the existing body of knowledge about the determinants of health, with a nutrition focus. The Nutrition Canada National Survey (1970-

1972) was the first comprehensive cross-sectional appraisal of the dietary status of Canadians. This survey was planned with the objective of collecting information about the nutritional well-being of Canadians by not only collecting demographic data, but also comprising data on food handling, food preparation and food frequency as well as anthropometric and laboratory tests (Health Canada 2005). Over 30 years later, the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2) was released, which was a nutrition focused survey which consisted of both a nutritional and general health component to estimate the distribution of usual dietary intake in terms of foods, food groups, dietary supplements, nutrients and eating patterns among a representative sample of Canadians at national and provincial levels using a 24-hour dietary recall (Health Canada 2012a). The Aboriginal Children's Survey (2006) measured social and living conditions among Canada's Aboriginal youth, and included data on vitamin and supplement intake as well as nutrition and developmental indicators (Statistics Canada 2007a). Additionally, the Canadian Health Measures Survey (CHMS) measured variables including height, weight and waist circumference, kidney and cardiovascular health and took respondents blood samples as well as a Food Frequency Questionnaire (FFQ) (Tremblay, Wolfson & Connor Gorber 2007, Bolger 2008). This thesis utilized both the CCHS 2.2 and the CHMS, and these surveys will be discussed in greater detail in the Methodology section of this dissertation.

1.2 General Objectives and Hypotheses

This thesis is comprised of a series of studies utilizing secondary analysis of the CCHS 2.2 and three cycles of the CHMS to: 1) explore the impact of a target food (pulse and soy) as a healthy food source in the Canadian diet, 2) examine the post-fortification folate intake and folate biomarker status of the average Canadian and 3) to explore new methods in estimating nutrient

intakes based on food frequency questionnaire data and parameters generated from dietary recall data by assessing the correlations of estimated dietary intake with biomarker status.

Study #1 (Chapters 3 and 4): The dietary implications of pulses and soy in the Canadian diet

Objective:

1. Assess the dietary patterns and intakes of Canadians who consume soy products in comparison with non-consumers, as well as to describe the typical soy consumer by socio-economic status as well as other demographic variables (including, but not limited to age, gender, household income and education, etc) in order to obtain a better understanding of the patterns of soy intake in Canada
2. Use pulse or soy consumption as an indicator to evaluate the healthy eating profile of young Manitobans

Hypothesis:

1. Canadians who consume soy or pulse products in their diet may have healthier eating profiles than their non-consuming counterparts

Study #2 (Chapter 5): Folate consumption in a post-fortification era: Folate intake and biomarker folate status of the Canadian population

Objective:

To use the nationally representative Canadian Community Health Survey, Cycle 2.2 and the Canadian Health Measures Survey to:

1. Determine the folate consumption levels in the Canadian population by both dietary sources as well as supplements

2. Measure the prevalence of folate inadequacy and toxicity among Canadians by:

- Dietary intakes
- Red blood cell (RBC) folate concentrations

Hypotheses:

1. There may exist potential subgroups in the Canadian population who are exceeding the Tolerable Upper Intake Levels of folic acid and who have elevated RBC folate

2. Supplement users may be at particular risk of folic acid toxicity and elevated RBC folate

Study #3 (Chapter 6): How to use population-based food frequency data to estimate nutrient intakes: methodology exploration and recommendations

Objective:

1. To explore new methods in estimating nutrient intakes based on food frequency questionnaire data and parameters generated from the 24 hour dietary recall data as well as portion sizes recommended by Canada's Food Guide to Healthy Eating.
2. To assess the correlations of estimated nutrient intakes (folate and vitamin B_{12}) and measured red blood cell folate and serum B_{12} status.

Hypotheses:

1. Portion sizes recommended by Canada's Food Guide to Healthy Eating or self-reported portions from the 24 hr dietary recall nutrition survey can be used in the population-based surveys with only FFQ data to estimate the nutrient intakes.
2. Using the parameters generated from age, sex, and culture group adjusted regression models based on 24 hr dietary recall data into the population-based survey with only FFQ

data, we will yield the best estimates of folate intake and achieve the best correlations between intake and status.

1.3 Organization of Thesis

This thesis is structured as a paper-based manuscript and includes the following chapters:

Chapter 1.0: Introduction

This chapter contains a general introduction to secondary analysis and describes the organization of the thesis as well as objectives and hypothesis of each Chapter.

Chapter 2.0: Review of the Literature, Hypothesis and Objectives

This section contains background information on folate, soy and pulse consumption and the analysis of dietary intake data, exploring the methodologies involved in linking two population-based nutrition surveys, and identifies key terms used in this thesis. This literature review chapter is comprised of three parts. Part 2.1 consists of a literature review on the health benefits of soy consumption, Part 2.2 consists of a review paper, titled "*Nutritional and health benefits of pulses"* which has been published (Mudryj, Yu & Aukema 2014). Part 2.3 contains information regarding folate and folic acid fortification. Section 2.4 includes a comprehensive explanation of the population surveys used for this research project: the Canadian Community Health Survey, 2.2 (CCHS 2.2) as well as the Canadian Health Measures Survey (CHMS). This section also contains detailed information about the dietary intake analysis components of the CCHS 2.2 (24-hour dietary recall) and the CHMS (Food Frequency Questionnaire) and a brief overview of the issues associated with these two methods. Finally, section 2.5 contains the objectives and hypotheses associated with each of these studies.

Chapter 3.0: Manuscript #1:

This paper, titled *"Intake patterns and dietary associations of soya protein consumption in adults and children in the Canadian Community Health Survey, Cycle 2.2"* presents the secondary analysis results regarding the prevalence and association of soy food consumption and nutrient intakes and dietary patterns of Canadians and addresses the objectives from Study #1. This paper has been published (Mudryj, Aukema & Yu 2015).

Chapter 4.0: Manuscript #2:

This paper is titled *"Nutrient Intakes of Manitoba Children and Youth: A population-based analysis by pulse and soy consumption status"* and presents results on pulse or soy consumption as an indicator to explore and evaluate the eating profile of Manitoban children and youth. This paper addresses the goals outlined in Study #1. This paper has been published by the Canadian Journal of Dietetic Practice and Research (Mudryj, Aukema, Fieldhouse & Yu, 2016).

Chapter 5.0: Manuscript #3:

This paper is entitled "*Folate intakes from diet and supplements may place certain Canadians at risk for folic acid toxicity*" and analyzes the folate intake and status of Canadians post-fortification. This paper addresses the objectives from Study #2. This manuscript has been published by the British Journal of Nutrition (Mudryj, de Groh, Aukema & Yu, 2016).

Chapter 6.0: Manuscript #4:

This paper has been written in publishable format and is titled "*How to use population-based food frequency data to estimate nutrient intakes: methodology exploration and*

recommendations". This paper seeks to clarify why correlations between dietary intake and nutrient status of vitamins such as folate have been weak by introducing two novel approaches to estimate folate and vitamin B_{12} intake when detailed 24 hr dietary recall data is unavailable. This paper addresses the objectives from Study #3.

Chapter 7.0: General Discussion, Conclusions and Future Directions:

This final chapter of the thesis provides a general summary of the overall findings, identifies the limitations associated with these studies and presents avenues for future directions and research.

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CHAPTER 2.0: Literature Review

2.1: Overview of the Nutritional Benefits of Soy Consumption

2.1.1 Introduction to Soy

According to the 2011 Census of Agriculture for Canada, the number of Canadian farms reporting production of soybeans was estimated at 27,215, with a 98.71% increase in production between 2004 and 2014 (Soy Canada 2016). As stated by the Soyfoods Association of North America, the American soyfood industry has increased in value by $1 billion dollars in under 20 years, reflecting the surge in popularity of soy based beverages and meat alternatives as well as foods such as edamame and tofu (Soyfoods Association of North America 2014). The United Soybean Board and their annual Report *Consumer Attitudes about Nutrition, Health and Soyfoods*, state that over 75% of Americans perceive soyfoods to be "healthy", with a 14% increase in soy consumption from 2011-2016 (64% and 78%, respectively) (United Soybean Board 2013). Indeed, soybeans are rich in nutrients such as magnesium, iron and potassium (Table 2.1) and contain compounds such as isoflavones and lecithins which have been associated with chronic disease reduction and improvement in bone health, particularly in women ((Lanou 2011, Montgomery 2003, Messina 2010, Webb 2011) In addition, soy protein is a complete protein, containing adequate quantities of the essential amino acids (Montgomery 2003).

Table 2.1. Nutritional value of 100 grams of selected soyfoods*

	Soybeans, mature, cooked, boiled	Tofu, salted and fermented (fuyu)	Soymilk, plain	Veggie burger patty, soyburger
Calories (kcal)	172	116	91.5	177
Protein (g)	18.2	8.9	2.9	15.7
Carbohydrate (g)	8.4	4.4	3.3	14.3
Fat (g)	9	0.4	1.6	6.3
Saturated Fat (g)	1.3	1.2	0.2	1.4
Cholesterol (mg)	0	0	0	0
Calcium (mg)	10	46	123	136
Dietary Fibre (g)	6	7.0	0.5	4.9
Iron (mg)	5.1	2.0	0.4	2.4
Magnesium (mg)	86	53	16	56
Phosphorus (mg)	245	73	32	206
Potassium (mg)	515	75	123	333
Sodium (mg)	1	2	49	569
Zinc (mg)	1.15	1.6	0.25	1.3
Dietary Folate Equivalent (DFE) (μg)	54	29	7	124

* Adapted from the Canadian Nutrient File (CNF), 2015

2.1.2 Health Benefits associated with Soy Consumption

Cardiovascular Disease

In 1999, the US Food and Drug Administration (FDA) approved a food labelling health claim for soy foods, based on their conclusion that soy protein coupled with a low fat diet may reduce the risk of cardiovascular disease (CVD), stemming from research which observed that 25 grams of soy protein per day in addition to a low fat diet may decrease one's risk of developing CVD (United States Food and Drug Administration 1999). Asian populations (where soy foods are a dietary staple) have reduced risk for CVD compared to their Western counterparts (Erdman 2000, Sacks et al. 2006, Zhang et al. 2003). Lipid lowering effects have been associated with consumption of fermented soy products supplemented with okara (a by-product from soybeans) in healthy Brazilian men (Bedani et al. 2015). A significant reduction (P=0.02) in serum

triglyceride levels was found after the consumption of soy milk among subjects with type 2 diabetes with nephropathy. Additionally, soy milk consumption was associated with better blood pressure control among type 2 diabetics, with consumption being linked to a significant reduction in systolic blood pressure when compared with cow's milk (-4.50% vs 5.89%) (Miraghajani et al. 2013). Small improvements in systolic and mean arterial pressures associated with soy protein consumption were also observed in animal models (Ibrahim et al. 2015).

Diabetes and Kidney Disease

Recent findings indicate that soy consumption, in particular, soy protein, may improve clinical indices of type 2 diabetes. A recent meta-analysis of clinical trials show that fasting plasma glucose ($P = 0.015$), fasting serum insulin ($P=0.002$), diastolic blood pressure ($P=0.033$) and low-density lipoprotein cholesterol ($P=0.000$) were all significantly reduced with soy protein supplementation when compared with a control group (Zhang, Zhang & Chi 2016). Additionally, intake of isoflavones was associated with a moderately lower risk of type 2 diabetes among US men and women who consumed low to moderate amounts of soy foods, based on results from Food Frequency Questionnaires (FFQ) ($P = 0.009$) (Ding et al. 2016). Soy consumption has also been associated with improved renal health in people with diabetes (Azadbakht et al. 2003) as well as animal models (Choi et al. 2010), as well as delaying polycystic kidney disease progression in soy protein fed animals (when compared with casein) (Aukema, Housini 2001)

Cancer

Epidemiologic studies have linked high consumption of soy foods with a decreased risk of cancer. A reduction in the risk of breast cancer in premenopausal Chinese women in Singapore who were high consumers of soy was first reported in 1991 (Lee et al. 1991). More recently, a study which examined breast cancer risk in Chinese women found that women with high soy isoflavone intakes had a decreased risk of breast cancer mortality, while women with high soy protein intakes had a lower risk of breast cancer compared to those with lower consumption (Kang et al. 2012). Similarly, intake of soy protein, isoflavones, soybeans and tofu as well as soy beverages and meat substitutes were found to be significantly lower among Caucasian and African American women in the US with breast cancer than those without cancer ($P = 0.03$) (Jaceldo-Siegl et al. 2015) and high intake of soy foods during adolescence was associated with a reduced risk of premenopausal breast cancer in Chinese women ($P < 0.001$) (Lee et al. 2009). It has been hypothesized that it is the isoflavones, a class of phytoestrogens containing anti carcinogenic properties, in particular daidzein and genistein, in soy that may produce cancer lowering effects (Khan et al. 2012). However, though research involving soy and breast cancer has been extensive, overall results have not been conclusive. In addition, the mechanism between soy intake and breast cancer risk reduction still remains unclear (Trock, Hilakivi-Clarke & Clarke 2006).

A meta-analysis by Yan and Spitznagel suggested that consumption of soy foods was associated with a reduction in prostate cancer risk in men based on available epidemiological studies on soy and isoflavone consumption and their association with prostate cancer (Yan, Spitznagel 2009). Again, plasma levels of isoflavone genistein (Kurahashi et al. 2008) and serum daidzein (Ozasa

et al. 2004) were found to be associated with a reduction in prostate cancer risk in Japanese men. As well, plasma equol, a metabolite of daidzein was significantly associated with a reduced risk of total prostate cancer (P=0.04) (Kurahashi et al. 2008). However, results from Western populations showed the opposite to be true: 2 case control studies based in the United Kingdom found no protective association between isoflavone intake and prostate cancer risk (Heald et al. 2007, Ward et al. 2008).

Bone Health

The phytoestrogens in soy and soy protein have been positively associated with bone-protective effects in women and with bone and calcium balance in postmenopausal women, respectively (Montgomery 2003, Arjmandi et al. 2003). Soy proteins may reduce calcium excretion due to their lower sulphur amino acid content (Messina, Messina 2000) In addition, soy isoflavones have been found to significantly increase bone mineral density in postmenopausal women (Wei et al. 2012), possibly due to cooperation with vitamin D in stimulating bone formation and reducing resorption (Weaver, Cheong 2005, Park, Weaver 2012).

Soy Consumption and Nutrient Intake

Studies have shown that replacement of meat and dairy products with soy products may improve diet quality (Tucker, Qiao & Maras 2010). For example, replacing a 3-ounce patty made from ground beef with a soy patty leads to a reduction of 12 grams of fat and 5 grams of saturated fat. Similarly, replacing a cup of whole milk with a cup of soymilk can reduce fat and saturated fat intake by 4 and 4.5 grams, respectively. The partial replacement of traditional protein ingredients with tofu led to an enhanced nutritional quality recipes used at American preschools. When tofu replaced cheese the amount of fat, cholesterol, sodium and energy was reduced; when it replaced

beef the amount of fat and cholesterol decreased and when it replaced egg or chicken the amount of cholesterol decreased. When cheese or egg was replaced by tofu the children ate more of the new dish but when beef or chicken was replaced they ate more of the original dish (Ashraf, Schoeppel & Nelson 1990). Soy consumption in the American diet was examined by Tucker and colleagues using NHANES data from 2003-2004 in which simulation analysis was performed where MyPyramid servings of meat were replaced with tofu and similar servings of milk were replaced with soy beverage. A simulation analysis showed that replacing meat with tofu would increase nutrient intake of folate, iron, calcium and magnesium by > 10% and lower intakes of saturated fat, cholesterol, protein, vitamins B_6 and B_{12}. If both meat and dairy were replaced with soy foods, intakes of fibre, folate, vitamin K, iron, calcium and magnesium also would be expected to increase, and saturated fat and cholesterol would decrease; however, protein and riboflavin intake also would be lower in men and women (Ashraf, Schoeppel & Nelson 1990, Tucker, Qiao & Maras 2010).

Recently published data explored the relationship between soy consumption in Iranian populations (Sadeghian et al. 2015). As aforementioned, soy protein (the edible portion of the soybean) contains adequate quantities of the essential amino acids (Montgomery 2003a). A cross-sectional study performed on subjects in Iran also observed increased intakes of whole grains and vegetables among those who consumed soy, which could define increased intakes of zinc, iron, and vitamin C in their diets (Sadeghian et al. 2015).

2.2: Nutritional and Health Benefits of Pulses

2.2.1 Abstract

Pulses (beans, peas, and lentils) have been consumed for at least 10,000 years and are among the most extensively used foods in the world. A wide variety of pulses can be grown globally, making them important both economically as well as nutritionally. Pulses provide protein and fibre, as well as a significant source of vitamins and minerals, such as iron, zinc, folate, and magnesium, and consuming half a cup of beans or peas per day can enhance diet quality by increasing intakes of these nutrients. In addition, the phytochemicals, saponins, and tannins found in pulses possess antioxidant and anti-carcinogenic effects, indicating that pulses may have significant anti-cancer effects. Pulse consumption also improves serum lipid profiles and positively affects several other cardiovascular disease risk factors, such as blood pressure, platelet activity, and inflammation. Pulses are high in fibre and have a low glycemic index, making them particularly beneficial to people with diabetes by assisting in maintaining healthy blood glucose and insulin levels. Emerging research examining the effect of pulse components on HIV and consumption patterns with aging populations indicates that pulses may have further effects on health. In conclusion, including pulses in the diet is a healthy way to meet dietary recommendations and is associated with reduced risk of several chronic diseases. Long-term randomized controlled trials are needed to demonstrate the direct effects of pulses on these diseases.

2.2.2 Introduction

Pulses are the edible seeds of members of the Leguminosae family, and are defined by the Food and Agricultural Organization (FAO) of the United Nations as "Leguminosae crops harvested exclusively for their grain, including dry beans, peas and lentils". This definition excludes legumes used for oil extraction, such as soybeans and peanuts or those harvested green for food, such as green beans and green peas. The FAO recognizes 11 primary pulses as follows: dry beans (including kidney, pinto, navy, azuki, mung, black gram, scarlet runner, ricebean, moth and tepary beans), dry broad beans (including the horse, broad and field bean), dry peas, chickpeas, black-eyed peas, pigeon peas, lentils, bambara groundnut, vetch, lupins and other "minor" pulses (jack, winged, velvet and yam beans) (FAO 1994; FAO 2010; Michaels 2004). For the purpose of this review, the FAO definition is used and the term "pulses" will refer to the dry, edible variety of beans, peas and lentils, and will not include soybeans, fresh beans or fresh peas.

It is estimated that pulses have been consumed for at least 10,000 years and are among the most extensively used foods in the world (Leterme and Munõz 2002). The Leguminosae family is second only in economic importance to the Poaceae (grass/cereal) family (International Legume Database and Information Service 2006). Pulses are primarily used for food and animal feed around the world, but are also emerging as an ethanol alternative fuel (Tigunova et al. 2013). Pulse crops such as peas and lentils are nitrogen fixing, requiring little or no nitrogen fertilizer (Burgess et al. 2012) and lower the carbon footprint of other crops grown in rotation by limiting the amount of greenhouses gases released in comparison to nitrogen fertilized systems (Gan et al. 2011; Harrison 2011). Because of the wide uses and recommendations of pulses as a nutritional

food source, as well as being an environmentally friendly crop, the objective of this review was to describe (1) the nutritional composition of pulses, (2) current dietary recommendations for pulse consumption, (3) the effects of their consumption on nutrient intakes, (4) the effect of pulses on chronic disease, and (5) emerging areas of pulse research in human health.

2.2.3 Nutritional Composition

Pulses are relatively low in energy density, providing 1.3 kcal/g (based on a cooked serving of pulses). They are characterized by a high carbohydrate content (~50-65%) yet are slowly digested, placing them lower on the glycemic index (GI) scale than other carbohydrate rich foods such as rice, white bread or potatoes (McCrory et al. 2010; Ofuya and Akhidue 2005). However the GI of pulses depends on type as well as cooking or processing, as the mean GI of canned beans is significantly higher than dry, cooked beans (Wolever et al. 1987). Examples of differences in GI of selected pulses are shown in Table 1. Pulses are also high in fibre, providing approximately 7 g of fibre per ½ cup serving, although this amount varies depending on type (Table 2). They contain mostly insoluble fibre as well as soluble fibre (Tosh and Yada 2010). Another healthy component of pulses is that they are a source of mono- and polyunsaturated fat (Lovejoy 2010) and contain plant sterols (Iqbal et al. 2006; Patterson et al. 2009). Furthermore, pulses also provide protein to the diet (Table 2). They are rich sources of the amino acid lysine, which is often low in cereal grains. Conversely, pulses are low in the essential amino acids methionine and tryptophan which are found in grain-based products. Thus when eaten with a cereal, pulses and grain-based products balance each other to provide a higher quality protein (Boy et al. 2010; Iqpal et al. 2006).

Pulses also are an excellent source of micronutrients (Winham et al. 2008). They are a good source of selenium, and are very high in thiamin, niacin, folate, riboflavin, and pyridoxine (CFIA 2011; USDA 2012). Pulses contain vitamin E and A, and while dried pulses do not contain vitamin C, their sprouted forms do (Raatz 2010). Pulses are a rich source of iron and zinc, and although iron content is one nutrient that can vary greatly depending on the variety (e.g. white beans contain almost twice as much iron as black beans), a ½ cup serving of beans provides nearly 10% of one's daily recommendation (Patterson et al. 2009; Winham et al. 2008). However, most of the iron contained in pulses is largely bound to phytates which reduces absorption (Sandberg 2002) and may contribute to iron deficiency in countries where pulses are a staple food (Petry et al. 2010). Steps such as soaking, germination and fermentation have been shown to significantly reduce the phytate content (Luo et al. 2013; Ologhobo and Fetuga 1984).

Table 2.2.1 Glycemic Index of Selected Pulses*[a]

Food	Glycemic Index (glucose = 100)
Black Beans	30
Chickpeas, average	10
Chickpeas, canned	38
Lentils, average	29
Navy Beans, average	31
Kidney Beans, average	29

* Adapted from Atkinson et al. 2008

[a] Per 150 grams except for white bread, where 2 slices (70 g) is used

Table 2.2.2 Nutritional value of 100 grams of selected pulses*

	Black beans, boiled	Lentils, boiled	Chickpeas, boiled	Chickpeas, canned, solids and liquid
Calories (kcal)	132	116	164	119
Protein (g)	8.7	9.0	8.7	5.0
Carbohydrate (g)	23.7	20.1	27.4	22.6
Fat (g)	<1	0.4	2.6	1.1
Saturated Fat (g)	0.1	0.05	0.3	0.1
Cholesterol (mg)	0	0	0	0
Calcium (mg)	27	19	49	32
Total Fibre (g)	7.0	4.2	4.6	4.6
Soluble fibre (g)	2.4**	1.4**	0.0**	0.0**
Iron (mg)	2.1	3.3	2.9	1.4
Magnesium (mg)	70	36	48	29
Phosphorus (mg)	140	180	366	90
Potassium (mg)	355	369	875	172
Sodium (mg)	1	2	7	299
Zinc (mg)	1.12	1.3	1.5	1.1
Dietary Folate Equivalent (DFE) (μg)	149	181	172	67
Copper (mg)	0.21	0.3	0.4	0.2
Selenium(μg)	1.2	2.8	3.7	2.8

* Adapted from the Canadian Nutrient File (CNF), 2010, except as indicated with ** Anderson 1990

2.2.4 Current Dietary Recommendations for Pulse Consumption

Pulses are recommended as part of a healthy diet by Canadian and US government agencies (Health Canada 2010; United States Department of Health and Human Services 2010). Both Canada's Food Guide (CFG) and the United States Department of Agriculture (USDA) MyPlate nutrition guides group pulses in the meat and alternative group (Health Canada 2007; USDA 2011), of which CFG can be viewed in Appendix B of this dissertation. Their high fibre content also allows them to be grouped within the vegetable group in the MyPlate guide (USDA 2011). The CFG recommends the consumption of pulses as good choices and the Dietary Guidelines for Americans suggest shifting food intake patterns to include cooked dry beans and peas. One of the Dietary Approaches to Stop Hypertension (DASH) diet goals is to increase pulse consumption to four to five times per week (Heart and Stroke Foundation 2013). Additionally, the USDA

recommends that Americans consume between 2.5 to 3.5 cups of pulses per week (United States Department of Health and Human Services 2010), although consumption in the Western world remains quite low, with only 7.9 to 13.1% of North Americans consuming pulses on any given day (Eihusen and Albrecht 2007; Mitchell et al. 2009; Mudryj et al. 2012; Schneider 2002).

Pulses are also a key component in a number of healthy diets, such as the Mediterranean diet, which is associated with reduced risks of developing heart disease, hypertension, type 2 diabetes, cancer, Parkinson's disease and Alzheimer's disease (Alcalay et al. 2012; Esposito et al. 2010; Fung et al. 2009; Scarmea et al. 2009; Willet et al. 1995). The Dietary Approaches to Stop Hypertension (DASH) diet for those with high blood pressure (Winham et al. 2008), as well as the Gluten-Free Diet (GFD) for people who suffer from Celiac disease (Kupper 2005) also include pulses as significant components of their diets. When combined with a complementary protein source, pulses are also an optimal food source for vegetarians who wish to find an alternative to meat (Winham et al. 2008).

2.2.5 Effect of Pulse Consumption on Nutrient Intake

Canadian adults who reported pulse consumption had enhanced micronutrient intake, resulting in fewer individuals who were below the Estimated Average Requirement (EAR) for thiamin, vitamin B_6, folate, iron, magnesium, phosphorus and zinc, compared to non-consumers (Mudryj et al. 2012). Similarly, US adults who consumed approximately ½ c beans or peas per day had higher intakes of fibre, protein, folate, zinc, iron, and magnesium as well as lower intakes of saturated fat and total fat than non-consumers (Mitchell et al. 2009). Preliminary findings have also shown that children who consumed beans had significantly greater intakes of fibre, magnesium, and potassium than those who did not eat them (Fulgoni et al. 2006). These values

are consistent with the nutrient profile of pulses. This evidence supports dietary advice that pulses be included in healthful diets. However, the majority of studies that examined the effect of pulse consumption on nutrient intake have relied on one to two days of intake.

Pulse consumers also reported increased intakes of sodium compared to non-consumers, likely due to the high sodium content of canned beans. The high sodium "brine" that accompanies most canned pulses likely contributes to this as well as the most frequently consumed foods that contain pulses, such as chili or Mexican dishes (Duyff et al. 2011; Mitchell et al. 2009; Mudryj et al. 2012). As well, foods such as hummus, which vary greatly in sodium content depending on commercial type, may influence sodium intake due to the addition of sodium chloride used in preparation (Al-Kanhal 1998). Cooked dry beans are more energy-dense, providing more protein, fibre, iron, potassium and magnesium and less sodium per gram than their canned alternatives ($P < 0.05$). Additionally, canned beans that are drained contain significantly more energy than un-drained canned beans, as well as more total carbohydrate, protein, fibre, iron, potassium, folate, magnesium, zinc and copper (Zanovec et al. 2011). Draining and rinsing of canned pulses is an effective means of reducing sodium content of canned beans: draining beans removes 36% of the sodium (from 503 mg/serving to 321 mg/serving), while both draining and rinsing removes, on average, 41% of the sodium (503 mg/serving to 295mg/serving, respectively) (Duyff et al. 2011; Zanovec et al. 2011). Future research directed at the long term benefits of pulse consumption on diet quality and nutrient intake would be critical to further understanding the influence pulses have on overall diet, as would studies observing the effect of low-sodium pulse products. As well, studies further examining the sources of increased sodium

in pulse consumers are required so that dietary advice to consume pulses can include ways to mitigate any negative effects of increased sodium intake.

2.2.6 Effects of Pulses on Chronic Disease

A number of nutrient and non-nutrient components of pulses have been connected to reduction of cancer risk (Dahl et al. 2012). Additionally, epidemiological evidence as well as animal studies support the protective link between pulse consumption and diseases such as cardiovascular disease (CVD) and diabetes (Iqbal et al. 2006; Winham et al. 2008).

2.2.7 Cancer

Currently the US Food and Drug Administration, Canadian Cancer Society and the World Cancer Research Fund recommend the consumption of pulses to reduce cancer risk (Guenther et al. 2006; Venter et al. 2001). The World Cancer Research Fund (WCRF) in conjunction with the American Institute for Cancer Research (AICR) published a report, entitled 'Food, Nutrition and the Prevention of Cancer: a Global Perspective', which offered a number of recommendations to help reduce cancer risk. Public health goals included increasing intake of pulses or legumes, which could help meet the recommended intake of 25 g of non-starch polysaccharides per day (World Cancer Research Fund/ American Institute for Cancer Research 2010).

The high fibre content of pulses has been negatively associated with colorectal cancers (World Cancer Research Fund, 2010). The non-digestible fibre component of many common beans has been shown to exhibit anti-proliferative activity as well as induce apoptosis in colon cancer cells (Campos-Vega et al. 2013; Haydé et al. 2012). More than three decades ago, Burkitt and Trowell (1977) highlighted the "virtual absence" of diseases such as colorectal cancer in native Africans

whose diet was rich in fibre. Similarly, a negative correlation was found between distal colon adenomas and dietary fibre intake in free-living subjects. Subjects in the highest quintile of dietary fibre intake had a 27% lower risk of adenoma than participants in the lowest quintile (Peters et al. 2003). A comprehensive review by The World Cancer Research Fund/American Institute for Cancer Research (AICR) weighed the strength of the evidence between diet and cancer at 19 locations in the body, concluding that diets high in fibre convincingly lowered risk of colorectal cancer, inferring that eating beans will likely reduce the risk of developing colon and rectal cancer (World Cancer Research Fund 2010). With respect to other cancers, the Nurse's Health Study II found that women who consumed pulses at least twice a week were 24% less likely to develop breast cancer (Adebamowo et al. 2005). However, the World Cancer Research Fund/ AICR expert panel that examined diet and cancer links concluded that evidence that fibre consumption decreased breast and other cancer risks (other than colon cancer) was not "probable" or 'convincing", rather, the data were "limited but suggestive" (World Cancer Research Fund 2010).

Other ingredients besides fibre also may be responsible for possible anti-cancer effects of pulses. Pulses also contain zinc, which is associated with decreased oxidative stress in cells (Eide 2011), improved function in immune cells (Ibs and Rink 2003), and depletion has been shown to cause DNA damage in peripheral blood cells in rats (Song et al., 2009). Additionally, selenium has been suggested to play a role in the prevention of breast, esophageal and stomach cancers, due to its ability to inhibit tumour cell development in mice with tumours (Greeder and Milner 1980). A case control study has shown that men with higher plasma selenium had a lower risk of developing prostate cancer (Brooks et al. 2001). Saponins, protease inhibitors, phytic acid and

tannins occur naturally in pulse crops and appear to have anticarcinogenic and antioxidant effects in cells (Dai and Mumper 2010; Kerem et al. 2005). Saponins inhibit the reproduction of cancer cells and also play a role in suppressing tumour growth in colon and lung carcinoma cells and leukemia cells (Fan et al. 2013; Shi et al. 2004). Protease inhibitors slow the division of cancer cells and prevent tumours from releasing proteases which destroy cells in close proximity. Specifically, pinto beans produce a trypsin inhibitor which possesses antiproliferative activity in vitro (Chan et al. 2013). Phytic acid also may suppress cancer by preventing oxidative DNA damage in cells (Midorikawa et al. 2001), while some tannins display antioxidant activity in vitro (Dai and Mumper 2010). Consumption of some saponins at high levels has been shown to be deleterious to health due to their hemolytic activity, although only a few have been shown to be toxic (Rochfort et al. 2011; Shi et al. 2004). Additionally, phytic acid and tannins can diminish mineral bioavailability (Sandberg 2002) and protein digestibility (Chung et al. 1998). However, dosage and type of compound strongly influence these potential effects, and there is no evidence that consumption of these compounds at levels found with current or recommended dietary pulse intakes poses any increased health risks. Future studies involving animal studies will help in identifying potential ingredients that mediate potential anti-cancer effects, and randomized controlled trials (RCT) with pulse consumption are required to directly examine the relationship between pulses and cancer risk.

2.2.8 Cardiovascular Disease

A growing body of evidence suggests that pulses may reduce risk of CVD by affecting several associated factors such as blood pressure, platelet activity, lipid profiles and inflammation. Data from the first National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study (NHEFS) showed an inverse relationship between dietary pulses and risk of coronary heart

disease (CHD) in American adults. Frequency of consumption was estimated using a three month food frequency questionnaire and after a 19 year follow-up period, consumption of pulses four times a week or more was associated with a 22 percent lower risk of CHD when compared with adults who consumed pulses at a rate of less than once per week (Bazzano et al. 2001). High fibre intakes from pulses have been associated with reduced blood pressure and reduced risk of CVD (Anderson et al. 1994; Papanikolaou et al. 2008). Extensive research has demonstrated that consuming pulses reduces total serum cholesterol and triglycerides, two leading factors in CVD (Bazzano et al. 2011; Reeves et al. 2007; Winham and Hutchins 2007). As aforementioned, pulses contain mono- and polyunsaturated fat and plant sterols, which aid in increasing HDL cholesterol and lowering both LDL and total cholesterol (Iqbal et al. 2006; Lovejoy 2010; Patterson et al.., 2009), and may lower inflammatory biomarkers related to CHD risk (Esmaillzadeh and Azadbakht 2012). Eating as little as a ½ cup of cooked dry beans daily has been shown to reduce total cholesterol by eight percent (Reeves et al. 2007). Pulse consumption also leads to an increase in high-density lipoprotein (HDL) cholesterol levels (Anderson and Major 2002). Results from eleven clinical trials found that intake of pulses caused decreases in fasting serum cholesterol and triacylglycerol levels as well as LDL cholesterol (Anderson and Major 2002). Similar results were observed in a meta-analyses conducted by Bazzano and colleagues (2011) and Ha et al. (2014), concluding that dietary pulses reduced LDL cholesterol.

Pulses also contain a variety of isoflavones, which possess antihypertensive, anti-atherosclerotic and antiplatelet activity (Hertog et al. 1993). The isoflavone content of pulses has been shown to differ by pulse type and species, as well as processing and cooking method (Mazur et al. 1998; Rochfort et al. 2011). Isoflavones found in kidney and black beans such as anthocyanins

stimulate adipocytes to secrete adiponectin, a cardioprotective hormone that exhibits anti-inflammatory properties in blood vessel cells and is linked to decreased risk of a heart attack when present in high levels in men (Pischon et al. 2008) and women (Teede et al. 2003).

An inverse association between pulse consumption and systolic blood pressure also has been observed in American adults (Papanikolaou and Fulgoni 2008). A recent meta-analysis of eight human isocaloric trials concluded that when pulses replaced other foods in the diet, systolic and mean arterial blood pressure were reduced (Jayalath et al. 2014). Similarly, increasing servings of beans was inversely associated with diastolic blood pressure among Costa Rican adults (Mattei et al. 2011). In a recent study, it was revealed that only lentils, compared to dried beans, peas and chickpeas, decreased blood pressure in spontaneously hypertensive rats, hinting that pulse type may play an important role in attenuating risk of CVD (Hanson et al. 2013). Currently the Heart and Stroke Foundation recommends following the DASH diet to lower blood pressure (Heart and Stroke Foundation 2013). Similarly, the American Heart Association recommends choosing high fibre foods and reducing intake of foods high in dietary cholesterol as part of a healthy strategy to reduce risk of heart disease (Lichtenstein et al. 2006). Recommendations from the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) include the use of functional foods such as legumes in their dietary recommendations (National Heart, Lung and Blood Institute 2013).

Therefore many studies support the relationship between pulse consumption and CVD risk reduction, and support recommendations to increase pulse consumption in the general population

as a simple dietary approach for primary CVD prevention. However, more studies examining the beneficial effects of pulses directly on heart health are still required in order to definitively link them to CVD risk reduction. Additionally, research which focuses on the specific cardioprotective qualities of different varieties of pulses also would be valuable in delineating the effects of individual pulses on cardiovascular health.

2.2.9 Diabetes

The low GI and high fibre content of pulses makes them an optimal food choice for people with diabetes (Jenkins et al. 1981; Rizkalla et al. 2002). The resistant starch in pulses assists in the improvement of glucose tolerance as well as insulin sensitivity, which reduce complications in diabetes (Jenkins et al., 2002). Intake of high GI foods has been shown to cause a rapid increase in blood glucose as well as insulin response after a meal (Mirza et al. 2011). Thus, inclusion of pulses in the diet may reduce the risk of type 2 diabetes by lowering the GI (Jenkins et al. 2012). Meta-analyses of RCT have concluded that pulses lower fasting blood glucose and insulin levels as well as glycosylated blood proteins and fasting blood glucose when coupled with low GI and high fibre diets (Sievenpiper et al. 2009). More than 30 published studies of postprandial effects have compared dry beans or other pulse products to controls (e.g. potatoes, rice, white bread, pasta, grains, glucose and isolated fibres) (Mitchell et al. 2012). While the majority of studies found significant reductions in postprandial glucose compared to the control (Jenkins et al. 2012; Olmedilla-Alonso et al. 2012; Shams et al. 2010; Thompson et al. 2012) some showed no difference between control diets and pulse enriched meals (Winham et al. 2007), and a study of American men found that a legume rich diet increased fasting blood glucose (Hartman et al. 2010), which is contradictory to most literature. A summary of the most recent studies (post

2009) can be found in Table 3. A comprehensive review by Sievenpiper and colleagues (2009) provides information on studies prior to 2009.

Currently, the Canadian Diabetes Association recommends eating greater amounts of high fibre foods such as whole grain breads and cereals, lentils, dried beans and peas, brown rice, vegetables and fruits (Canadian Diabetes Association 2012). Similarly, the American Diabetes Association also suggests that people with diabetes include dried beans (like kidney or pinto beans) and lentils into meals (Bantle et al. 2008), since diets rich in fibre and low GI foods are useful in controlling and ameliorating type 2 diabetes. However, future research assessing the health improvements associated specifically with quantity and frequency of pulse consumption is necessary in order to demonstrate benefits in both the prediabetic and/or diabetic population.

Table 2.2.3 Characteristics of studies examining the effect of pulse consumption on diabetes and glucose control

Study (Author, date)	Sample Size	Study Type	Treatments	Pulse Dosage	Main Results
Hartman et al. 2010	n = 64	RCT	High legume, low GI diet Control: high GI, healthy American diet	250 g/d pulses	Neither diet ↓ fasting insulin Control diet ↓ fasting glucose concentration ($p = 0.012$) Legume diet ↑fasting glucose concentrations ($p = 0.001$)
Shams et al. 2010	n = 30 (with DM2)	Crossover RCT	Normal diet with added beans, cheese, and canola oil Control: Normal diet	50 g cooked lentils	Lentil diet ↓ total cholesterol and fasting blood glucose ($p < 0.05$)
Jenkins et al. 2012	n = 121 (with DM2)	Parallel RCT	Low-GI legume diet Control: High wheat fibre diet	Target: 1 cup/day of cooked beans, chickpeas or lentils (adherence assessed by 7 day FR)	Low-GI legume diet ↓ HbA_{1c} (−0.5%), body weight (-2.7 kg), waist circumference (-1.4 cm) and total cholesterol (-8 mg/dL) Blood pressure and heart rate reduced on Low-GI legume diet in comparison with control
Olmedilla-Alonso et al. 2012	n = 12 (with DM2)	Crossover	2 Spanish varieties of white beans Control: white bread with olive oil	275 g	Beans elicited lower glycemic responses than control during the first 2 h and there was a slow decline in the blood glucose after bean intake than with control The insulin responses to both bean intakes were similar ($p = 0.686$) and more than twofold lower than that observed with control ($p = 0.000$).

Thomson et al. 2012	n = 17 (with DM2)	Crossover, single dose	Pinto beans/rice, black beans/rice, and dark red kidney beans/rice Control: white rice	Amount of beans standardized to provide 50 g of carbohydrate (139 g kidney, 115 g black beans and 177 g pinto beans)	Glucose responses were significantly lower for the pinto, black, and dark red kidney bean and rice meals than control ($p < 0.01$) Pinto and black bean/rice combinations produced a lower glycemic response overall than the dark red kidney bean/rice meal

Abbreviations used: d day, DM2 2 diabetes mellitus, FFQ Food Frequency Questionnaire, FR food record, RCT randomized controlled trial

2.2.10 Other Areas of Research

Weight Management

Pulses contain components which have been shown to benefit weight control. Dietary fibre may interfere with caloric intake by increasing chewing time and satiety (Heaton 1973; McCrory et al. 2010; Slavin 2005), and a recent study found that bread made with pea fibre increased the duration of satiety in subjects when compared with regular bread (Lunde et al. 2011). Similarly, consuming lentils with pasta and sauce were found to lower food intake in human subjects when compared to consuming pasta and sauce only or consuming chickpeas with pasta and sauce (Mollard et al. 2012). Results from the NHANES cohort suggested that adults who consumed beans on a regular basis were less likely (-22%) to be classified as obese and to have a higher waist circumference (-23%) than their non-consuming counterparts (Papanikolaou 2006). An RCT that examined the effect of consumption of pulses in conjunction with whole grains in a sample of obese adults found that the group that consumed two servings of pulses and four servings of wholegrain foods per day as substitutions for more refined carbohydrates had higher intakes of fibre, and a greater reduction in waist circumference (-2.8 cm after 18 months) than the control group (Venn et al. 2010). In addition, US bean consumers aged 12-19 weighed significantly less than non-consumers and had smaller waist measurements when compared with their non-consuming counterparts (Fulgoni et al. 2006).

In contrast to these findings, however, Canadian and American epidemiological data show that adult pulse consumers actually eat more calories than non-consumers (Mitchell et al. 2009; Mudryj et al. 2012). It would be expected that this higher energy intake by pulse consumers would be associated with an increased body mass. However, although mean BMI was higher in Canadian pulse consumers (28.0 and 27.3 kg/m^2, respectively), the difference was not

statistically significant (Mudryj et al. 2012). Physical activity was not examined, which could potentially be a confounder, but there is no evidence to suggest that pulse consumers are less active than non-consumers. The fact that the BMI is not different by pulse consumption status is unexpected, given the previous work by Papanikolaou and colleagues (2006) and other papers cited above. Under-reporting of energy intake by those with a higher BMI has been described (Johansson et al. 2001), but if this is true then both consumers and non-consumers would be expected to under-report their food intake almost equally as the BMI levels are not significantly different. The increased caloric consumption by pulse consumers may be due to the foods that conventionally accompany pulses, as the epidemiological data suggest that when examining the entire dietary pattern, pulse consumers typically eat more calories. Further studies are needed to elucidate this apparent discrepancy.

Human immunodeficiency virus (HIV)

Lectins, which occur naturally in pulses, are primarily a form of self-defense for plants and cannot be digested by humans. Developing in vitro research suggests that lectins (carbohydrate binding proteins) found in legumes may inhibit HIV-1 reverse transcriptase, the enzyme responsible for generating the DNA copy of the viral RNA that will be integrated into the human DNA (Ye et al. 2001). In addition, red bean lectins may enhance immune function by increasing the phagocytosis activity of macrophages (Hou et al. 2010). The antifungal peptides present in pinto beans also inhibit HIV-1 reverse transcriptase in vitro (Ye et al. 2001). These studies are preliminary, but may have potential impact and warrant further investigation.

Aging and Stress

In addition to disease prevention, it has been suggested that pulse intake may increase longevity and potentially enhance mental health. Research suggests that pulse consumers may have a longer life expectancy than non-consumers. A study of five cohorts of older adults to identify protective dietary predictors of long-lived elderly people (aged 70 +) examined intakes from nine major food groups. In these studies, high legume intake was the only food group to show consistent and significant protective effects among the elderly. For every 20 gram increase in daily intake of legumes there was a seven to eight percent reduction in mortality hazard ratio (with or without controlling for ethnicity) (Darmadi-Blackberry et al. 2004). Additionally a recent study found that frequent bean consumption in older adults was associated with reduced stress, anxiety and depression as well as somatic symptoms (Smith 2012). This novel area of pulse research should also be further explored.

2.2.11 Conclusions and Future Directions

Pulses are nutrient-dense foods which have been recommended by many major health organizations as a way to reduce the risk of chronic diseases. Published data have provided indirect evidence supporting the role of pulse consumption in disease risk reduction, but are based more on nutrient composition, and remain limited. While the overall literature indicates that pulses possess many nutritive and non-nutritive factors which have been shown to possess anticarcinogenic, antioxidant and satiating effects, long term RCTs specifically on pulses are necessary to clarify their role in health. As well, research which focuses on the specific cardioprotective effects of different varieties of pulses would be valuable in further understanding the relationship between dietary pulses and heart health. Current consumption

levels are fairly low, indicating a potential for pulses to make a significant contribution to maintaining overall health and preventing disease. Future research should also include studies on the innovative health benefits of pulses, such as HIV and aging.

Part 2.3: Folate, Folic Acid Fortification and Supplementation

2.3.1 Introduction

Functions of Folate in the Body

Folate (vitamin B_9) is an essential, water-soluble B vitamin that plays a role as co-enzyme in the metabolism of one-carbon groups, and involved in the prevention of chromosome breakage and DNA synthesis (Selhub 2002, Fenech 2001) and is essential for numerous functions in the body, such as aiding in rapid cell division, making it crucial during the pregnancy period (Chidambaram 2012, Coutts 1998).

2.3.2 Dietary Sources of Folate

Folate occurs naturally in foods such as leafy green vegetables (spinach, mustard and turnip greens etc.), fruits and legumes. Folic acid (FA) is the synthetic form of folate, and is found in fortified foods such as ready-to-eat cereals, white flours and pastas (National Institutes of Health. Office of Dietary Supplements 2012). For the purpose of this dissertation, the term *food folate* will refer to the naturally occurring form found in food. *Folic acid* (FA) will refer to the synthetic form of the vitamin, found in supplements, multivitamins or fortified foods such as breads, ready-to-eat cereals, pasta, etc. *Dietary folate equivalent* or DFE will refer to total folate occurring in food (i.e. food folate plus the synthetic folic acid). The Institute of Medicine (IOM) suggests using the following equation to account for the differences in bioavailability of the different forms of folate:

DFE = µg food folate + (µg dietary folic acid x 1.7) + (µg supplemental folic acid x 2)*

*A factor of 2 is used if supplemental folic acid is consumed on an empty stomach. However, if supplemental folic acid is not consumed on an empty stomach, 1.7 is used (Allen 2010, Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998).

2.3.3 Deficiency and Neural Tube Defects

In 1976, work by Smithells and colleagues showed that the diets of women who had given birth to a child with NTD were deficient in several nutrients. Additionally, their postpartum blood levels of these nutrients were also low (Smithells, Sheppard & Schorah 1976). Five years later, intervention studies showed that FA (or a multivitamin containing FA) consumed during the peri-conceptional period (3-4 weeks after conception) reduced the risk of Neural Tube Defects (NTDs) (such as spina bifida and anencephaly) in women who had had a previous NTD-affected pregnancy (Laurence et al. 1981). In 1991, the Medical Research Council Vitamin Study Research Group conclusively showed that FA supplementation lowered the risk of NTD recurrences (MRC Vitamin Study Research Group 1991). A deficiency of folate is thought to compromise the ability of cells to methylate (transfer of a methyl group into a compound) important compounds such as proteins and lipids, leading to impaired cellular function (Scott et al. 1994). It has been suggested that folate acts to protect these “disruptions” in methylation that cause NTDs (Blom et al. 2006). Animal studies show that embryos with low methionine concentrations causes NTDs to occur, supporting the mechanism that it is the defects in methylation that may result in the failure of the neural tube to close, which occurs 21-28 days after conception (Coelho, Klein 1990).

The failure of neural tube closure leads to NTDs. *Anencephaly* is fatal; infants born with this defect are either stillborn, or die shortly after birth (Blom et al. 2006) and occurs when the cerebral cortex and calvarium (bony, upper portion of the skull) fail to develop (Pitkin 2007). *Spina bifida* (an incomplete closing of the membranes around the spinal cord) occurs in the other 2/3 of NTDs, and leads to paraplegia and paralysis of lower extremities as well as impaired bladder/bowel functions and is usually not fatal (Pitkin 2007). Evidence also suggests that other factors may also play a role in NTD development besides folate deficiency. Environmental factors which may affect risk of NTDs include maternal obesity (Ray et al. 2005, Rasmussen et al. 2008) and diabetes (Chang et al. 2003). Females of certain ethnic backgrounds, such as Celtic (Shurtleff 2004) or Sikh (Baird 1983), as well as those residing in the Northern parts of China have increased risks of bearing children with NTDs (Moore et al. 1997)

Worldwide, the incidence of NTDs ranges from 1.0 to 10.0 per 1000 births (Au, Ashley-Koch & Northrup 2010). In Canada, the 1997 national NTD birth prevalence was 0.75 per 1000 births (Health Canada 2000). Geographically, the Maritime Provinces have had higher rates of NTDs, with Newfoundland reporting an average yearly rate of 3.4 per 1000 births (1976-1997) (Crane et al. 2001) and Nova Scotia, which reported mean annual rates of 2.55 and 2.61 per 1000 births in the periods between 1991-1994 and 1995-1998, respectively (Persad et al. 2002).

Several studies have corroborated the effect of FA supplementation on lowering the prevalence of NTDs and other birth defects (De Wals et al. 2007, Hewitt et al. 1992, Werler, Shapiro & Mitchell 1993). As such, adequate intakes of folate are essential for women of childbearing age (WCBA) and the IOM recommends that WCBA consume 400 μg (0.4 mg) a day of FA in addition to consuming foods naturally rich in folate (Centres for Disease Control and Prevention

2012a, Liu et al. 2004b). However, public health promotion of the use of FA-containing supplements in WCBA has been considerable, but limited in its success; as findings show only 20-40% of WCBA take supplements containing FA (De Wals et al. 2007).

2.3.4 Folic Acid Fortification

In 1996, Oman became the first country to implement mandatory folic acid fortification after the approval of adding 5 mg/kg of folic acid to white flour (World Public Health Nutrition Association 2013). Later that year, the United States Food and Drug Administration (USFDA) announced that it would allow for the fortification of flour and other cereal grain products with FA, deeming fortification mandatory in 1998. That same year Canada also mandated the addition of FA to white flour and enriched pasta and cornmeal at 150 μg (0.15 mg) FA per 100 g of flour and 0.20 mg FA per 100 g of pasta (Liu et al. 2004b) predicting an overall intake increase of 100 μg/d (Choumenkovitch et al. 2002). Post fortification results showed a dramatic decrease (~40%) in national rates of NTDs (House et al. 2006, De Wals et al. 2007) and an improvement in folate status (Ray et al. 2002, Shuaibi, House & Sevenhuysen 2008). The goal of fortification was to increase the daily FA intake by 30-70% among WCBA without compromising the health of the general public (Health Canada 2013b). However, while FA intake has increased and NTDs have decreased nationally since fortification (De Wals et al. 2007), it has also resulted in exposure of the general population to unprecedented levels of FA.

Globally, 86 countries have legislation to mandate fortification of at least *one* industrially milled cereal grain, of which 66 have legislation for wheat flour, 14 for wheat flour and maize, 3 for

wheat flour and rice, 2 for wheat flour, maize flour and rice, and 1 for rice (Food Fortification Initiative 2016).

2.3.5 Folic Acid Overconsumption

Accumulating evidence suggests FA intakes above the recommended upper limit is linked to adverse health outcomes and that the general population of the United States has been exposed to unprecedented levels of FA above the established Tolerable Upper Intake Levels (UL) established by the IOM (Kalmbach et al. 2008) [Table 2.6]. This may be attributed to the fact that high plasma folate has been associated with an exacerbation of both clinical and biochemical signs of B_{12} deficiency, potentially permitting cognitive impairment to occur (Selhub, Rosenberg 2016, Smith et al. 2016). A recent review article outlined the increased risk of anemia and cognitive issues seen in older adults with high serum folate but poor B_{12} status, implying that excessive FA intake is not safe and was associated with adverse clinical outcomes in the elderly (Selhub, Rosenberg 2016, Smith et al. 2016). This is of a particular concern in older adults who are more susceptible to vitamin B_{12} deficiency (Baik, Russell 1999). However, older adults are not the only ones at risk for adverse health outcomes: an increase in insulin resistance has been linked to a high maternal RBC folate among Indian mothers (Yajnik et al. 2008). Additionally, animal studies have shown that excessive FA may be harmful, reducing natural killer cell cytotoxicity in mice (Sawaengsri et al. 2016) and also disturbing their immune response and resistance to malaria (Meadows et al. 2015). Trends also suggest that post fortification, North America has experienced a “reversal” of the downward trend in the incidence of colorectal cancer at a statistically significant rate of 4 to 6 cases per 100,000 individuals, although causality cannot be inferred (Mason et al. 2007). However, even though there is evidence that excess FA

may be associated with advanced colorectal adenomas (Cole et al. 2007) and recurrent colorectal adenomas (Mason et al. 2007, Wu et al. 2009), a recent meta-analysis study does not support this (Vollset et al. 2013). Still, results from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial observed that women who reported consuming greater ≥ 400 µg/d of FA had a 20% greater risk of developing breast cancer (Kim 2006). The hypothesized mechanism behind these phenomena suggests that while FA aids in normal DNA replication, in malignant cells excess amounts may cause cell proliferation (Siu et al. 2012). As it passes through the intestinal wall, FA is converted to a natural biological form of the vitamin, resulting in the circulating form, 5-methyltetrahydrofolate (Morris et al. 2010, Mason 2009). It has been shown that doses of FA in physiologic quantities can "saturate" this conversion mechanism, resulting in measurable levels of circulating FA, which may be detrimental (Mason 2009).

Table 2.3.1. Estimated Average Requirement (EAR) and Tolerable Upper Intake Level (UL) for folate by life stage group by the Institute of Medicine (IOM) (National Institutes of Health. Office of Dietary Supplements 2012)

Life Stage Group (Males and Females)	**EAR[a] (µg)**	**UL[b] (µg)**
1-3 y	120	300
4-8 y	160	400
9-13 y	250	600
14-18 y	330	800
19-30 y	320	1000
31-50 y	320	1000
51-70 y	320	1000
> 70 y	320	1000

[a] Based on Dietary Folate Equivalent
[b] Based on synthetic Folic Acid (from fortified foods and supplements)

It has been observed that increased concentrations of plasma FA in elderly women who took FA-containing supplements (> 400 µg) were inversely associated with decreases in the cytotoxicity of circulating natural killer cells (Troen et al. 2006). Although the biochemical and physiologic consequences of overconsumption remain unclear, there remains concern over the adverse effects of high levels of FA (Mason et al. 2007, Selhub et al. 2009). Previous work has indicated that it is permissible for manufacturers to fortify at higher levels than the mandated amount, and Shakur and colleagues have estimated that the amount of FA in Canada's fortified foods may be as high as 50% more than the quantity expected based on the government authorized levels (Quinlivan, Gregory 2003, Shakur et al. 2009, Shakur et al. 2010). A summary of the most recent studies regarding potential adverse outcomes post-fortification (post-2011) can be found in Table 2.3.2.

Table 2.3.2. Characteristics of research examining potential adverse effects associated with Folic Acid (FA) fortification

Authors	Subjects/Study Design	Notes on Folate intake	Main Results	Conclusions
		Cancer		
(Chuang et al. 2011)	Nested case-control study using EPIC cohort (463 cases of pancreatic cancer, 464 controls)	High folate described as > 20nmol/L	Folate deficiency (5-10 nmol/L) and high folate were associated with increased risk of pancreatic cancer, but this was not statistically significant	Mechanisms warrant further investigation
(Kennedy et al. 2011)	Review paper (meta- analysis) of 27 articles observing colon/rectal cancers and dietary or total folate intake	Dietary folate intake described as folate from food. Total folate intake included food and nutritional supplements	Results suggest that there is an inverse association between folate intake and colorectal cancer incidence. There is an apparent 15% protective effect with high total folate intake in the case control studies, 13% decrease for high dietary folate intake in the case control, and 18% decrease for high dietary intake in the cohort studies	Higher folate intakes reduce one of the risks associated with colorectal cancer
(Wien et al. 2012)	Review of 19 RCTs	Studies on folic acid fortification of foods was not included	No significant difference in prostate cancer incidence was shown between groups receiving folic acid and placebo/control group, for any other cancer type. A meta-analysis of the RCTs did not show any significant difference in prostate cancer mortality in folic acid supplemented groups	A borderline significant increase in frequency of overall cancer in the folic acid group compared to controls was seen. Prostate cancer was the only cancer type found to be increased after folic acid supplementation (meta-analyses of six RCTs).

Authors	Subjects/Study Design	Notes on Folate intake	Main Results	Conclusions
			compared to controls	
(He, Shui 2013)	Meta-analysis of 13 epidemiological studies observing the association between folate intake and bladder cancer	Dietary and supplemental folic acid	Results support an inverse association between folate intake and bladder cancer	Further research with large samples and well-designed cohort studies are needed before definitive conclusions can be drawn.
(Lin et al. 2013)	Review of ten studies observing the association between folate intake and risk of pancreatic cancer	Dietary and supplemental folic acid, blood folate	A 100 μg/day increment in dietary folate intake was associated with a 7% decreased risk for pancreatic cancer No increased risk of pancreatic cancer observed with supplemental folic acid	Findings suggest that dietary folate may play a protective role in carcinogenesis of pancreatic cancer
(Qin et al. 2013)	Meta-analysis of RCTs observing association between folic acid supplementation and cancer	Varying amounts of folic acid by study	FA supplements associated with significant reduced risk of melanoma	Folic acid supplementation has no significant effect on total cancer incidence, colorectal cancer, prostate cancer, lung cancer, breast cancer or hematological malignancy, but may reduce the risk of melanoma National incidence rates of cancer do not support a substantial, population-wide adverse effect of folic acid supplementation
(Nan et al. 2013)	Evaluation of the association between alcohol consumption and colorectal cancer by	Dietary folate assessed via FFQ Supplemental folic acid also		Folic acid fortification may reduce the adverse effect of high alcohol consumption on the risk of colorectal cancer

Authors	Subjects/Study Design	Notes on Folate intake	Main Results	Conclusions
	fortification period (before 1998 vs. after 1998) in 2 prospective cohort studies	assessed		
(Vollset et al. 2013)	Meta-analysis of 13 trials that compared folic acid versus placebo, had scheduled treatment duration at least 1 year, included at least 500 participants, and recorded data on cancer incidence	Varying depending on study	Even in the trial of 40 mg/day of folic acid, which produced more than a hundred-fold increase in plasma folate, no apparent increase was noted in overall cancer incidence	No significant effect of folic acid supplementation on the incidence of cancer of the large intestine, prostate, lung, breast, or any other specific site
(Keum, Giovannucci 2014)	Although there was a momentary increase in the incidence of colorectal cancer with the initiation of folate fortification, there now exists as downward trend in the incidence rates of colorectal cancer in the US		Evidence suggests that the increase in CRC incidence rates in the later 1990s was unlikely due to folic acid fortification	More studies are needed to provide definitive evidence to address the potential benefits and risks of folate on colorectal cancer incidence rates and mortality
(Zhang et al. 2014)	Meta-analysis of 19 studies observing the association	Varying definitions on “high” folate	A dose-response meta-analysis findings did not suggest any association	Folate intake had little or no effect on the risk of breast cancer

Authors	Subjects/Study Design	Notes on Folate intake	Main Results	Conclusions
	between folate intake and breast cancer	intake dependent on study	between the risk of breast cancer and a 100 µg/day increase in folate intake Daily folate intake of 200–320 µg was associated with a lower breast cancer risk; but risk increased with a daily folate intake >400 µg	
Cognitive Decline, unmetabolized FA, other				
(Crider, Bailey & Berry 2011)	Review of potential concerns arising from fortification		Unmetabolized folic acid has been found among many groups examined—from older U.S. adults to the cord blood from newly delivered infants. It has been hypothesized that unmetabolized folic acid is related to cognitive impairment among seniors	Currently, there are no definitive studies that have found health effects from exposure to unmetabolized folic acid
(Mikael et al. 2013)	Animal study	20 mg/kg diet FA given to pregnant mice	Maternal FA supplementation was associated with embryonic loss, embryonic delays, a higher incidence of ventricular septal defects, and thinner left and right ventricular walls	Moderately high levels of FA supplementation may adversely affect fetal mouse development. Additional studies are warranted to evaluate the impact of high folate intake in pregnant women
(Qi et al. 2014)	B12 deficiency observed among adults aged >50 y using cross-		After fortification, higher folic acid intake was associated with a lower prevalence of low serum B-12 status in the	Results suggest that the prevalence of low serum B-12 status in the absence of anemia or macrocytosis among older U.S. adults did not increase after

Authors	Subjects/Study Design	Notes on Folate intake	Main Results	Conclusions
	sectional data from the NHANES 1991–1994 (pre-fortification) and 2001–2006 (post-fortification).		absence of anemia or macrocytosis	fortification. No evidence to support concerns that FA adversely affected the clinical presentation of vitamin B-12 deficiency among older adults.
(Selhub, Rosenberg 2016)	Review article describing peer reviews literature showing harm in excessive FA intake	Depending on study	Excess FA may lower natural killer cell activity in elderly females, increase insulin resistance in children of mothers with high FA intake and increase incident of breast cancer is women with polymorphism of dyhydrofolate gene	This article counters others put forth which deem that “high folic acid intake has not reliably been shown to be associated with negative health effects” by concluding that excessive FA intake may be particularly harmful among the elderly

2.3.6 B_{12} and Folate Interactions

Vitamin B_{12} (cobalamin) is a water-soluble vitamin found in most animal derived food, such as fish, shellfish, meat, dairy and eggs which is important for proper red blood cell formation, DNA synthesis and neurological function and acts as a cofactor for methionine synthase and L-methylmalonyl-CoA mutase (National Institutes of Health. Office of Dietary Supplements 2016). Table 2.8 shows the Recommended Dietary Allowances (RDA) currently in place for vitamin B_{12}.

Table 2.3.3. Recommended Dietary Allowances (RDA) and Estimated Average Requirements (EAR) for Vitamin B_{12} (National Institutes of Health. Office of Dietary Supplements 2016)

Life Stage Group (Males and Females)	**RDA (µg)**	**EAR (µg)**
0-6 mos.	0.4	NA
7-12 mos.	0.5	NA
1-3 y	0.9	0.7
4-8 y	1.2	1.0
9-13 y	1.8	1.5
≥ 14 y	2.4	2.0

The metabolism of folate and vitamin B_{12} interact significantly, and this interaction is responsible for the megaloblastic anemia that occurs during deficiency (Stabler 2013). Thus, the nutritional status of one can influence that of the other. Vitamin B_{12} deficiency can result in megaloblastic anemia (which occurs when DNA synthesis is inhibited during red blood cell formation), neurodegeneration and, when left undiagnosed, cognitive decline (Stabler 2013, Doets et al. 2014, Hunt, Harrington & Robinson 2014). High folate status has been shown to possibly exacerbate these clinical outcomes, particularly in the elderly population. A recent review article outlined the increased risk of anemia and cognitive issues seen in older adults with high serum folate but poor B_{12} status, implying that excessive FA intake is not safe and was associated with adverse clinical outcomes in the elderly (Selhub, Rosenberg 2016, Smith et al.

2016). This is of a particular concern in older adults who are more susceptible to vitamin B_{12} deficiency (Baik, Russell 1999). Thus, in the context of fortification, and perhaps excessive intakes, it is essential to determine the nature of the effect of high FA intake on B_{12} status and the consequences of this.

2.3.7 Estimating Folate and Vitamin B_{12} Intake and Exposure

Dietary Intake

There have been numerous methods used to estimate folate intake, ranging from extensive dietary records, weighed food records (where the participant measures both the portion sizes of the foods, as well as any food waste) and in-depth assessment of the intake of folate rich foods to short folate screeners and standard food frequency questionnaires (FFQs) to capture dietary patterns. Regardless of the method used, most correlations of estimated intake with RBC folate (or serum folate) have been within the range of r=.20 to r=.35 among non-supplement users (Clifford et al. 2005, Baric et al. 2009, Flood et al. 2004). Correlations have typically increased if supplement use as a source of FA is included, though the magnitude of the association with folate status has remained modest. Studies that have looked at the relationship of B_{12} intake and status, have also typically found modest correlations (Verkleij-Hagoort et al. 2007, Henríquez-Sánchez et al. 2009).

2.3.8 Red Blood Cell Folate

Red blood cell folate concentrations respond slowly to changes in folate intake, due to the fact that erythrocytes have a 120 day lifespan, and accumulate folate only during the formation of red blood cells (erythropoiesis) (Large 1988). Thus, RBC folate is the preferred measurement of long-term folate status (Piyathilake, Robinson & Cornwell 2007). The reference range of RBC

folate varies by age, but generally falls between 317-1422 nmol/L, with a level of 305 nmol/L deemed by the IOM to denote a deficiency state, based on its association with megaloblastic anemia (Fischbach, Dunning 2009, Crider et al. 2014). Researchers have used quantiles of study populations to postulate cut-offs for RBC folate (Colapinto, O'Connor & Tremblay 2011, MacFarlane, Greene-Finestone & Shi 2011, Quinlivan, Gregory 2007). Macfarlane and colleagues proposed an RBC folate cut-off of 1090 nmol/L (MacFarlane, Greene-Finestone & Shi 2011) based on data from Quinlivan and Gregory, who determined the relationship between dietary folate intake and RBC folate to be 2.1 mg DFE per 1090 nmol/L(Quinlivan, Gregory 2007). This reflects a combined intake of 0.4 mg DFE (based on Recommended Dietary Allowance) and a 1 mg FA supplement (bioavailability 1.7 mg). Using results from the Canadian Health Measures Survey (CHMS) 2007-2009, they observed that 63.5% of Canadians had RBC folate levels above the 1090 nmol/L cut-off (MacFarlane, Greene-Finestone & Shi 2011). However, a more stringent cut-off of 1360 nmol/L, which was based on the concentration at the 97^{th} percentile of the National Health and Nutrition Examination Survey (NHANES) 1999-2004 suggested by Colapinto, reduced this proportion to 40% (Colapinto, O'Connor & Tremblay 2011). Pfeiffer and colleagues suggested 3 cut-offs using NHANES data (RBC folate concentrations obtained via microbiological assay) using upper RBC folate concentrations at the 90^{th}, 95^{th} and 97.5^{th} percentiles of 1820, 2140 and 2490 nmol/L(Pfeiffer et al. 2012). Colapinto mimicked these percentiles using converted CHMS RBC folate data, obtaining slightly lower cut-offs than Pfeiffer. In comparison to Macfarlane's results, using the conversion factor and more stringent high RBC folate cut-offs decreased the proportion of Canadians with high RBC folate status to 16.4% (Colapinto et al. 2015).

Recently it has been suggested that RBC folate concentrations differ depending on the assay used, depending on certain variations or calibrations (Colapinto et al. 2014). Using a sample size of 152 individuals and two different assays (Immulite 2000 and the microbiological assay), Colapinto and colleagues used the Deming regression method to create the equation as follows: *Predicted microbiological assay concentration = -22.95 x (0.81) x Immulite 2000 assay concentrations*

2.3.9 Factors Contributing to RBC Folate Concentrations

Folate deficiency manifested as low RBC folate may be caused by a many factors. Low-carbohydrate intake (in a post-fortification era) due to weight loss trends such as the Atkins® diet, may contribute to low intakes or deficiency (Cioffi et al. 2014). Additionally, since folate is a water-soluble vitamin, naturally occurring food folate is prone to leeching into cooking water, as well as being susceptible to oxidation (McKillop et al. 2002). Individuals who smoke also have been shown to have lower folate levels than non-smokers (Piyathilake et al. 1992) as do persons who abuse alcohol or have alcohol-related liver disease (Wu et al. 1975, Medici, Halsted 2013) and those with malabsorption issues (due to inflammatory bowel disease (Allen 2008) or hereditary factors (Diop-Bove, Kronn & Goldman 1993).

2.3.10 Serum B_{12}

There is currently no UL set for dietary vitamin B_{12} intake, and no "gold standard" for laboratory confirmation of a "clinically relevant vitamin B_{12} deficiency (Gröber, Kisters & Schmidt 2013). However, blood serum below 200 ng/L (148 nmol/L) has been set to diagnosis clinically significant/severe B_{12} deficiency, with a normal range falling between 200-1000 ng/L.

"Functional" B_{12} deficiency may also be present as levels < 450 ng/L (Gröber, Kisters & Schmidt 2013). Causes of B_{12} deficiency may include dietary deficiency (in particular, vegetarians (Pawlak et al. 2013), the elderly (Andres et al. 2004), or individuals with alcoholism (Gröber, Kisters & Schmidt 2013) who are at an increased risk), those with malabsorption problems, those suffering from intestinal diseases such as Crohn's disease or Zollinger-Ellinger syndrome (Gröber, Kisters & Schmidt 2013, Allen 2008).

2.4: Food and Nutrition Surveillance in Canada

2.4.1 Introduction

This dissertation used data from the Canadian Health Measures Survey (CHMS) and the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2).

2.4.2 Canadian Community Health Survey (CCHS)

The Canadian Community Health Surveys (CCHS) are based on collaborative efforts from Health Canada, Statistics Canada and the Canadian Institute for Health Information (CIHI), with the main goals of:

- *Gathering and providing reliable, detailed and timely estimates of health determinants, health status and health system utilization across Canada*
- *Assembling data at the sub-provincial level of geography*
- *Creating a flexible survey instrument that meets specific health region data gaps, develops focused survey content for key data and deals with emerging health/health care issues as they arise*
- *Informing and guiding future policies and guidelines of both government and local health agencies (Statistics Canada 2007b)*

The CCHS surveys are cross-sectional and are comprised of two types of surveys, an annual component on general health, and more focused surveys on specific health topics. Collection periods usually occur between the months of January and December. The first year of the survey (originally ending in 'x.1'), is a "large sample, general population health survey designed to provide reliable estimates at the health region level." Since 2007, data collection takes place on an ongoing basis, and as of 2008, the CCHS data is released annually, and every two years, a

combined cycle of the 2 years samples is released. The second type of survey cycle (prior to 2007, these ended in 'x.2', eg. CCHS 2.2, and now are designated by the topic covered in the survey), have a smaller sample size (~35,000) and are designed to deliver province-level results on specific health topics.(Statistics Canada 2007b). The CCHS 2.2 consisted of both a nutritional as well as general health component and the main objectives were to estimate the distribution of usual dietary intake in terms of foods, food groups, dietary supplements, nutrients and eating patterns among a representative sample of Canadians at national and provincial levels using a 24-hour dietary recall. The CCHS 2.2 also measured the prevalence of household food insecurity among a variety of Canadian population groups, collected anthropometric measurements (body height and weight) as well as information on physical activity, selected health conditions and socio-demographic characteristics. Prior to its release, the last population based dataset that examined national food consumption and nutrition assessment was the Nutrition Canada National Survey (NCNS) which took place between 1970 and 1972. Although various provincial nutrition surveys were undertaken by Health Canada in the 1990s, national food consumption data was lacking. Development for the CCHS 2.2 began in 2002 and data collection began in January 2004, continuing for 12 months (Health Canada 2010a).

What follows is a brief overview of the CCHS 2.2 (Garriguet 2010). Further details on any of the methods used in the CCHS 2.2 are available on the Statistics Canada Website (Statistics Canada 2007b, Statistics Canada 2008).

2.4.3 Sampling Processes in the CCHS 2.2

Household Sampling

This CCHS 2.2 is a cross-sectional sample survey targeted respondents from all age groups living in private occupied dwellings in the ten provinces and excluding reserve occupants, residents of the three territories (Nunavut, Yukon and the Northwest Territories), persons residing in institutions, members of the Canadian Forces and residents of some remote areas. In total, the CCHS 2.2 had 35,107 respondents (Health Canada 2010a). Prior to data collection, the provinces of Manitoba, Ontario and Prince Edward Island provided extra funding in order to obtain a larger sample, and more funding was provided to obtain a greater sample of off-reserve aboriginals. According to Health Canada, the purpose of these "buy-ins" was to achieve sufficient sample sizes in order to "provide reliable estimates for sub-provincial areas for key domains of interest for Manitoba and Ontario." Prince Edward Island's rationale for their "buy-ins" was to increase the targeted number of respondents for various age/sex categories (Health Canada, 2010).

Sampling of Individual Respondents

Individual respondents were selected based on the goal of obtaining the target number of respondents in each area of interest per province as well as region. To obtain the minimum 80 respondents per domain of interest, Statistics Canada selected one person per household based on parameters that would "guarantee the minimum number of individuals in each domain of interest in each province and/or region without generating extreme sampling weights at the end." An example provided for a three person household (containing 2 adults aged 31 + and a 15 year old) makes the teenager 3 times more likely to be selected than the two adults. The selected dwellings were randomly assigned to four collection periods based on their PSU (Quarter 1: January to

March 2004, Quarter 2: April and May 2004, Quarter 3: June to August 2004 and Quarter 4: September to November 2004), with data collection continuing into January 2005 to improve response rates (Health Canada, 2010).

2.4.4 Data Collection

Interviewers administering the CCHS 2.2 questionnaires were given 3.5 days of training by a senior interviewer or a collection manager. Test questions and mock interviews were conducted and the Instructor's Manual was reviewed to ensure familiarity with the procedures and survey concepts (Health Canada, 2010). The questionnaires used in the CCHS 2.2 were administered using a computer-assisted interviewing application (CAPI). During the summer of 2003, the draft questionnaire was pilot-tested using a sample of 700 units to test survey length and assess respondent's reaction and evaluate the efficacy of the interviewer's training and procedures (Health Canada, 2010).

Data collection took place between January 2004 and January 2005. Prior to data collection, a letter and brochure was delivered to all dwellings providing potential respondents with information about the survey. Interviewers visited each household on the primary visit and collected information on household members (including a listing of all members residing in the dwelling, their relationship to each other and demographic information including gender, age and marital status). Following this, one member of the respective household was selected to participate in the CCHS 2.2. The first interview was comprised of two parts: the "24 hour dietary recall" and the "general health questionnaire" and took place in the respondent's home. "Proxy interviews" were required for respondents aged l1 years or younger wherein parents or guardians

assisted with the interview. For respondents under 6 years of age, the parent or guardian provided the responses (Health Canada, 2010).

To ensure privacy, interviews were conducted in private unless otherwise allowed. Variables and flags were set up to indicate whether or not another person was present during the interview, and whether the interviewer felt that this presence affected the interview. Statistics Canada also ensures protection of respondents through suppression of individual values, variable grouping, and variable capping. Further confidentiality is guaranteed by limited access to Statistics Canada's "Master File" which is only available through custom tabulations, remote access service or Research Data Centres (Health Canada, 2010).

24-Hour Dietary Recall

A grand total of 35,107 adults and children completed the initial 24-hour dietary recall. Following this, a subsample of 10,786 (or approximately 30 percent of respondents) completed a secondary recall three to ten days later to account for intra-individual variability which may increase the variance of distribution of intakes, potentially impairing the estimation of "at-risk" individuals. The recall constituent used CAPI originally put in place by the United States Department of Agriculture (USDA). This program was then updated by Health Canada to account for differences in foods available to Canadians in regards to ethnic foods as well as how the food was prepared, and was set up in both English and French (Health Canada, 2010). According to Statistics Canada, this application contains approximately 27,000 foods within look-up lists. A major advantage to this system was that a trained nutritionist was not required to perform the interview (Garriguet 2010, Statistics Canada 2008).

Twenty-four hour dietary recalls were collected primarily by face-to-face interviews by trained interviewers (unless the respondent refused to be surveyed in person or travel was prohibited in which case the interview was conducted by telephone) (Health Canada 2010a).

Interviewers collected information on the respondent's food and beverage consumption during the previous 24 hours (from midnight to midnight) and were comprised of 5 steps (these can also be viewed in further detail in Appendix C of this thesis):

1. **Quick list:** A listing of all foods and beverages consumed by the respondent in the previous 24 hours.
2. **Forgotten foods:** Respondent is asked a series of questions about forgotten foods from nine categories
3. **Time and Occasion:** Respondent is asked details regarding the time they began consuming the food or beverage as well as the eating occasion (example breakfast, lunch)
4. **Detail Cycle:** The respondent is asked more detailed questions regarding their food and beverage intake including food descriptions, preparation methods, food additions, amounts, and location of preparation. A '*Food Model Booklet*' is used as a measuring guide to assist respondents describes the amount of food or beverage they have consumed.
5. **Final Review:** A final series of questions are asked for anything else that the respondent may have consumed.

The overall length of the first interview (including the dietary recall) was 60 minutes. The second day recall was performed over the phone in an interview (unless no phone was available or

preferred and in-person interview) and lasted approximately 30 minutes in length (Garriguet, 2010; Health Canada, 2010).

Health Canada used the Canadian Nutrient File, version 2001b (CNF) to report the nutrient content of the foods reported by respondents. The CNF is a continuously updated bilingual database that contains information on 150 nutrients in over 5,807 foods. The CNF also contains data from the USDA Food and Nutrient Databases for Dietary Studies, version 1.0 for corresponding foods (in particular for mixed dishes) as well as modified data which reflects the Canadian food supply and unique Canadian dishes. Further details on both the CNF database (including information on serving size) and the USDA Food and Nutrient Database are available on their respective websites (Health Canada 2012b, United States Department of Agriculture 2011).

Data Quality and Survey Response Rates

Overall, a national response rate of 76.5% was achieved. To minimize non-response, interviewers were directed to make "all reasonable attempts" to obtain a completed interview. Rescheduling and call-backs were made to ensure participation and respondents who refused to participate were sent letters from Statistics Canada's Regional Offices which "stressed the importance of the survey and the household's collaboration" followed by a second call or visit from a senior interviewer to encourage the respondent to participate. During the final months of data collection, non-responders were again approached and persuaded to take part in the survey. As mentioned, if a special circumstance prevented an interview from taking place, and adequate information was not available the household was treated as a non-response (Health Canada 2010a).

Statistics Canada recruited interviewers with a vast knowledge of languages to lessen language barriers. In cases where the interviewer could not complete the interviewer in the respondent's language another member of the household (if present) was permitted to translate the interviewer's questions and respondent's answers, (it should be noted that this may present a future limitation in that certain questions or answers may be lost in translation). To ensure data quality, Statistics Canada set up a monitoring system at the interviewer level and weekly feedback was given. Completed interviews were transferred daily to Statistics Canada's Head Office and verified for accurateness (Statistics Canada, 2010).

2.4.5 Weighting and Bootstrapping

The process of weighting allows the data collected from surveys to be considered "representative of the covered population". A survey weight is given to each respondent which corresponds to the number of individuals in the *entire* population represented by that respondent. According to Statistics Canada, the weighting strategy for the CCHS 2.2 was developed by treating the sampling frames independently and then integrating them into a single set of weights (Health Canada 2010a).

Because the CCHS 2.2 is a multi-stage survey design, it requires a more complex formula to calculate variance estimates. The approximation recommended when analyzing data from the CCHS 2.2 is called 'bootstrapping'. This variance estimation technique is used to estimate standard errors, coefficients of variation and confidence intervals. Bootstrapping is an approach used to estimate distribution from a sample's statistics. It can also be defined as "sampling within a sample" and involves the selection of random samples known as replicates, and the calculation

of the variation in the estimates from replicate to replicate. In the CCHS, PSUs were randomly chosen from each stratum and the original sampling weights were adjusted to reflect the probability of selection into the subsample (Rao, Wu & Yue 1992, Rust, Rao 1996). A macro program "Bootvar" was developed to give users access to the bootstrap method and is available in both SAS and SPSS formats. The bootstrapping method was used in all the data analyses for this study via STATA software. Version 1.11 of the SIDE-IML program was used in conjunction with SAS to generate an estimation of the usual dietary intake by using both the first and second dietary recall along with bootstrapping weights to estimate variance (Dodd et al. 2006, Statistics Canada 2007c).

2.4.6 Overview of the Canadian Health Measures Survey (CHMS)

The CHMS is a cross-sectional comprehensive direct health measures survey which includes blood, urine and anthropometric measures and banks specimens for future measurements and genetic research. It is the result of collaborative efforts by members of Statistics Canada, Health Canada and the Public Health Agency of Canada as well as other specialists. The main objectives of the CHMS are to collect health information via a household interview as well as by direct physical measures at a mobile clinic (Statistics Canada 2014a).

2.4.7 Data Collection

This thesis used data from the following available CHMS cycles: Cycle 1 (2007-2009), Cycle 2 (2009-2011) and Cycle 3 (2012-2013). Each cycle had a minimum of 500 respondents for each gender from ten age groups on Canadians 6-79 y (Cycle 1) and 3-79 y (Cycles 2 and 3). Cycles 1,2 and 3 surveyed 5604, 6395 and 5785 respondents, respectively (Bolger 2008, Tremblay,

Wolfson & Connor Gorber 2007, Health Canada 2015a). Questions were designed for CAPI interviewing, much like the CCHS, and Census lists were used as a frame to stratify dwellings and occupants to obtain the target number of respondents by age group (Statistics Canada 2014a). Interviews took between 45 and 60 minutes, after which an appointment was set up for the physical measures component of the survey (Statistics Canada 2014a).

Prior to the collection period, an introductory letter and brochure was sent out to respondents, outlining the different steps of the survey and well as information regarding how the results would be used. These documents were available in English, French, Punjabi and Chinese (Statistics Canada 2014b). The CHMS collected physical measures, such as height, weight, hip and waist circumference as well as blood and urine tests that assessed infectious disease such as Hepatitis B and C as well as kidney function and environmental exposure to toxins such as tobacco, triclosan, and certain metals from consenting respondents (Day 2013). The CHMS also included a targeted food frequency questionnaire (FFQ) intended to provide estimates of relative nutrient intake linked to specific CHMS nutrient biomarkers, including red blood cell folate and serum B_{12} and captured the intake of vitamin and mineral supplements. Respondents were asked whether they had taken any "vitamins, minerals, fish oils, other oils, botanical or homeopathic preparations which people use to prevent illness or to improve or maintain their health." For each product taken in the last month, interviewers recorded the Drug Identification Number (DIN), the exact name and dosage of the product, and the last time the product was used (Bolger 2008, Tremblay, Wolfson & Connor Gorber 2007). Biomarker analysis was performed by a certified phlebotomist (and measured using the Immulite 2000 assay) (Colapinto, O'Connor & Tremblay 2011). As with the CCHS 2.2, the bootstrapping method was used in CHMS analyses for this

study via STATA software, and protocols put in place by the Manitoba Research Data Centre were followed when combining the three cycles.

Data Quality and Survey Response Rates

To remove language as a barrier, respondents were interviewed in their official language of choice (English or French), and interviewers were recruited for translation if necessary (Statistics Canada 2014b). To reduce the number of non-response cases as well as to ensure consistency, interviewers were extensively trained by Statistics Canada, provided with detailed interviewer manuals, and were subject to regular observations. A combined response rate of 51.7% was observed for cycle 1 of the CHMS (Statistics Canada 2014b). Cycle 2 and 3 had response rates of 53.5% and 52.0%, respectively (Manuel et al. 2014).

2.4.8 Assessment of Dietary Intake

Measuring dietary intake allows for the assessment of nutritional adequacy of individuals and groups. It also can provide information about nutrient intakes, including energy, food, and eating patterns. However, the majority of dietary assessment method validation studies indicate a degree of misreporting, compared to the 'gold standard' doubly labelled water method (DLW) used to estimate energy intake. A recent review of current food intake methods found that under-reporting by food records varies from 19% to 41% (n=5) with over-reporting most often associated with 24-hour recalls (7% to 11%, n=4), diet history (9% to 14%, n=3), and food frequency questionnaires (FFQ) (2% to 59%, n=2) (Burrows et al, 2010). Additionally, it has been shown that at least 3 24-hr multiple pass dietary recalls (which include weekdays and weekends) are necessary in order to correctly describe an individual's energy intake (Burrows et al, 2010; Ma et al, 2009).

Food frequency questionnaires (FFQ), which focus on consumption of specific food items, are cost effective and convenient. However, they also possess limitations, such as a heavy reliance on memory as well as dependence on the ability of respondents to estimate amounts consumed. However, the FFQ has been shown to demonstrate good relative validity in the estimation intake of some of the major nutrients in dietary intervention trials. Additionally, the FFQ has proved to be a reasonably valid tool in assessing overall food and/or food group intake. At the individual level, moderate to high degrees of correlation existed between the FFQ and the reference method for most of the food and food groups (Segovia-Siapco et al, 2008). However, at the group level, the FFQ has been shown to overestimate "healthy" foods and underestimate foods considered "less healthy" or "unhealthful", implying that social approval and social desirability may influence the quality of a subject's responses to a dietary assessment tool (Segovia-Siapco et al, 2008). Overall, dietary intake is difficult to measure, and any single method cannot assess dietary exposure perfectly. As Shim and colleagues point out "while certain nutritional biomarkers are valid for objective estimates of dietary exposures in clinical or anthropometric assessments, others are subjective estimates" (for example dietary recalls/histories and FFQ) (Shim et al, 2014). Although progress has been made in the area of accuracy, overall, there exist both strengths and limitations with each method of dietary intake, and continued efforts will be required to improve both accuracy and feasibility (Willett 2012).

Positive correlations between the FFQ and diet recalls have been shown for total energy ($r = 0.34$), total carbohydrate ($r = 0.42$), vegetable protein ($r = 0.43$), total fat ($r = 0.51$), polyunsaturated fat ($r = 0.77$), total fibre ($r = 0.60$), linoleic acid ($r = 0.78$) and α-linolenic acid ($r = 0.79$) (Segovia-Siapco et al, 2007). Additionally, mean nutrient estimates have been shown to

be “nearly identical” in FFQ and dietary recalls (Kumanyika, 2003; Verkleij-Hagoort et al, 2007). However these correlations are not consistent and other work has shown mixed results. European studies comparing the FFQ and dietary recalls have found correlations ranging from 0.54 for dietary fibre to 0.86 for alcohol (Kroke et al, 1999). Similarly, varying correlations were observed comparing intake of certain foods in FFQs and dietary recalls, ranging from 0.15 for pizza to 0.80 for tea (Haftenberger et al, 2010). A recent study has even shown cultural differences in dietary assessment methods, reporting lower correlations in African American respondents when compared to Caucasians (Arab et al, 2011).

2.5: Hypotheses and Objectives

This thesis is comprised of a series of studies utilizing secondary analysis of the CCHS 2.2 and three cycles of the CHMS, with the overall objective of 1) exploring the impact of a target food (pulse and soy) as a healthy food source in the Canadian diet, 2) examination of the post-fortification folate intake and folate biomarker status of the average Canadian and 3) exploration of new methods in estimating nutrient intakes based on food frequency questionnaire data and parameters generated from dietary recall data by assessing the correlations of estimated dietary intake with biomarker status. The following section will describe in detail the objectives and hypotheses associated with each section of the dissertation.

2.5.1: The dietary implications of pulses and soy in the Canadian diet (Chapters 3 and 4)

Published data observe an enhanced micronutrient intake among adult pulse consumers (Mitchell et al. 2009; Mudryj et al. 2012) and preliminary findings showing greater intakes of fibre, magnesium and potassium among young consumers (Fulgoni et al. 2006). Additionally, studies show that replacement of meat and dairy products with soy products may improve diet quality (Tucker, Qiao & Maras 2010) and show an increase in intakes of whole grains and vegetables among those who consume soy products, resulting in increased intakes of zinc, iron, and vitamin C in their diets (Sadeghian et al. 2015). Although existing literature shows an improvement in diet quality associated with pulse consumption, there is limited data on North American soya eating habits. Recent reports suggest that Canadians do not receive adequate intakes of vitamin D and calcium and that many adults do not meet the requirements for magnesium, calcium, potassium, fibre and vitamin D, all nutrients present in high amounts in soya foods.

Poor eating habits in children have been linked to many negative health outcomes. Although past evidence supports dietary advice that pulses and soy foods be included in healthful diets, there is

limited data on nutrient intakes of children and youth, with respect to pulse and soy consumption; particularly in the province of Manitoba, where the rate of overweight and obesity exceeds the national level.

Objectives and Hypotheses

The purpose of these chapters was to describe the demographic characteristics of Canadian soya consumers and compare the dietary patterns and nutrient profiles of soya-consumers in comparison to non-consumers as well as to use pulse or soy intake as indicator to evaluate the eating profile of Manitoba's children and youth in order to obtain a better understanding of this area of research. Our hypothesis is that young Canadians who consume pulses or soy foods in their daily diet may have healthier eating profiles than their non-consuming counterparts. Additionally, it is hypothesized that nutrient intakes such as protein, fibre, magnesium, iron and potassium will be significantly higher among pulse or soy consumers.

2.5.2 Folate Intake and Status of Canadians (Chapter 5)

Previous research has shown an improvement in the overall folate intakes of Canadians and suggests that FA intakes above UL are considered "safe" (Shakur et al, 2010). However, when evaluating our current Canadian intake levels and status, it is important to keep in mind that differences may exist among FA supplement users as well as by "overage" amounts, as previous work has indicated that manufacturers may fortify at higher levels than those mandated by the government (Shakur et al, 2009, Shakur et al, 2010).

Objectives and Hypotheses

With this in mind, the objectives will be to utilize results from two nationally representative Canadian surveys (Canadian Community Health Survey, Cycle 2.2 and the Canadian Health Measures Survey) to calculate folate consumption levels in the Canadian population by both dietary sources as well as supplements, as well as with additional "overage" factors accounted for (using data from previous work by Shakur and colleagues). We hypothesize that there may exist potential subgroups in the Canadian population who exceed the Tolerable Upper Intake Levels of FA, and that more than likely overconsumption will be more prevalent among supplement users. It is also hypothesized that the groups who are consuming high levels of dietary FA from dietary recalls (from CCHS 2.2 data) will have elevated levels of RBC folate (using CHMS data). This component of the dissertation will present a unique contribution to this area of research, in that it will utilize both 24 hour dietary recalls as well as blood samples to observe folate inadequacy and potential toxicity. Another novel objective of this project will be to explore new methods in estimating nutrient intakes based on food frequency questionnaire data and parameters generated from 24 hour dietary recall data in order to assess the correlations of estimated nutrient intakes (folate and vitamin B_{12}) and red blood cell folate and serum B_{12} status. The main hypothesis from this section is that there may exist potential subgroups in the Canadian population who are exceeding the Tolerable Upper Intake Levels of folic acid and who have elevated RBC folate. In particular, we hypothesize that supplement users may be at particular risk of folic acid toxicity and elevate RBC folate

2.5.3 How to use population-based food frequency data to estimate nutrient intakes: methodology exploration and recommendations. Combining Methods: A Novel Approach (Chapter 6)

Available Canadian nutrition surveys usually involve only one method to measure dietary intake. For example, the CCHS 2.2 measured dietary intake using one 24-hr recall, while the CHMS (Canadian Health Measures Survey) employed FFQ and blood samples for biomarkers. It is methodologically challenging to explore the relationship between nutrition (i.e. dietary intake, especially detailed nutrient intake) and frequency of intake certain foods, and the biomarkers of those nutrients in our body based on non-linkable surveys.

Objectives and Hypotheses

As previously mentioned, a component of this dissertation will present a unique contribution to secondary analysis research, in that it will utilize both 24 hour dietary recalls from the CCHS 2.2 as well as blood samples from three cycles of the CHMS to observe folate intake, status and subsequent inadequacy and potential toxicity. Another novel objective of this project will be to to explore new methods in estimating nutrient intakes based on food frequency questionnaire data of the CHMS and parameters generated from 24 hour dietary recall data from the CCHS 2.2 in order to assess the correlations of estimated nutrient intakes (folate and vitamin B_{12}) and red blood cell folate and serum B_{12} status. This will be accomplished by utilizing a novel method taking into account that dietary intake may be influenced by a person's food culture, age, and sex. As such, these hypothesized differences will be taken into account by creating unique regression models for specific foods by gender and cultural background (both variables which are assessed by the same parameters in both the CCHS 2.2 and CHMS) as well as respondent's age in order to minimize confounding factors linked to these characteristics. Additionally, we will also calculate anticipated nutrient intakes and status using portion size data from the CCHS

2.2 recall as well as Canada's Food Guide to Healthy Eating, hypothesizing that the parameters generated from adjusted regression models will obtain the best estimates of folate intake and achieve the best correlations between intake and status.

2.6 Chapter 2.0 References

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CHAPTER 3.0: Intake patterns and dietary associations of soya protein consumption in adults and children in the Canadian Community Health Survey, Cycle 2.2

3.1 Abstract

Soya foods are one of the recommended alternatives to meat in many dietary guidelines. While this is expected to increase the intake of some nutrients, potential concerns regarding others have been raised. The purpose of the present study was to examine the prevalence and the association of soya food consumption with nutrient intakes and dietary patterns of Canadians (age ≥ 2 years). Cross-sectional data from the 2004 Canadian Community Health Survey (Cycle 2.2; n 33 218) were used to classify soya consumers and non-consumers. Soya consumers were further divided into two groups based on their soya protein intake. Sample weights were applied and logistic regression analysis was used to explore the association between nutrient intakes and soya consumption, with cultural background, sex, age and economic status being included as covariates. On any given day, 3·3% (n 1085) of Canadians consume soya foods, with females, Asian Canadians and adults with post-secondary education being more likely to be soya consumers. As a whole, adolescent and adult respondents who had consumed at least one soya food during their 24 h dietary recall had higher energy intakes, as well as increased intakes of nutrients such as protein, fibre, vitamin C, vitamin B6, naturally occurring folate, thiamin, Ca, P, Mg, PUFA, Fe and K and lowered intakes of saturated fat. These data indicate that soya food consumption is associated with improved diet quality of Canadians. However, future research is necessary to investigate the association between increased energy intake and soya consumption.

3.2 Introduction

Soyabeans are rich in nutrients such as calcium, iron, riboflavin and potassium and contain compounds such as isoflavones and lecithins which have been associated with chronic disease reduction and improvement in bone health, particularly in women[1-6]. In addition, soya protein contains adequate quantities of the essential amino acids[2]. In 1999, the US Food and Drug Administration (FDA) approved a food labelling health claim for soya foods, based on their conclusion that 25 grams of soya protein per day, coupled with a low fat diet may reduce the risk of cardiovascular disease (CVD) by lowering blood cholesterol levels[7]. Asian populations in which soya foods are a staple have reduced risk for CVD compared to their Western counterparts [8-10]. A decreased risk of breast cancer in premenopausal Chinese women who were high consumers of soya was first reported by Lee and colleagues[11]. More recently, a study which examined breast cancer risk in Chinese women found that women with high soya isoflavone intakes had a decreased risk of breast cancer mortality, while women with high soya protein intakes had a lower risk of breast cancer compared to those with lower consumption[12]. Although research involving soya and breast cancer has been extensive, overall results are not conclusive. In addition, the mechanism between soya intake and breast cancer risk reduction remains unclear [13].

The phytoestrogens in soya and soya protein have been positively associated with bone-protective effects in women and with bone and calcium balance in postmenopausal women, respectively [2,14]. Soya proteins may reduce calcium excretion due to their lower sulphur amino acid content [15]. In addition, soya isoflavones have been found to significantly increase bone mineral density in peri and post-menopausal women[16], possibly due to its interaction with

vitamin D in stimulating bone formation and reducing resorption[17]. Although many studies have been conducted to investigate the beneficial effects of soya consumption on the prevention of bone loss or osteoporosis in post-menopausal women due to soya isoflavones, results remain inconclusive, and further randomized controlled trials are necessary to better elucidate the relationship between isoflavones and bone loss[18-20].

Studies have shown that replacement of meat and dairy products with soya products may improve diet quality. For example, replacing a 3-ounce patty made from ground beef with a soya patty leads to a reduction of 12 grams of fat and 5 grams of saturated fat. Similarly, replacing a cup of whole milk with a cup of soyamilk can reduce fat and saturated fat intake by 4 and 4.5 grams, respectively. The partial replacement of traditional protein ingredients with tofu led to an enhanced nutritional quality recipes used at American preschools. When tofu replaced cheese the amount of fat, cholesterol, sodium and energy was reduced; when it replaced beef the amount of fat and cholesterol decreased and when it replaced egg or chicken the amount of cholesterol decreased. When cheese or egg was replaced by tofu the children ate more of the new dish but when beef or chicken was replaced they ate more of the original dish[21]. A simulation analysis in which servings of meat were replaced with tofu showed that this replacement would increase nutrient intakes of folate, iron, calcium and magnesium by > 10% and lower intakes of saturated fat, cholesterol, vitamins B_6 and B_{12}. If both meat and dairy were replaced with soya equivalents(i.e. tofu or soyamilk) , intakes of fibre, folate, vitamin K, iron, calcium and magnesium also would be expected to increase, and saturated fat and cholesterol would decrease [22]. On the other hand, concerns regarding compromised nutrient intake in soya consumers also

have been raised. With these substitutions, protein, riboflavin and vitamins B_6 and B_{12} would be lowered[21,22].

Previously published data from the European Prospective Investigation into Cancer and Nutrition (EPIC) study described soya consumption patterns in Europe[23]. Similarly, Messina and colleagues analyzed soya protein intake in four Asian nations[9,24]. However, there is limited data on North American soya eating habits. Recent reports suggest that Canadian children do not receive adequate intakes of vitamin D and calcium and that many adults do not meet the requirements for magnesium, calcium, potassium, fibre and vitamin D[25,26], all nutrients present in high amounts in soya foods. The purpose of this analysis was to describe the demographic characteristics of Canadian soya consumers and compare the dietary patterns and nutrient profiles of soya-consumers in comparison to non-consumers.

3.3 Methods

This analysis used data from the Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) conducted by Statistics Canada and methods similar to those used in previous analysis of bean, pea and lentil consumption patterns[27]. The CCHS 2.2 was completed in 2004 and targeted respondents from all age groups living in the ten provinces. The main objectives were to gather information on the nutritional status of Canadians and to estimate the distribution of dietary intake in terms of foods, food groups, dietary supplements, nutrients and eating patterns among a representative sample of Canadians at national and provincial levels using a 24-hour dietary recall. A total of 35,107 adults and children completed the initial 24-hour dietary recall. Following this, a subsample of 10,786 completed a second recall three to ten days later. Twenty-

four hour dietary recalls were collected primarily by face-to-face interviews by trained interviewers[28,29]. Further details on the methods used in the CCHS 2.2 are available on the Health Canada Website[28].

Although the main outcome variable was dietary intake, the CCHS 2.2 also collected physical measurements, demographic characteristics and socioeconomic data from respondents. The main demographic variables that were examined in this analysis included gender, age, provincial location and cultural background. Income and education also were examined (for those aged ≥19 y), splitting the respondents into four groups based on their household income adequacy or highest level of education attained[28,29].

Data for the current analysis was limited to Canadians aged ≥2 years (*n* 33,941). Children under 2 years of age were excluded due to the dramatic change in food sources during the first 2 years of life, and the fact that conventional foods (rather than breast milk based diets) are introduced into the diet around 2 years of age[30,31]. Although second day consumption levels were examined, they only assessed a small subset of the original survey, thus nutrient intakes assessments were limited to one day recalls only. Respondents who did not report consuming any food, only reported consuming breast milk, or whose recalls were considered to be unreliable according to Health Canada were removed, leaving a total sample size of 33,218. Pregnant and breastfeeding women were included in this study. Although vitamin and mineral supplementation were collected in the CCHS 2.2, this analysis considers nutrient intakes from food only, which were obtained using values from the Canadian Nutrient File (CNF)[32]. Consumers were identified as individuals who had reported eating at least one soya product

during their recall period. Food sources included soyabeans, soya beverages, soya flour, soya protein powders and isolates, soya bread, tofu and other fermented products, soya based dairy products (cheeses, ice cream), and soya based meat alternatives (such as patties or wieners). Respondents who reported soya sauce and soya based margarines as their only soya consumption were not considered soya consumers. Soya sauce and soya based margarines were excluded. The amount of soya protein per 100 g of soya product was calculated using the Canadian Nutrient File (CNF), 2001b recipe database and the United States Department of Agriculture (USDA) Food and Nutrient Databases for Dietary Studies, version 1.0, and can be viewed in Appendix D. The CNF is a continuously updated bilingual database that contains information on 150 nutrients in over 5,807 foods, which was utilized by Health Canada to report the nutrient content of the foods reported by CCHS 2.2 respondents. The CNF also contains data from the USDA Food and Nutrient Databases for Dietary Studies, version 1.0 for corresponding foods (in particular for mixed dishes) as well as modified data which reflects the Canadian food supply and unique Canadian dishes[32]. Further details on both the CNF database (including information on serving size) and the USDA Food and Nutrient Database are available on their respective websites[32-34]. Food group intake data were obtained from the Canada's Food Guide File which contained previously calculated food group servings for each survey respondent[29,33].

Soya consumers were divided into 3 age groups, 2- 8y (*n* 128), 9-18 y (*n* 226) and ≥ 19 y (*n* 731), totalling 1085 soya consumers overall. Consumers in each age group were then further divided into 2 groups based on the median level of soya protein consumed in grams in their respective group, resulting in 2 equal sized groups of consumers. Soya consumers age 2-8 y were split into two groups of 64, those 9-18 y into two groups of 113, and those age ≥ 19 into 2 groups

of 366 and 365. Those who consumed soya protein in amounts less than the median value were referred to as “low consumers” while those who ate soya protein above the median were grouped as “high consumers”. Logistic regression was used to determine whether any demographic variables (gender, age, cultural background, province of residence, income adequacy and education level) increased the likelihood of being classified as a soya consumer and odds ratios were calculated. Results are reported as odds ratios with 95% confidence intervals (OR (95% CI)). Data for macro and micro nutrients were expressed as absolute values, as well as quantity per 4184 kJ (1000 kcal). General linear models were used to analyze macronutrient and micronutrient intakes and to compare nutrient intakes and other variables between non-consumers and consumers as well as between non-consumers and consumers at each of the two levels of consumption. In addition, similar analyses were conducted for each of the food groups using the data from the CCHS`s Canada Food Guide file. The significance level was set at $P < 0.05$ for differences between groups and $0.05<P<0.10$ for trends. It is important to note that because the CCHS 2.2 is a population based survey, results represent population estimates and do not represent individual or chronic dietary exposure.

The bootstrapping method was used in all the data analyses for this study. This approximation technique is recommended by Statistics Canada for use with the CCHS 2.2 to estimate standard errors, coefficients of variation and confidence intervals. Using SUDAAN software, bootstrapping was used to estimate distribution from a sample’s statistics and involves the selection of random samples known as replicates, and the calculation of the variation in the estimates from replicate to replicate[28]. All analyses were performed using PASW SPSS

Statistics, IBM, version 18 and SUDAAN Statistical Analysis Software Package, RTI International, version 10.0.1.

3.4 Results

Demographics of the Canadian soya consumer

The median age of soya consumers and non-consumers were very similar among all age groups: for 2-8 y olds it was 5 y, for 9-18 y olds it was 15 y for soya consumers and 14 y for non-consumers, and for adults ≥ 19 y the median age of soya consumers was 47, while for non-consumers it was 52 y. Adult females were significantly more likely to consume soya products than male adults ($p < 0.05$), yet females consumed less soya protein overall, regardless of age. Geographically, British Columbia had higher proportions of both paediatric and adult soya consumers than the rest of Canada. In addition, Ontario youth aged 9-18 y were also more likely to be soya consumers. Ethnicity was also an important factor of soya consumption status, with both adult and paediatric Asian Canadian respondents being significantly more likely to consume protein than any other ethnic group (Table 3.1). In adults, income was not a significant factor influencing soya consumption status, but respondents who had completed post-secondary education were more likely to be soya consumers than their counterparts with lower education levels.

Table 3.1. Demographic characteristics (% of total Canadian population) of all soy consumers based on 1-day intakes from the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2), 2004.(Odds ratios and 95% confidence intervals; mean soy protein consumed (g) and standard errors)

Characteristic	2-8y (*n* 128)				9-18 y (*n* 226)				≥ 19 y (*n* 731)			
	% of total	OR (95% CI)	Mean	SE	% of total	OR (95% CI)	Mean	SE	% of total	OR (95% CI)	Mean	SE
Gender												
Male	2.2	Reference	12.0	4.2	3.1	Reference	7.5	1.6	4.3	Reference	14.0	3.5
Female	3.6	1.68 (0.72-3.96)	7.0	1.9	3.7	1.20 (0.71-2.02)	7.1	1.7	5.9	1.40 (1.02-1.91)	9.0	1.2
Provincial Location												
Maritimes	1.6	Reference	7.0	5.7	1.2	Reference	3.0	1.3	9.8	Reference	13.0	4.8
Quebec	1.6	0.98 (0.18-5.46)	7.0	1.6	2.7	2.38 (0.78-7.20)	5.0	1.6	3.7	1.33 (0.63-2.79)	8.0	2.7
Ontario	3.1	1.97 0.52-7.41)	5.0	2.7	3.7	3.26 (1.35-7.83)	8.0	1.8	5.3	1.96 (0.58-6.63)	11.0	3.0
Prairies	4.0	2.0 (0.31-5.30)	21.0	12.0	1.9	1.64 (0.62-4.36)	7.0	2.7	10.0	1.48 (0.80-2.74)	9.0	2.3
British Columbia	6.6	4.34 (1.07-17.57)	11.0	3.8	7.4	6.86 (1.87-25.18)	8.0	2.0	10.2	4.00 (1.5-10.68)	13.0	2.9
Overall	3.4		9.0	2.2	3.4		7.0	0.9	5.2		10.8	6.5
Cultural Background												
African, Latin, Arabic, Aboriginal or Other	1.4	Reference	27.0	35.5	2.2	Reference	5.0	5.09	1.4	Reference	10.0	2.1
Caucasian	2.3	0.58 (0.03-10.27)	10.0	2.7	2.7	0.97 (0.28-3.39)	6.0	0.9	3.0	1.03 (0.30-3.59)	7.0	6.0
Asian Canadian	6.9	3.15 (1.03-9.66)	5.0	1.8	11	4.26 (2.39-7.58)	10.0	2.0	8.7	4.38 (1.56-12.35)	13.0	1.7
Income*												
Lowest		NA				NA			4.7	Reference	7.0	3.9
Lower Middle									5.5	1.18 (0.50-2.79)	12.0	5.0
Upper Middle									4.0	0.91 (0.47-1.78)	12.0	3.0
Highest									6.0	0.98 (0.47-2.03)	9.0	2.0
Education*												
< Secondary School		NA				NA			2.5	Reference	10.0	3.4
Secondary School									4.7	1.94 (0.71-5.34)	10.0	2.8
Post-Secondary School									5.1	2.12 (1.02-4.44)	13.0	7.1
Post-Secondary Degree /Diploma									6.3	2.68 (1.58-4.57)	11.0	2.1

NA, not determined.

*Income and Education were not examined among respondents aged < 19 years.

Food sources

On any given day, 3.3 (95% confidence interval = 3.1-3.5%) percent of Canadian adults and youths consume soya products. The main sources of soya protein in the adult Canadian diet were as follows: soya flour, tofu products, soya beverages, soya protein isolate, soyabeans, soya meat substitutes, soyabean soups and fermented products, soya cheeses and yogurt and soya bread or cereal products. Canadian youth data showed similar results in terms of the types of soya foods consumed, with the exception that 9-18 y olds consumed less soya flour and more soya beverages than adults (Fig. 3.1).

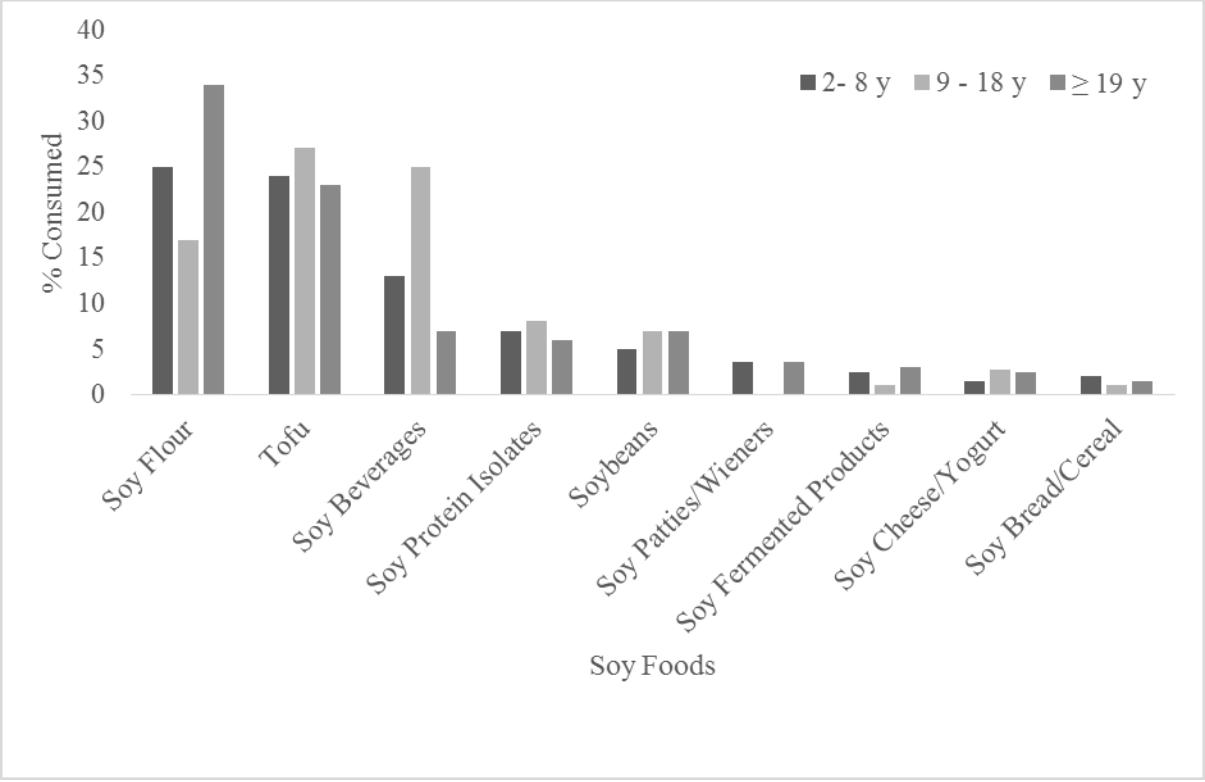

Fig. 3.1. Most Commonly Consumed Food Sources of Soy Products by Age Group in the Canadian Diet (1 d, 24 h dietary recall of the Canadian Community Health Survey, Cycle 2.2 (2004)).

Dietary Associations of Soya Protein Consumption

In children aged 2-8y, nutrient intakes differed between consumers and non-consumers only when expressed relative to energy intake. Consumers at the highest level of soya protein consumption consumed more fibre (29%), calcium (23%), magnesium (18%), iron (14%) and protein (16%) per 4184 kJ (1000 kcal) consumed and had lower intakes of saturated fat at both levels of soya consumption (21% less in both groups) (Tables 3.2).

Table 3.2a. Soy protein amount and nutrient intakes per day for soy-consumers and non-consumers age 2-8 y based on 1-day intakes from the Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) 2004 (*n* 4105).

Intake category	Non-Consumers (*n* 3977)		Low Consumer (*n* 64)		High Consumer (*n* 64)	
	Mean	SE	Mean	SE	Mean	SE
Mean Soy Protein Intake (g)	0		1.5	0.5	16.6	4.1
Food amount (g)	1922	26	1846	140	1964	131
Energy						
kcal	1777	37	1598	173	1679	103
kJ	7435	154	6686	724	7025	431
Protein (g)	65	1.4	59	7.8	71	5.1
Protein per (g/4184 kJ)	37	0.5	37	3.3	43*	3
Carbohydrate (g)	245	5	231	33	233	20
Carbohydrate (g/4184 kJ)	141	1	145	10	139	6
Fibre (g)	12.4	0.3	15.5	4.7	14,2	1.3
Fibre per (g/4184 kJ)	7	0.1	10	2.3	9*	0.7
Sugar (g)	117	2	86**	11	104	13
Total Fat (g)	62	1.6	51	7	55	6.2
Total Fat (g/4184 kJ)	34	0.5	32	2.6	32	2.3
Saturated Fatty Acid (g)	23	0.6	18**	1.8	19**	1.5
Saturated Fat (g/4184 kJ)	10	0.3	10	0.7	9	0.6
Monounsaturated Fatty Acid (g)	23	0.8	19	3.1	19	3.1
Monounsaturated Fatty Acid (g/4184 kJ)	7.0	0.2	7.2	1.1	6.1	0.7
Polyunsaturated Fatty Acid (g)	9	0.3	9	1.7	11	2.1
Polyunsaturated Fatty Acid (g/4184 kJ)	2.8	0.07	3.2	0.4	3.3	0.3
Linoleic Acid (g)	7.6	0.3	7.4	1.7	9.4	1.7
Linoleic Acid (g/4184 kJ)	4.2	0.1	4.5	0.8	5.3	0.6
Linolenic Acid (g)	1.2	0.05	1.1	0.2	1.3	0.3
Linolenic Acid (g/4184 kJ)	0.7	0.02	0.7	0.1	0.8	0.1
Linoleic: Linolenic Ratio	6.3 :1		6.7:1		7.2:1	
Cholesterol (mg)	201	7.6	175	33.2	169	28.5
Cholesterol (mg/4184 kJ)	113	2.7	111	22.4	107	22.4
Vitamin A (µg)	576	13	471†	55	627	132
Vitamin A (µg/4184 kJ)	333	7.2	301	53	387	68.5
Vitamin C (mg)	145	4	122	23	170	461
Vitamin C (mg/4184 kJ)	84	2.9	81	15	101	20
Thiamin (mg)	1.5	0.03	1.4	0.3	1.6	0.13
Thiamin (mg/4184 kJ)	0.9	0.01	0.9	0.1	1.0*	0.06
Riboflavin (mg)	2.0	0.05	1.7	0.2	1.8	0.2
Riboflavin (mg/4184 kJ)	1.1	0.03	1.0	0.1	1.1	0.09

Intake category	Non-Consumers (*n* 3977)		Low Consumer (*n* 64)		High Consumer (*n* 64)	
	Mean	SE	Mean	SE	Mean	SE
Niacin (mg)	27.7	0.6	25.8	4.1	29.8	2.1
Niacin per (mg/4184 kJ)	15.7	0.2	16	1.3	18†	1.2
Vitamin B_6 (mg)	1.4	0.02	1.3	0.18	1.5	0.1
Vitamin B_6 (mg/4184 kJ)	0.8	0.02	0.8	0.05	0.9†	0.05
Vitamin B_{12} (µg)	3.5	0.1	3.0	0.4	3.3	0.3
Vitamin B_{12} (µg/4184 kJ)	2.0	0.07	1.9	0.3	2.1	0.3
Naturally Occurring Folate (µg)	163	4.8	199	50	193	19
Folic Acid (µg)	110	3.4	107	20.4	97	21.6
Folate (from food in dietary folate equiv.) (µg)	363	14	351	78	374	41
Folate (µg/4184 kJ)	205	3.9	216	32.8	219	16.5
Vitamin D (µg)	6.0	0.2	6.2	0.8	6.0	0.7
Vitamin D (µg/4184 kJ)	3.5	0.1	3.9	0.8	3.7	0.5
Calcium (mg)	1028	20.5	1019	158	1213	136
Calcium (mg/4184 kJ)	594	16	651	82	731*	64*
Phosphorus (mg)	1209	19.8	1122	124.6	1183	79.1
Phosphorus (mg/4184 kJ)	692	11	706	53	726	55
Magnesium (mg)	245	3.6	238	27.7	273	19.3
Magnesium (mg/4184 kJ)	141	2.7	151	8.2	166**	9.3**
Iron (mg)	12	0.3	10	1.2	14	1.5
Iron (mg/4184 kJ)	6.7	0.1	6.3	0.4	8.1*	0.6
Zinc (mg)	9	0.2	8	0.9	9	0.7
Zinc (mg/4184 kJ)	4.9	0.06	5.0	0.3	5.5	0.4
Sodium (mg)	2480	78	1991	276	2377	205
Sodium (mg/4184 kJ)	1396	21	1241†	78	1439	42
Potassium (mg)	2523	38	2333	211	2607	166
Potassium (mg/4184 kJ)	1455	26.1	1477	86	1617	118
Grain Products (servings)	5.2	0.1	5.6	1.1	4.8	0.7
Fruit and Vegetable Products (servings)	4.3	0.1	4.0	0.7	5.0	1.0
Milk and Milk Products (servings)	2.4	0.06	1.9	0.5	2.2	0.3
Meat and Alternatives (servings)	2.3	0.1	2.5	0.5	3.5	0.5

Values are mean ± SE unless otherwise indicated
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, †$0.1 < p < 0.05$, compared to non-consumers

In Canadian youths 9-18y, high soya consumers consumed more food (g) (21%) and had higher intakes of fibre (25%), protein (26%), vitamin B_6 (33%), naturally occurring folate (43%), folacin (43%), calcium (34%), phosphorus (21%), magnesium (31%), iron (24%), zinc (25%)

and potassium (24%). When nutrient intakes were calculated relative to total energy intake, vitamin B_6, calcium and magnesium remained significantly higher. Soya consumers aged 9-18 y also had higher intakes of fruits and vegetables and meat and alternatives than their non-consuming counterparts (Table 3.2b).

Table 3.2b. Soy protein amount and nutrient intakes per day for soy-consumers and non-consumers age 9-18 y based on 1-day intakes from the Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) 2004 (*n* 8957).

Intake category	Non-Consumers (*n* 8731)		Low Consumer (*n* 113)		High Consumer (*n* 113)	
	Mean	SE	Mean	SE	Mean	SE
Mean Soy Protein Intake (g)	0		0.8	0.12	12.3	1.5
Food amount (g)	2701	34	2830	284	3288*	223*
Energy						
kcal	2360	41	2332	265	2662	203
kJ	9874	172	9757	1109	11138	849
Protein (g)	86	1.5	87	8.7	109*	8.5*
Protein per (g/4184 kJ)	37	0.35	38	2.1	41†	2.6
Carbohydrate (g)	320	5.7	309	39.0	366	33.4
Carbohydrate (g/4184 kJ)	137	0.5	135	4.3	139	4.0
Fibre (g)	15.7	0.2	17.4	2.1	19.7*	1.5
Fibre per (g/4184 kJ)	6.9	0.08	8.1	0.91	7.9†	0.62
Sugar (g)	146	3.0	136	20.2	157	11.4
Total Fat (g)	84	1.4	86	10.6	88	7.1
Total Fat (g/4184 kJ)	35	0.19	36	1.80	33	1.30
Saturated Fatty Acid (g)	29	0.6	29	3.5	29	2.6
Saturated Fat (g/4184 kJ)	14	0.7	12	0.3	10	0.4
Monounsaturated Fatty Acid (g)	33	0.5	34	4.8	34	2.8
Monounsaturated Fatty Acid (g/4184 kJ)	6.1	0.1	6.2	0.6	4.7***	0.3
Polyunsaturated Fatty Acid (g)	14	0.28	14	2.2	17	1.8
Polyunsaturated Fatty Acid (g/4184 kJ)	3.6	0.06	2.6	0.3	2.4	0.2
Linoleic Acid (g)	11	0.3	12	1.9	14†	1.4
Linoleic Acid (g/4184 kJ)	4.8	0.06	5.1	0.50	5.2	0.30
Linolenic Acid (g)	1.8	1.7	1.9	0.4	2.1	0.2
Linolenic Acid (g/4184 kJ)	0.8	0.01	0.76	0.12	0.79	0.06
Linoleic: Linolenic Ratio	6.1:1		6.3:1		6.7:1	
Cholesterol (mg)	255	6.4	282	53.2	330†	39.4
Cholesterol (mg/4184 kJ)	108	1.6	120	19.6	123	15.6
Vitamin A (µg)	652	13	756	146	783	104
Vitamin A (µg/4184 kJ)	286	5.0	354	75.3	312	44.7
Vitamin C (mg)	152	3.6	175	41.6	221†	34.1
Vitamin C (mg/4184 kJ)	68	1.4	83	23.3	89	14.1
Thiamin (mg)	1.9	0.06	1.9	0.21	2.3†	0.2

Intake category	Non-Consumers (*n* 8731)		Low Consumer (*n* 113)		High Consumer (*n* 113)	
Thiamin (mg/4184 kJ)	0.83	0.01	0.81	0.05	0.88	0.05
Riboflavin (mg)	2.2	0.05	2.2	0.28	2.5	0.19
Riboflavin (mg/4184 kJ)	0.97	0.01	0.95	0.12	0.97	0.07
Niacin (mg)	38.4	0.7	37.8	3.8	45.0†	3.4
Niacin per (mg/4184 kJ)	16.5	0.17	16.4	0.83	17.1	0.92
Vitamin B_6 (mg)	1.8	0.03	1.7	0.18	2.4**	0.21
Vitamin B_6 (mg/4184 kJ)	0.8	0.01	0.8	0.06	1.0*	0.09
Vitamin B_{12} (µg)	4.2	0.12	4.0	0.57	6.2	2.13
Vitamin B_{12} (µg/4184 kJ)	1.8	0.03	1.8	0.19	2.1	3.4
Naturally Occurring Folate (µg)	205	4.4	248	34.0	295***	23.3
Folic Acid (µg)	146	2.5	148	24.9	295	23.2
Folate (from food in dietary folate equiv.) (µg)	480	11	495	54	544	40
Folate (µg/4184 kJ)	208	2.2	219	22.2	211	12.3
Vitamin D (µg)	6.3	0.13	6.5	1.87	7.1	0.93
Vitamin D (µg/4184 kJ)	2.7	0.04	2.8	0.74	2.6	0.26
Calcium (mg)	1097	17	1085	181	1472**	139
Calcium (mg/4184 kJ)	474	5.4	480	70.7	568*	42.3
Phosphorus (mg)	1450	23	1476	172	1763*	125
Phosphorus (mg/4184 kJ)	621	4.8	647	47	673	45
Magnesium (mg)	301	3.8	318	39.2	395***	24.8
Magnesium (mg/4184 kJ)	131	1.4	145	15.5	157**	10.3
Iron (mg)	15.5	0.38	14.9	1.3	19.3*	1.7
Iron (mg/4184 kJ)	6.7	0.06	6.6	0.50	7.4	0.05
Zinc (mg)	11.5	0.2	12.1	1.3	14.4*	1.3
Zinc (mg/4184 kJ)	4.9	0.05	5.3	0.40	5.5	0.40
Sodium (mg)	3387	64	3700	632	3650	368
Sodium (mg/4184 kJ)	1458	12.2	1574	92.7	1390	115.0
Potassium (mg)	3017	46	3116	358	3771*	293
Potassium (mg/4184 kJ)	1312	13.2	1394	105.2	1474	107.0
Grain Products (servings)	6.7	0.14	6.3	0.7	7.6	0.7
Fruit and Vegetable Products (servings)	4.7	0.09	4.9	0.5	7.5**	1.05
Milk and Milk Products (servings)	2.4	0.04	2.4	0.05	2.5	0.3
Meat and Alternatives (servings)	3.5	0.07	3.6	0.5	4.8**	0.5

Values are mean ± SE unless otherwise indicated
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, †$0.1 < p < 0.05$, compared to non-consumers

Adult soya consumers in the highest category of soya protein intake consumed 16.5 ± 2.0 g of soya protein and had higher intakes of many nutrients. Compared to non-consumers, high soya consumers had 10% higher energy intakes (kJ/d) and consumed a greater amount of food overall (g/d). Compared to non-consumers, high soya consumers in this group also consumed significantly higher amounts of carbohydrate (9%), fibre (36%), polyunsaturated fatty acid (44%), linoleic acid (48%), linolenic acid (32%), protein (27%), vitamin C (25%), thiamin (29%), vitamin B_6 (26%), naturally occurring folate (33%), folacin (32%), calcium (53%), phosphorus 14%), magnesium (32%), iron (35%) and potassium (15%). When total energy intake was accounted for, intakes were significantly higher than non-consumers for thiamin, vitamin B_6, calcium, phosphorus, magnesium and iron, as well as lower for saturated fat (-20%). No major differences were found comparing adult soya consumers in the low soya consumption group (who consumed on average 1.5 g of soya protein) with non-consumers, with the exception of higher fibre per 4184 kJ, and lower intakes of vitamin D per 4184 kJ. In terms of food group intake, adult consumers in both soya groups consumed ~1 serving greater of fruits and vegetables than their non-consumer counterparts. Soya consumers in the high soya group took in 1.5 servings more of meat and alternatives than both the non-consumers and low soya consumers (Table 3.2c).

Table 3.2c. Soy protein amount and nutrient intakes per day for non-consumers and soy consumers (≥ 19 y) based on 1-day intakes from the Canadian Community Health Survey Cycle 2.2 (CCHS 2.2) 2004 (*n* 20,156).

Intake category	Non-consumers (*n* 19425)		Low Consumer (*n* 365)		High Consumer (*n* 366)	
	Mean	SE	Mean	SE	Mean	SE
Mean Soy Protein Intake (g)	0		1.5	0.12	16.5	2.0
Food amount (g)	3232	130	3485	264	3563**	193
Energy						
kcal	2088	183	1993	125	2298	260
kJ	8736	766	8339	523	9615	1088
Protein (g)	85	7	72	8	108*	19
Protein per (g/4184 kJ)	41.8	0.3	42.1	2.7	47.2†	3.1
Carbohydrate (g)	256	21	256	13	279*	24
Carbohydrate (g/4184 kJ)	125	1.3	131†	2.9	125	4.3
Fibre (g)	17.1	1.2	18.9	0.9	23.3***	1.1
Fibre per (g/4184 kJ)	8.7	0.2	10.0***	0.4	10.5	1.4
Sugar (g)	104	7	94	12	101	7
Total Fat (g)	76.3	7.5	67.3	7.1	80.2	9.9
Total Fat (g/4184 kJ)	36	0.47	32	2.13	34	1.02
Saturated Fatty Acid (g)	24.9	2.8	22.0	3.3	23.0	4.4
Saturated Fat (g/4184 kJ)	11.6	0.3	10.4	0.9	9.7**	0.9
Monounsaturated Fatty Acid (g)	30.8	2.9	26.0	3.1	30.1	3.7
Monounsaturated Fatty Acid (g/4184 kJ)	14.2	0.2	12.9	0.9	12.4	1.0
Polyunsaturated Fatty Acid (g)	13.4	1.2	11.7	0.8	19.3***	1.7
Polyunsaturated Fatty Acid (g/4184 kJ)	6.3	0.06	6.3	0.35	5.5	0.6
Linoleic Acid (g)	10.7	1.0	9.3	0.6	15.8***	1.3
Linoleic Acid (g/4184 kJ)	5.0	0.05	5.0	0.37	7.0	1.21
Linolenic Acid (g)	1.9	0.2	1.7	0.2	2.5***	0.2
Linolenic Acid (g/4184 kJ)	0.9	0.02	0.8	0.07	1.1**	0.07
Linoleic: Linolenic Ratio	5.6:1		5.5:1		5.3:1	
Cholesterol (mg)	282	27	266	48	285	79
Cholesterol (mg/4184 kJ)	138	2.4	140	28.1	122	20
Vitamin A (µg)	699	114	613	49	733	130
Vitamin A (µg/4184 kJ)	357	34	321	21	331	35
Vitamin C (mg)	126	15	150	13	157*	14

Intake category	Non-consumers (*n* 19425)		Low Consumer (*n* 365)		High Consumer (*n* 366)	
	Mean	SE	Mean	SE	Mean	SE
Vitamin C (mg/4184 kJ)	66	2.6	79†	6.6	70	6.9
Thiamin (mg)	1.7	0.1	1.6	0.3	2.2**	0.3
Thiamin (mg/4184 kJ)	0.85	0.04	0.83	0.09	0.98*	0.05
Riboflavin (mg)	1.9	0.2	1.9	0.2	2.2	0.4
Riboflavin (mg/4184 kJ)	0.96	0.01	0.96	0.05	1.00	0.1
Niacin (mg)	40	3.5	38	3.6	46†	6.6
Niacin per (mg/4184 kJ)	19.7	0.1	19.9	1.6	20.5	0.9
Vitamin B_6 (mg)	1.9	0.2	1.9	0.2	2.4***	0.2
Vitamin B_6 (mg/4184 kJ)	0.94	0.1	0.97	0.04	1.05*	0.05
Vitamin B_{12} (µg)	4.4	0.2	3.9	0.7	6.0	2.1
Vitamin B_{12} (µg/4184 kJ)	2.2	0.1	1.9	0.2	2.5	0.6
Naturally Occurring Folate (µg)	234	27	262	25	312**	16
Folic Acid (µg)	121	22	123	27	154	42
Folate (from food in dietary folate equiv.) (µg)	462	54	461	24	482	26
Folate (µg/4184 kJ)	231	6.9	237	8.9	219	16.6
Vitamin D (µg)	5.7	0.4	4.1	1.0	6.3	1.4
Vitamin D (µg/4184 kJ)	2.8	0.1	2.1**	0.3	2.8	0.4
Calcium (mg)	855	87	788	115	1309**	234
Calcium (mg/4184 kJ)	423	5	394	42	582**	53
Phosphorus (mg)	1339	113	1271	112	1705**	235
Phosphorus (mg/4184 kJ)	658	5	647	17	748***	23
Magnesium (mg)	326	26	336	23	431***	50
Magnesium (mg/4184 kJ)	166	3	176†	6	191***	5.3
Iron (mg)	14.1	0.9	13.2	0.8	19.0***	2.1
Iron (mg/4184 kJ)	7	0.3	6.9	0.4	8.6***	0.3
Zinc (mg)	11.3	0.7	11.1	0.9	13.7†	2.0
Zinc (mg/4184 kJ)	5.5	0.2	5.6	0.3	6.0	0.2
Sodium (mg)	3103	243	2908	349	3690	606
Sodium (mg/4184 kJ)	1530	28	1493	134	1655	141
Potassium (mg)	3114	222	3118	201	3579***	290
Potassium (mg/4184 kJ)	1587	36	1629	73	1591	91

Intake category	Non-consumers (*n* 19425)		Low Consumer (*n* 365)		High Consumer (*n* 366)	
	Mean	SE	Mean	SE	Mean	SE
Grain Products (servings)	5.8	0.6	5.8	0.5	6.1	0.8
Fruit and Vegetable Products (servings)	5.2	0.5	6.1*	0.6	6.2	0.4
Milk and Milk Products (servings)	1.7	0.2	1.4	0.2	1.7	0.2
Meat and Alternatives (servings)	4.2	0.3	4.1	0.7	5.7**	0.6

Values are mean ± SE unless otherwise indicated
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, †$0.1 < p < 0.05$, compared to non-consumers

3.5 Discussion

On any given day, 3.3% of Canadians consume soya products. This is similar to US data from two 24-h recalls on soya consumption patterns in respondents aged 9-70y, in which case there were 226 mentions of soya products by 5510 individuals[22]. If it is assumed that each mention was made by separate respondents, the consumption rate using this NHANES data would be 4.1 %; this is similar to the CCHS 2.2 results using both 1 and 2 day recalls (4.2 %, data not shown). In a 1-d recall interview in the EPIC study of the European population, only 1.9% of respondents consumed soya. However, gender results from the EPIC study were similar to the CCHS 2.2 analysis, with females being more likely to be soya consumers than males[23]. The difference in gender consumption patterns may be due to adult females consuming soya products for their hypothesized bone protective effects[2,14,15].

Differences between European and Canadian consumption rates may be attributed to Canada's large Asian population, which is in the order of 10%, making up approximately 66% of Canada's visible minority population[35], while Asians represent a much smaller proportion of the European population[36]. Indeed, Asian Canadian adults and children were significantly more

likely to be soya consumers than any other cultural group, and the province of British Columbia has a high Asian population[37].

Previous studies have suggested that socioeconomic status may influence soya consumption patterns[38-40]. This study found that those with a higher level of education were more likely to consume soya, consistent with previous research which has shown that the nutrition and health perceptions of soya foods increased with education level[41] and knowledge of soya as a functional food[42]. Similarly, lack of knowledge (preparation techniques, associated health benefits) has been cited as a hindrance to soya consumption[43]. Although income level did not influence the consumption of soya products in this study, research has indicated that the cost of soya foods is a major barrier to soya consumption, particularly among low income adults[39]. Western soya consumption differs greatly with Asian soya consumption which is higher in "traditional" soya foods such as tofu, tempeh, miso and natto (boiled and fermented soyabeans) [24]. The types of soya foods consumed also differed in this cohort when compared to US and European studies. Soya flour (all forms) accounted for more than a third of the soya products consumed by adults in the CCHS 2.2, while in Europe it was the third most reported item (grouped with other soya grain products), and did not even appear in the NHANES results. In the US population, the most common form of soya consumed was soya sauce (which was excluded in this study), followed by meat replacement products (which were not highly consumed among Canadians regardless of age group), soya replacement drinks/bars, soya milk and tofu. Tofu consumption was also more prevalent in Canada than in the US. Comparably, Western European consumers ate more soya dairy substitutes (milk, cream, drinks, cheese and yogurt)[22,23].

Canadian soya protein intake averaged between 7 and 14 g/d depending on age and gender. Messina and colleagues observed soya protein consumption in adults in 4 Asian countries and found that soya protein intake varied depending on age, gender and region. Older Japanese adults consumed between 6-11 g of soya protein while residents of Shanghai had a daily mean soya protein intake of 8.8 g. Conversely, Singapore data show lower soya protein intake (≤ 5.1 g/day) among residents[24].

The current analyses show that Canadians that consume soya products have higher nutrient intakes than non-consumers. Previous simulation analyses predicted that dietary intakes of some nutrients would be increased, while others would be compromised if soya foods replaced milk and meats in the diet[22]. Many soya products are good sources of carbohydrates and soya protein is a high quality protein source[2], which is evidenced in the higher intakes of these macronutrients by adult soya consumers. In their simulation, Tucker et al predicted that replacing milk with soya dairy beverage would result in greater magnesium, iron and fibre intakes, all of which were observed in our study, likely due to the fact that soya is rich in these nutrients[22]. Thiamin, which was also found to be significantly higher in the soya consumer group, is also found in soya beverages[44], with one cup providing nearly ¼ of the recommended dietary allowance (RDA)[45]. Tucker's simulation also correctly predicted that replacing meat with soya based alternatives would lower the intake of saturated fat, which was observed in the current analysis[22]. However, it should be noted that the nutrient composition of soya depends dramatically on the type of soya food[46]. For example, fresh or dried soyabeans, as well as foods made with fermented soya (miso, tempeh, natto) are more nutrient dense and less processed[47],

while soya foods which have been processed at a high temperature (soya patties and other soya-based meat alternatives) may have reduced nutrient quality, such as increased sodium[46] as well as lowered levels of isoflavones[46]. Data from the CNF shows that enriching soya milk adds 250 mg more calcium per serving when compared to its unenriched counterpart[32]. Additionally, the quality of soya products is affected by the attributes of the soyabean variety as well as the environmental conditions it was grown in. For example, soyabean varieties which are higher in phosphorus, protein and fat produce tofu with higher amounts of these nutrients[48].

In contrast to concern over levels of vitamins B_6 and B_{12} due to reduced meat consumption, the current analyses reveal that B_{12} intakes were actually higher among soya consumers and vitamin B_6 intake was enhanced among adolescent and adult soya consumers. The reason for this is not apparent, as soya foods are not rich in these vitamins, and further research is required to examine this finding. A possible explanation is that consumers with the highest soya intakes consume approximately one more serving from the meat and alternatives food group, which may account for the higher intake of vitamin B_6. Another reason that these nutrients are not compromised in soya consumers may be because they are not replacing dairy and meat products with soya substitutes, but rather consuming them in conjunction with each other. For example, an average soya patty contains 14 grams of soya protein[49], which if compared to the approximately 16 grams of soya protein that adult high soya consumers are eating, would likely contribute to the 1.5 more servings of meat and alternatives that was observed in this study. However this can only be speculated, because the total amount of soya protein may not be solely attributed to soya-based meat alternatives. The CCHS 2.2 did not account for specific dietary habits or food exclusions (such as vegetarianism). To examine whether or not soya was consumed as a meat

substitute, each respondent's 24 hr dietary recall was examined for presence of meat products. The analysis showed that 83% of soya consumers ate at least one meat product on the day of their dietary recall, confirming that the majority of soya and meat items were consumed together, and not as a substitution.

There are several health implications of these results. Only 40% of Canadians report eating 5 or more servings of fruits and vegetables a day[50]. Results from this study show that Canadian soya consumers display a higher intake of fruits and vegetables. There also have been concerns raised over sufficient intakes of protein, vitamin $B_{6,}$ vitamin $B_{12,}$ vitamin D, riboflavin, calcium and iron in vegan or vegetarians[51-54]. Although information on specific diets was not gathered in the CCHS 2.2 survey, intake of these nutrients was enhanced in soya consumers who also tend to be meat consumers, suggesting that soya consumption along with meat appears to improve the nutrient status of the diet. Nonetheless, the higher energy intakes and the increased amount of food eaten among soya consumers may be a cause for concern if this increase in intake is sustained over time. Interestingly, body mass index did not differ among consumers and non-consumers, nor did physical activity status, with similar proportions of respondents in both groups reporting regular physical activity. As such, it remains unclear as to why soya consumers consume more food yet do not weigh more than non-consumers. Future research involving long term assessment of soya consumption is required to elucidate this trend.

Another potential benefit of soya consumption may be related to bone health. Although studies show conflicting effects of soya isoflavones on bone health, intakes of magnesium and calcium

were significantly higher among soya consumers of all ages. A study in Italy using 3-day dietary recalls found that osteoporotic patients had significantly lower levels of these nutrients than controls ($p<0.05$). They showed that magnesium intake greater than 350 mg/d was correlated to normal bone mineral content [55]. In the CCHS 2.2 cohort, soya consumers in the higher intake category attained intakes of magnesium greater than 350 mg. High soya consumers also consumed more than 1200 mg of calcium, a value which has been linked with an increased rate of bone mineralization in pubertal girls[56] as well as a decreased risk of osteoporosis and diabetes in women[57]. In addition, the Framingham Children's Study observed that children over 12 who consumed ≥ 2 servings of dairy products a day coupled with ≥ 4 servings of meat and alternatives had higher bone mineral content in their later teen years[58], levels of intake which were met by high soya consumers in the 9-18 y age group. Phosphorus, which is important in bone health and maintenance, is crucial during puberty due to its role in growth and formation of bones[59,60] and was higher in the diets of soya consumers.

With respect to cardiovascular disease (CVD), numerous clinical studies demonstrate the benefits of diets high in fibre and fruits and vegetables and low in saturated fat[30], a dietary pattern which was evident in soya consumers. However, even the average high soya consumer did not consume sufficient soya protein to derive the protective cardiovascular effect of soya as specified by the FDA health claim of 25 g of soya protein per day[7] , and the increased energy intake associated with soya consumption could be deleterious to cardiovascular health.

As the CCHS 2.2 is a cross-sectional survey, a limitation of this study is that this 1-d dietary recall may not be a true representation of individual's habitual eating habits and there is potential

for over or under estimation of soya consumption habits. Additionally, the CCHS 2.2 is a self-reported survey and non-sampling errors such as non-response, recall bias and social desirability may affect the validity of results. Although the five step multiple pass method utilized during the 24 hr recall has been shown to enhance accuracy and assist the respondent in remembering what and how much food they consumed [61], it has been reported that the average under-reporting of energy intake in the CCHS 2.2 is estimated at 10%, with a greater under-reporting rate among respondents who were overweight or obese, adults compared with teenagers, and women compared with men [62]. Additionally, frequency of soya consumption cannot be determined. However, this large survey included 35,107 individuals, providing greater validity to this type of survey. Information was not collected on specific types of diets (i.e. low carbohydrate, vegetarian or vegan) which may have been useful in further observation of the average Canadian soya consumer. This study only looked at food intake and not use of supplements among respondents. Further, cause and effect relationship cannot be assumed with these results, as soya consumption may be a constituent of an overall healthier lifestyle. As well, there are methodological issues which arise when observing small populations, such as soya-consumers. The overall number of consumers is low, and when further split to account for age groups, the resulting estimates may exhibit increased variability and as a result be less efficient [63]. Furthermore, it must be cautioned that nutrient content of various soya products may differ by manufacturer, variety and brand. As this study relied on values from the CNF database, it should be noted that the accuracy of these databases are not always perfect and nutrient composition may vary from product to product.

Soya consumers have higher intakes of fibre and lower intakes of saturated fat, potentially reducing the risk of CVD. They also have higher intakes of calcium, magnesium, phosphorus and potassium, which are crucial in preventing bone loss and maintaining bone health [55,57,59]. The reasons for the effects of soya consumption on these potential dietary improvements need to be clarified. In particular, the implied increase in energy intake among soya consumers needs to be further explained using long term studies to determine what dietary habits other than soya consumption are contributing to this pattern.

3.6 Chapter 3.0 References

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CHAPTER 4.0: Nutrient and Food Group Intakes of Manitoba Children and Youth: A population-based analysis by pulse and soy consumption status

4.1 Abstract

Poor eating habits among children are associated with negative health outcomes. The objective of this study was to use pulse/soy consumption as an indicator to evaluate the eating profile of young Manitobans. Data from the Canadian Community Health Survey Cycle 2.2 was used for analysis and restricted to Manitoba residents 2 to 18 y (n= 1840). Consumers were identified as individuals who reported eating at least one pulse/soy product during their recall. On any given day, 8.2% of Manitobans reported consumption of pulses/soy. Intakes of fibre, protein, magnesium and zinc were higher in consumers only when expressed relative to total caloric intake. Consumers also reported increased intakes of meat and alternatives. Total intakes of vitamin D, fibre and fruit and vegetable consumption were low among all groups. Sodium intakes in both groups were high when compared to levels recommended by health professionals. These results indicate that there are many dietary issues affecting Manitoba children, suggesting the need for more research targeting dietary habits of children and youth, the quality of the food supply and effective strategies in nutrition education.

4.2 Introduction

Poor eating habits have been linked to a multitude of negative health outcomes such as increased risk of childhood obesity, type 2 diabetes and increased risk of developing adult obesity [1]. The rate of childhood overweight/obesity in Manitoba is 31%, a significantly higher proportion than Canada's national average of 26% [2].

Recent data explored the relationship between pulse consumption (beans, peas, and lentils) and nutrient intakes of North Americans [3, 4]. Pulses are a significant source of many nutrients and epidemiological evidence supports the protective link between pulse consumption and diseases [5]. Among adults, consuming at least one pulse product during their dietary recall resulted in improved nutrient intakes [3, 4]. Similarly, there have also been substantial amounts of research devoted to the health benefits of soy consumption. Although by definition soybeans are an oil seed, they are considered a legume and are grown like a pulse, and Manitoba Pulse Growers considers them a part of their 'pulse portfolio' [6, 7]. With this in mind, and due to small sample sizes, pulses and soy foods were combined in this study.

In 2015, Health Canada approved a health claim linking soy consumption to lowered cholesterol, based on evidence that consumption ≥25 g of soy protein per day helps reduce cholesterol [8]. Soy consumption in the American diet was examined using NHANES data in a simulation analysis in which MyPyramid servings of meat were replaced with tofu and milk was replaced with soy beverage. This replacement simulation showed an increase in folate, iron, calcium and magnesium and lowered intakes of saturated fat, cholesterol, protein and vitamins B_6 and B_{12} when soy products replaced dairy and meat [9]. Comparably, results from the Canadian Community Health Survey (CCHS) Cycle 2.2 24 hour dietary recall found that adolescent and adult respondents who had consumed at least one soy food during their recall had increased intakes of energy, protein, fibre, vitamin C, vitamin B_6, folate, thiamin, calcium, potassium, phosphorus, magnesium and iron and lowered intakes of saturated fat [10].

4.3 Purpose

Although the rate of overweight and obesity in Manitoba exceeds the national level, there is limited data on the nutrient intakes of Manitoba's children and youth. Therefore, the objective of this study was to use pulse/soy consumption as an indicator to evaluate the eating profile of Manitoba's children and youth.

4.4 Methods

Participants

Data from the CCHS 2.2 was used for this study and restricted to respondents 2 to 18 years residing in Manitoba. Children under 2 years of age were excluded due to the changes in food sources during the first 2 years of life [11-13]. Twenty-four hour dietary recalls were collected using the USDA's multiple step approach [14]. Details on the CCHS 2.2 survey methodology and sampling designed can be found elsewhere [14, 15]. Pulse/soy consumers were identified as individuals who had reported eating at least *one* soy or pulse food product during their recall period. Serving sizes were not observed. Respondents who did not report consuming any food, consumed only breast milk or had unreliable recalls (according to Health Canada) were excluded, resulting in a sample size of 1840. Food and ingredient and recipe files were utilized to obtain all sources of pulse/soy foods. Food sources included dry beans, peas and lentil dishes, soybeans, soy flour, soy protein powders, tofu and other fermented products, soy based dairy products and beverages, and soy based meat alternatives (patties or wieners). Soya sauce and soy based margarines were excluded.

Statistical Analysis

Food group intake data was obtained from the Canada's Food Guide File that contained previously calculated data for respondent's food group servings [11, 14]. Data for nutrients were expressed as absolute values and quantity per 1000 kcal. General linear models were used to analyze macronutrient, micronutrient and food group intakes and to compare nutrient intakes and other variables between non-consumers and consumers. Logistic regression was used to determine whether any demographic variables increased the likelihood of being classified as a pulse/soy consumer and odds ratios were calculated. The significance level was set at $P < 0.05$ for differences and $0.05<P<0.10$ for trends. All analyses were performed using PASW SPSS Statistics, version 22.0 (2013) and SUDAAN Statistical Analysis Software Package 10.0 (2008).

4.5 Results

Overall, 8.2% of Manitobans age 2-18 y (n=150) reported consumption of pulse/soy on any given day. No demographic differences were shown (Table 4.1). Wholly, the energy and nutrient intake profiles of non-consumers and consumers did not differ significantly, except when nutrients were expressed relative to energy intake. Per 1000 kcal, pulse/soy consumers had significantly higher intakes of fibre (17%), protein (14%), magnesium (10%) and zinc (15%), and significantly lower intakes of carbohydrate (5%) and vitamin C (22%). Food group intakes between the two groups were not significantly different with the exception of the meat and alternative food group, where pulse/soy consumers ate 1 more daily serving than non-consumers (Table 4.2).

Table 4.1. Demographic characteristics of pulse or soy consumers in Manitoban youth 2-18 years based on 1 day dietary recalls from the Canadian Community Health Survey, Cycle 2.2

	% Pulse/Soy Consumers [N= 1840]	OR[a]	95% CI
Gender			
Male	7.3	1	Reference
Female	9.0	1.23	0.51-2.41
Age [years]			
2 to 8	7.4	1	Reference
9 to 13	8.6	1.16	0.75-2.56
14 to 18	4.4	1.13	0.61-2.56
Residential Location			
Burntwood/Norman/Churchill	7.9	1.21	0.56-2.64
Assiniboine/Parkland/Brandon	9.3	1.45	0.71-2.90
North Eastman/ South Eastman/Interlake/Central	9.1	1.42	0.57-1.97
Winnipeg	6.4	1	Reference
Urban or Rural			
Urban	8.0	1	Reference
Rural	8.3	1.04	0.56-2.00
BMI Classification			
Normal	8.4	2.90	0.70-6.90
Overweight	9.2	3.39	0.78-14.73
Obese	2.9	1	Reference

[a]Logistic regression was used to determine whether any demographic variables [gender, age, geographic location or BMI] increased the likelihood of being classified as a pulse/soy consumer and odds ratios were calculated

Table 4.2. Macronutrient, Micronutrient and energy intakes[a] per day of Manitoban youth 2-18 years based on 1 day dietary recalls from the Canadian Community Health Survey, Cycle 2.2

	Overall [n=1840]	Non-Consumers [n=1690]	Pulse/Soy Consumers [n =150]
Food amount [g]	2332 ± 59	2335 ± 49	2294 ± 341
Energy [kcal]	2123 ± 71	2136 ± 93	1964 ± 246
Carbohydrate [g]	294 ± 12	296 ± 16	262 ± 42
Carbohydrate per 1000 kcal [g]	140 ± 2	140 ± 2	133 ± 6*
Fibre [g]	13.7 ± 0.3	14.1 ± 0.3	14.2 ± 2.0
Fibre per 1000 kcal [g]	6.6 ± 0.3	6.5 ± 0.2	7.6 ± 0.6*
Sugar [g]	140.0 ± 9.1	142.1 ± 12.2	117.3 ± 35.1
Total Fat [g]	74.3 ± 2.0	75.2 ± 3.0	69.1 ± 7.2
Total Fat per 1000 kcal [g]	34.2 ± 0.5	34.1 ± 0.7	35.0 ± 1.5
Saturated Fatty Acid [g]	29.1 ± 0.7	26.4 ± 1.4	24.3 ± 2.7
Saturated Fat per 1000 kcal [g]	10.8 ± 0.1	12.1 ± 0.4	12.0 ± 0.6
Monounsaturated Fatty Acid [g]	28.1 ± 2.6	29.1 ± 0.9	28.4 ± 2.6
MUFA per 1000 kcal [g]	13.3 ± 0.3	13.3 ± 0.4	14.1 ± 0.7
Polyunsaturated Fatty Acid [g]	11.9 ± 0.3	12.2 ± 0.3	11.3 ± 1.3
PUFA per 1000 kcal [g]	5.5 ± 0.2	5.5 ± 0.2	5.7 ± 0.3
Linoleic Fatty Acid [g]	9.3 ± 1.1	10.0 ± 0.3	9.1 ± 1.1
Linoleic Fatty Acid per 1000 kcal [g]	4.6 ± 0.2	4.6 ± 0.2	4.7 ± 0.2
Linolenic Fatty Acid [g]	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.3
Linolenic Fatty Acid per 1000 kcal [g]	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.1
Cholesterol [mg]	239 ± 19.3	225 ± 10	240 ± 19
Cholesterol per 1000 kcal [mg]	108 ± 4	107 ± 6	131 ± 32
Protein [g]	75.6 ± 2.0	75.1 ± 2.5	79.2 ± 8.2
Protein per 1000 kcal [g]	36.7 ± 0.7	36.2 ± 0.6	41.3 ± 2.0*
Vitamin A [RAE]	594 ± 19	597 ± 20	558 ± 101
Vitamin A per 1000 kcal [RAE]	291 ± 9	291 ± 9	293 ± 19
Vitamin D[mg]	6.5 ± 0.2	6.5 ± 0.2	6.4 ± 1.7
Vitamin D per 1000 kcal [mg]	3.2 ± 0.1	3.2 ± 0.1	3.5 ± 0.4
Vitamin C [mg]	131 ± 7	132 ± 7	110 ± 30†
Vitamin C per 1000 kcal [mg]	67 ± 5	67 ± 5	55 ± 11**
Thiamin [mg]	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.1
Thiamin per 1000 kcal [mg]	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
Riboflavin [mg]	2.2 ± 0.1	2.2 ± 0.1	2.0 ± 0.2
Riboflavin per 1000 kcal[mg]	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
Niacin [mg]	33.0 ± 0.7	33.2 ± 0.8	34.1 ± 3.7
Niacin per 1000 kcal [mg]	15.9 ± 0.4	15.8 ± 0.4	17.5 ± 1.2
Vitamin B_6 [mg]	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.2
Vitamin B_6 per 1000 kcal [mg]	0.7 ± 0	0.7 ± 0.0	0.8 ± 0.1
Vitamin B_{12} [mg]	3.8 ± 0.2	3.8 ± 0.2	3.9 ± 2.9
Vitamin B_{12} per 1000 kcal [mg]	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1

Folic Acid [µg]	134 ± 15	136 ± 16	127 ± 11
Folic Acid per 1000 kcal [µg]	66 ± 6	66 ± 6	67 ± 9
Folate [from food in dietary folate equiv.] [µg]	426 ± 21	428 ± 26	400 ± 47
Folate per 1000 kcal [µg]	206 ± 4	205 ± 5	207 ± 12
Calcium [mg]	1078 ± 45	1080 ± 58	1062 ± 155
Calcium per 1000 kcal [mg]	518 ± 7	517 ± 8	519 ± 7
Phosphorus [mg]	1358 ± 41	1357 ± 54	1365 ± 164
Phosphorus per 1000 kcal [mg]	652 ± 7	648 ± 8	708 ± 29†
Magnesium [mg]	268 ± 5	267 ± 6	273 ± 38
Magnesium per 1000 kcal [mg]	130 ± 3	129 ± 3	142 ± 7*
Iron [mg]	13.9 ± 0.6	14.1 ± 0.8	14.2 ± 2.4
Iron per 1000 kcal [mg]	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.5
Zinc [mg]	10.1 ± 0.3	10.3 ± 0.4	11.2 ± 0.9
Zinc per 1000 kcal [mg]	4.9 ± 0.1	4.8 ± 0.1	5.5 ± 0.3*
Sodium [mg]	3028 ± 62	3027 ± 86	3031 ± 588
Sodium per 1000 kcal [mg]	1445 ± 48	1436 ± 48	1568 ± 80
Potassium [mg]	2669 ± 56	2669 ± 54	2762 ± 387
Potassium per 1000 kcal [mg]	1303 ± 44	1297 ± 45	1397 ± 68
Grain Products [servings]	6.1 ± 0.4	6.1 ± 0.5	5.7 ± 0.6
Vegetable and Fruit Products [servings]	3.8 ± 0.1	3.8 ± 0.1	3.3 ± 0.5
Milk and Alternatives [servings]	2.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.4
Meat and Alternatives [servings]	2.9 ± 0.1	2.9 ± 0.1	4 ± 0.4***

[a] Intakes ± SD

Note: Comparing to non-consumers * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, †$0.1 < p < 0.05$

Vegetable and fruit intake of Manitobans 2-18 years fell below the recommended level (range of 4 to 8 servings/day) of Canada's Food Guide to Healthy Eating. Servings of milk and alternatives were at the low end of the recommended 2 to 4 servings/day range. The average intake of sodium in both groups, as well as overall, exceeded the established Tolerable Upper Intake Levels (UL), which falls between 1500 and 2300 mg/day, depending on age. Vitamin D intakes were below the recommended Estimated Average Requirement (EAR) intake of 10 µg. Similarly, overall intake of fibre (13.7g ± 0.3), regardless of consumption status, fell below the established Adequate Intake (AI) of 19-38 g/d (Table 4.2) [16].

4.6 Discussion

Our results show that consumption of pulse/soy foods are lower in young Manitobans than previously reported studies using adult populations [3, 4, 17]. In comparison to soy consumption patterns among Americans 9-18 years in NHANES, only 4.5% of respondents reported consuming soy products [9], while 2.8% of Canadians 2-18 years reported similar consumption using CCHS data [17]. Pulse/soy consumption in the current study may appear to be higher due to the inclusion of pulses in addition to soy, as well as the wider age ranges used. According to an Alberta survey, factors that limit pulse consumption may include disliking the taste, lack of preparation knowledge, and the presence of gastro-intestinal side effects [18]. Although this survey was based on adult respondents, among children [who can be finicky [19]], the "dislike of the taste or texture" was one of the most frequent top-of-mind reasons for not eating pulses [18] and a recent study on lentil consumption in families with young children found that caregiver's/parent's lack of acceptance of lentils was a major barrier to their use/consumption [20].

Previous data on adult pulse intake patterns observed numerous differences in dietary intakes between pulse consumers and non-consumers and soy consumers and non-consumers, attributed mainly to the fact that pulses and soy foods contain high levels of these nutrients [3, 4]. However, carbohydrate, fibre, protein, zinc and magnesium, which were significantly higher among adult pulse consumers at the highest levels of consumption (when pulse intake was quantified by gram) were only significantly different when calculated per 1000 kcal. These differences may be attributed to the fact that the previous study on Canadian adults expressed nutrient intake on four levels of pulse consumption [4], while the current study simply placed

consumers into one group due to small sample sizes. Indeed, the majority of nutrient differences in our previously published study on adult pulse consumers were significant only at the highest 2 levels of pulse consumption (consuming > 99 g of pulses during recall) [4]. It is possible that grouping Manitobans by amount of pulse or soy product consumed could provide a different picture, although the small sample size of the current study population precluded further sub-categorization. Another potential area that may account for differences in intake is the fact that our study includes soy products. For example, 100 g of dark red kidney beans contains 25 g of fibre and 60 g of carbohydrate while the same amount of soybeans contains 6.3 g of fibre and 10 g of carbohydrate [21]. However, based on previous work, both adult soy and pulse consumers reported similar increases in protein as well as fibre intake with consumption [4, 10].

The excessive intakes of sodium observed in this study are consistent with adult levels, as Canadians consume ~ 3400 mg per day [22]. Adult pulse consumers consume higher amounts of sodium when compared to non-consumers [4], likely not due to the composition of the pulses, but perhaps reflecting an increased intake of pulse dishes traditionally high in sodium [Mexican dishes, canned bean soups] [23]. Similarly, soy foods such as miso and certain tofu have high levels of sodium, and are a major source of dietary sodium in Asian, American and British diets [24]. Observing the dietary intakes and patterns of all Manitobans 2-18 years [regardless of consumption status], it is evident that the majority of Manitoba's youth are not consuming optimal diets. Levels of sodium intake exceed the recommended intake [1000-1500 mg/d], as well as the UL set by the Institute of Medicine (IOM) [16]. High sodium intake may lead to development of hypertension and CVD later in life, as sodium intake has been positively associated with high blood pressure among US children and adolescents [25]. Targeted health

goals, such as lowering blood pressure/CVD risk, show greater impact when started early as eating habits are being formed [26].

Low vitamin D intakes in this study were similar to intakes reported by a recent Finnish study, and may lead to compromised bone health later in life [27]. Additionally, cross-sectional and cohort studies have shown that higher intake of dairy, fruits and vegetables and cereals in children are associated with increased bone mass, when compared with diets high in processed foods [28], and children with low dietary fibre intakes have increased body fat [29]. The low fibre intakes of Manitoban youth may play a role in their unhealthy weight status, particularly since both pulse/soy consumers and non-consumers had low intakes of vegetables and fruit. It is uncertain why pulse/soy consumers had lower vitamin C intakes, although marginally lower intake of vegetables and fruit may contribute to this result.

Study Limitations

As the CCHS 2.2 is a cross-sectional survey, a limitation of the present study is that this 1 day dietary recall may not be a true representation of individual's habitual eating habits, and there exists potential for over- or underestimation of pulse or soy consumption habits. The CCHS 2.2 is a self-reported survey, and although methods were utilized during the 24 hour dietary recall to enhance accuracy [30], the average under-reporting of energy intake in the CCHS 2.2 is estimated at 10% [31]. Additionally, some studies have shown that proxy-assisted interviews, which were used for respondents under 11 years, inaccurately reflect actual food portions, types of foods, and nutrients consumed [32-34]. The small sample size of this study and the combination of soy and pulse foods may limit the generalizability of the results, as did the fact

that portions were not observed. Similarly, comparing consumption rates should be viewed with caution, as most previous studies have observed pulse or soy consumption, and not a combination of the two.

4.7 Relevance to Practice

Although pulse or soy consumption does not appear to relate to the nutrient intake profile of Manitobans 2-18 years, results from this study shed light on the poor eating habits of Manitoba's children and youth overall. High intakes of sodium and low intakes of vegetables and fruit, fibre, and vitamin D signal a potential public health issue for their future cardiovascular and bone health. The rate of childhood obesity in Manitoba exceeds the national average, leading to a variety of negative health outcomes and incurring high health care costs. Children who are at an unhealthy weight or practice poor dietary habits are also more likely to continue these habits throughout their life, suggesting that there exist opportunities to specifically target 'at risk' groups. The addition of pulse or soy foods in the diet has shown benefits to the overall diet in both adults (pulse) and adults/children (soy). Therefore, these data are relevant to childhood obesity and dietary intake patterns of children and youth. Efforts targeting the dietary habits of young Canadians should be paired with others aimed at improving the quality of the food supply (e.g. reducing sodium, increasing vitamin D and vegetable and fruit intake) and nutrition education in children.

4.8 Chapter 4.0 References

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CHAPTER 5.0: Folate intakes from diet and supplements may place certain Canadians at risk for folic acid toxicity

5.1 Abstract

To examine the prevalence of folate inadequacy and toxicity based on usual intakes from food and supplements as well as biomarkers of folate, secondary data analyses were performed using cross-sectional, nationally representative data from the Canadian Community Health Survey, Cycle 2.2 (N=32,776) as well as biomarker data from the Canadian Health Measures Survey cycles 1, 2 and 3 (N=15,754). Based on unfortified food sources, Canadians would struggle to consume adequate amounts of folate. When folate intakes from all food sources are considered, the overall prevalence of folate inadequacy was low across all age/gender groups, with the exception of females > 70 y. However, > 10% of supplement users were above the Tolerable Upper Intake Level, climbing to almost 18% when overage factors were accounted for. Additionally, between 20 and 52% of supplement users had elevated red blood cell folate concentrations, depending on cut-off used. Results from this study suggest that insufficient dietary intakes of folate in Canadians have been ameliorated due to the fortification policy, although folate inadequacy still exists across all age groups. However, supplement users appear to be at an increased risk of FA overconsumption as well as elevated red blood cell folate. As such, the general population should be informed of the potential risks of FA overconsumption resulting from supplement use. This study suggests a need for more careful assessment of the risks and benefits of food fortification, particularly fortification above mandated levels, and FA supplement use in general population.

5.2 Introduction

Folate (vitamin B_9) is a water soluble vitamin, necessary for cell division and growth, which occurs naturally in foods such as leafy green vegetables, fruits and legumes. Its synthetic form, folic acid (FA), is found in supplements and fortified foods[1]. Folate deficiency during the peri-conceptional period (3-4 weeks after conception) has been associated with an increased risk for Neural Tube Defects (NTDs) such as spina bifida and anencephaly[2-4] and several studies have corroborated the effect of FA supplementation on lowering the prevalence of NTDs and other birth defects[5-7]. As such, adequate intakes of folate are essential for women of childbearing age (WCBA) and the Institute of Medicine (IOM) recommends that WCBA consume 400 μg (0.4 mg) a day of FA in addition to consuming foods naturally rich in folate [8,9]. In 1996, the United States Food and Drug Administration (USFDA) announced that it would allow for the fortification of flour and other cereal grain products with FA, deeming fortification mandatory in 1998. That same year Canada also mandated the addition of FA to white flour and enriched pasta and cornmeal at 0.15 mg FA per 100 g of flour and 0.20 mg FA per 100 g of pasta[9] predicting an overall intake increase of 100 μg/d [10]. Post fortification results showed a dramatic decrease (~40%) in national rates of NTDs[11,12] and an improvement in folate status[13,14].

In spite of this success, there exists some controversy regarding possible health concerns associated with FA overconsumption. The Tolerable Upper Intake Level (UL) is defined as the "maximum level of chronic nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population" [15,16]. Based on the metabolic interactions between folate and vitamin B_{12}, the Food and Nutrition Board (FNB) and the IOM established an

UL for the *synthetic* forms of folate (µg of FA) available in dietary supplements and fortified foods for each life stage group[1].

Recently, FA fortification has come under scrutiny due to accumulating evidence which suggests that the general population of the United States has been exposed to unprecedented levels of FA above the UL[17]. This may be attributed to the fact that high plasma folate has been associated with an exacerbation of both clinical and biochemical signs of B_{12} deficiency, potentially permitting cognitive impairment to occur[18,19]. A recent review article outlined the increased risk of anemia and cognitive issues seen in older adults with high serum folate but poor B_{12} status, implying that excessive FA intake is not safe and was associated with adverse clinical outcomes in the elderly[18,19]. This is of a particular concern in older adults who are more susceptible to vitamin B_{12} deficiency[20]. However, older adults are not the only ones at risk for adverse health outcomes: an increase in insulin resistance has been linked to a high maternal RBC folate among Indian mothers[21]. Additionally, animal studies have shown that excessive FA may be harmful, reducing natural killer cell cytotoxicity in mice[22] and also disturbing their immune response and resistance to malaria[23]. Trends also suggest that post fortification, North America has experienced a "reversal" of the downward trend in the incidence of colorectal cancer at a statistically significant rate of 4 to 6 cases per 100,000 individuals, although causality cannot be inferred[24]. However, even though there is evidence that excess FA may be associated with advanced colorectal adenomas[25] and recurrent colorectal adenomas[24,26], a recent meta-analysis study does not support this[27]. Still, results from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening trial observed that women who reported consuming greater $\geq$ 400 µg/d of FA had a 20% greater risk of developing breast cancer[28]. The hypothesized mechanism behind

these phenomena suggests that while FA aids in normal DNA replication, in malignant cells excess amounts may cause cell proliferation[29]. As it passes through the intestinal wall, FA is converted to a natural biological form of the vitamin, resulting in the circulating form, 5-methyltetrahydrofolate[30,31]. It has been shown that doses of FA in physiologic quantities can "saturate" this conversion mechanism, resulting in measurable levels of circulating FA, which may be detrimental[31].

Mandatory fortification has resulted in increased exposure to circulating FA[17], and it has been observed that increased concentrations of plasma FA in elderly women who took FA-containing supplements (> 400 µg) were inversely associated with decreases in the cytotoxicity of circulating natural killer cells[32]. Although the biochemical and physiologic consequences of overconsumption remain unclear, there remains concern over the adverse effects of high levels of FA[24,33]. Previous work has indicated that it is permissible for manufacturers to fortify at higher levels than the mandated amount, and Shakur and colleagues have estimated that the amount of FA in Canada's fortified foods may be as high as 50% more than the quantity expected based on the government authorized levels[34-36]. Thus it is critical to not only observe the dietary intake of folate, but also to measure the red blood cell folate concentrations to obtain a clearer picture of the folate status of the Canadian population in a post-fortification era. The aim of this study was to determine if there existed any groups who were at risk for toxicity or deficiency of this vitamin. Additionally, we assessed intake of supplement users separately from non-supplement users, as this particular group could be at an increased risk of FA overconsumption as well as elevated levels of RBC folate[37,38].

5.3 Methods

Data Source

This study used data from the Canadian Community Health Survey, Cycle 2.2 2004 (CCHS 2.2) as well as data from the Canadian Health Measures Survey, Cycles 1, 2 and 3 (CHMS). In total, the CCHS 2.2 surveyed 35,107 respondents, each of whom completed a general health questionnaire as well as a 24 hour dietary recall, administered by trained interviewers. A sub-sample of 10,786 respondents completed a second 24 hour dietary recall three to ten days later [39]. This study utilized the CCHS 2.2 Share File, restricting the analysis to the 95.3% respondents who agreed to share their information for research purposes[39,40]. Further details on the CCHS 2.2 survey methodology and sampling design can be found elsewhere[40]. In addition to demographic details, the general health component of the CCHS 2.2 captured information on vitamin and mineral supplement use during the 30 days prior to the interview[41]. Exclusion criteria for this study included women who were pregnant or breastfeeding at the time of the survey, those who had an invalid or missing dietary recall, those who reported consuming only breast milk and those who did not report any food items consumed during the 24 hour recall period, resulting in a final sample size of N = 32,776. CCHS 2.2 data was used for folate intake analysis.

The CHMS is a comprehensive direct health measures survey which includes blood, urine and anthropometric measures and banks specimens for future measurements and genetic research. The CHMS has collected data in 3 cycles (2007-2009, 2009-2011 and 2012-2013) with a minimum of 500 respondents for each gender from ten age groups of Canadians 6-79 y (Cycle 1) and 3-79 y (Cycles 2 and 3). Cycles 1, 2 and 3 surveyed 5604, 6395 and 5785 respondents, respectively. The CHMS also captured the intake of vitamin and mineral supplements 30 days

prior to the clinic visit[42,43]. CHMS data was used for the frequency of supplement use and RBC folate level estimates.

Folate Intake Estimates

In order to determine folate consumption habits of Canadians, folate intake was categorized as follows: naturally occurring folate from foods (food folate), the synthetic form from supplements or from fortification (FA). The dietary folate equivalent (DFE) for synthetic FA sources were calculated, a method introduced in 1998 by the FNB and IOM to take into account the higher bioavailability of synthetic FA compared to natural folate[44]. The DFE for specific foods was calculated as the amount of food folate (μg) plus 1.7 times the amount of synthetic FA (μg) from the 24 hour dietary recall thus taking into account both natural folate and synthetic FA. Because supplements taken in the CCHS 2.2 were assumed to have been consumed on an empty stomach, the aforementioned calculation was modified for supplemental FA, resulting in a conversion factor of 2. The DFE values for natural folate and FA from food fortification were also kept as separate values to estimate intake from these different sources.

Folate intakes were calculated for separate Dietary Reference Intakes (DRI) life stage groups including by FA supplement use (total folate intake from all sources was also calculated). As well, the amount of folate from fortified sources was recalculated with an "overage" factor, defined as the "potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage" [36]. For key food groups (breads, baked goods, etc.) the amount of folate in the product, plus overage was calculated by multiplying initial amounts by an adjustment factor based on the following food group categories

determined by Shakur and colleagues: Bread 1.34, Buns and Rolls 1.17, Cookies 1.66, Ready-to-eat Cereal 1.87, Pre-packaged desserts 1.84, Pasta 1.38 and Crackers 1.31[36]. Folate intakes for the three sources and for all the sources, combined, were then compared with the Estimated Average Requirement (EAR) for DRI life stage groups, which is the amount of a nutrient expected to meet the needs of 50% of the population, and the UL[15,16]. Nutrient and food intake data were analyzed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC). Version 1.11 of the SIDE-IML program was used in conjunction with SAS to generate an estimation of the usual dietary intake by using both the first and second dietary recall[45,46] along with bootstrapping weights to estimate variance.

Blood Folate Estimates

Three cycles of CHMS data were pooled to provide a larger dataset for statistical evaluation of red blood cell folate (RBC) analysis. Biomarker analysis is described briefly here and in detail elsewhere[47]. RBC folate status was assessed using cut-offs for elevated blood levels that have been proposed in the literature while the RBC folate values were taken by a certified phlebotomist and measured using the Immulite 2000 assay[42,43]. Participants who did not give blood or had unusable samples were excluded from the analysis, as were respondents under 6 y, to ensure compatibility between cycles (only cycles 2 and 3 had participants age 3 y and above), resulting in a final sample size of 15,754. SPSS, version 22 and STATA 13 software were used to generate an estimation of Canadians with elevated red blood cell folate concentrations along with bootstrapping weights to estimate variance, by life stage group as well as by supplement use.

Recently it has been suggested that RBC folate concentrations differ depending on the assay used, depending on certain variations or calibrations[48]. Using a sample size of 152 individuals and two different assays (Immulite 2000 and the microbiological assay), Colapinto and colleagues used the Deming regression method to create the equation as follows:

Predicted microbiological assay concentration = -22.95 x (0.81) x Immulite 2000 assay concentrations

Given that results from differing assay methods can be large, and that the microbiological assay is deemed the gold standard for determining RBC folate concentrations, it seemed prudent to utilize the adjustment method proposed by Colapinto to convert Immulite 2000 assay results to those on par with the microbiological assay used in NHANES or "NHANES level". However, results must be interpreted with caution, as converting CHMS Immulite 2000 assay to microbiologic assay values lowers RBC folate concentrations, and thus may underrepresent the number of Canadians who have elevated levels of RBC folate.

5.4 Results

Overall Dietary Folate Equivalent Intake

The average DFE intake for Canadians, excluding supplements, was 442 ug. When overage factors were added to the total DFE, intake increased to 487 μg. The highest intakes were seen among males 14-50 y, ranging from 521 to 576 μg (Table 5.1). Among all life stage groups, average DFE intake surpassed the EAR values, irrespective of calculation method. When DFE intake was calculated with the addition of FA from dietary supplements, overall intake was significantly higher for the supplement user group ($p < 0.001$) compared to non-supplement users

by a difference of approximately 900 μg. Intake was also significantly higher among supplement users in all age gender groups when compared to non-supplement users in the same group (Table 5.1). Overall, non-supplement using WCBA had mean DFE intakes of 417 ug ± 113 while those women who consumed supplements had an intake of 1474 ug ± 501.

Table 5.1. Folate intake of Canadians by source and supplement use based on results from the CCHS 2.2

Life Stage Group	Natural Folate	DFE	DFE + Overage[a]	Folic Acid	Folic Acid + Overage[a]
Overall (N = 32776)					
1 to 3 (n= 2117)	144 ± 59	280 ± 103	320 ± 117	114 ± 76	137 ± 83
4 to 8 (n=3235)	173 ± 59	381 ± 105	444 ± 114	162 ± 72	196 ± 79
Male 9 to 13 (n= 2080)	213 ± 69	466 ± 123	541 ± 136	182 ± 81	222 ± 90
Female 9 to 13 (n= 1980)	181 ± 58	403 ± 108	470 ± 119	161 ± 81	196 ± 88
Male 14 to 18 (n= 2288)	255 ± 98	563 ± 182	651 ± 208	231 ± 131	275 ± 144
Female 14 to 18 (n= 2256)	198 ± 77	432 ± 150	495 ± 177	198 ± 136	231 ± 148
Male 19 to 30 (n=1804)	272 ± 101	576 ± 171	653 ± 189	259 ± 170	298 ± 181
Female 19 to 30 (n= 1854)	210 ± 82	410 ± 124	456 ± 133	232 ± 216	254 ± 219
Male 31 to 50 (n= 2596)	262 ± 90	521 ± 155	588 ± 173	241 ± 197	279 ± 209
Female 31 to 50 (n= 2686)	228 ± 99	406 ± 137	446 ± 147	279 ± 286	300 ± 285
Male 51 to 70 (n= 2550)	263 ± 109	463 ± 155	515 ± 169	279 ± 276	305 ± 281
Female 51 to 70 (n= 3200)	223 ± 82	381 ± 113	418 ± 125	281 ± 329	300 ± 329
Male 71 and over (n= 1520)	224 ± 87	394 ± 123	447 ± 139	267 ± 305	292 ± 309
Female 71 and over (n=2610)	195 ± 75	327 ± 102	363 ± 110	268 ± 351	286 ± 352
Overall (n=32776)	230 ± 96	442 ± 160	487 ± 181	217 ± 215	274 ± 238
Supplement Users (N = 8390)[b]					
1 to 3 (n= 603, 28%)	163 ± 61	523 ± 168***	566 ± 169***	191 ± 73 ***	215 ± 74***
4 to 8 (n=1243, 38%)	171 ± 54	580 ± 162***	643 ± 170***	221 ± 73 ***	254 ± 79***
Male 9 to 13 (n= 462, 22%)	218 ± 64	728 ± 256***	810 ± 264***	275 ± 120***	317 ± 128***
Female 9 to 13 (n= 425, 22%)	196 ± 54	694 ± 302***	767 ± 308***	271 ± 146***	308 ± 155***
Male 14 to 18 (n= 293, 15%)	266 ± 91	1227 ± 586***	1318 ± 603***	502 ± 272***	548 ± 282***
Female 14 to 18 (n= 340, 13%)	223 ± 69	1178 ± 638***	1249 ± 640***	501 ± 301***	537 ± 301***
Male 19 to 30 (n= 313, 15%)	290 ± 112	1497 ± 484***	1585 ± 519***	632 ± 231***	678 ± 250***
Female 19 to 30 (n= 452, 24%)	213 ± 70	1328 ± 652***	1370 ± 645***	578 ± 326***	599 ± 327***
Male 31 to 50 (n= 541, 21%)	263 ± 81	1451 ± 652***	1517 ± 667***	616 ± 337***	652 ± 344***

Female 31 to 50 (n= 812, 30%)	220 ± 82	1506 ± 814***	1533 ± 805***	659 ± 408***	675 ± 404***
Male 51 to 70 (n= 631, 25%)	287 ± 113	1654 ± 767***	1702 ± 762***	702 ± 372***	727 ± 370***
Female 51 to 70 (n= 1065, 33%)	238 ± 86	1589 ± 1014***	1623 ± 1002***	299.5 ± 329.4	688 ± 506***
Male 71 and over (n= 372, 25%)	268 ± 98	1644 ± 885***	1703 ± 886***	291.5 ± 308.7	704 ± 432***
Female 71 + (n= 838, 32%)	214 ± 85	1560 ± 1042***	1595 ± 1035***	286.3 ± 351.5	685 ± 517***
Overall (n= 8390, 26%)	235 ± 95	1344 ± 824***	1395 ± 819***	274.3 ± 238.1	546 ± 395***
Non-supplement Users (N = 24386)					
1 to 3 (n= 1514)	136 ± 58	269 ± 111	307 ± 127	78 ± 42	100 ± 53
4 to 8 (n=1992)	173 ± 58	387 ± 102	451 ± 114	126 ± 37	160 ± 48
Male 9 to 13 (n= 1618)	212 ± 70	470 ± 127	543 ± 138	152 ± 46	191 ± 56
Female 9 to 13 (n= 1555)	176 ± 59	396 ± 111	461 ± 122	129 ± 35	164 ± 40
Male 14 to 18 (n= 1995)	252 ± 98	565 ± 180	653 ± 205	186 ± 59	231 ± 75
Female 14 to 18 (n= 1916)	193 ± 77	426 ± 146	487 ± 171	138 ± 51	171 ± 67
Male 19 to 30 (n=1491)	269 ± 98	568 ± 159	644 ± 165	177 ± 55	215 ± 65
Female 19 to 30 (n= 1402)	209 ± 83	417 ± 136	465 ± 147	122 ± 50	148 ± 64
Male 31 to 50 (n= 2055)	262 ± 88	527 ± 163	594 ± 180	155 ± 63	189 ± 76
Female 31 to 50 (n= 1874)	231 ± 100	419 ± 138	464 ± 142	111 ± 37	135 ± 44
Male 51 to 70 (n= 1919)	253 ± 101	445 ± 139	498 ± 151	114 ± 46	140 ± 58
Female 51 to 70 (n= 2135)	215 ± 77	378 ± 103	418 ± 116	94 ± 26	116 ± 40
Male 71 and over (n= 1148)	208 ± 79	371 ± 116	418 ± 131	97 ± 42	122 ± 53
Female 71 and over (n=1772)	185 ± 68	317 ± 97	352.7 ± 107	78 ± 37	97 ± 46
Overall (n=24386)	228 ± 95	442 ±168	503 ± 182	112 ± 55	600 ± 399

[a] Overage is defined as the potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage

[b] Percentages in brackets reflect the proportion of supplement users in each age group

*p < 0.05, ** p < 0.01, *** p < 0.001, †0.1 < p < 0.05, compared to non-supplement users

Supplemental Folate Use

Both CCHS 2.2 and CHMS data showed comparable proportions of Canadians who take FA containing supplements (Table 5.2). Overall the CCHS reported 25% of Canadians consumed a supplement containing FA, ranging by age group from 13% to 38%. Slightly lower results were observed by age group using the combined CHMS cycles (22%). The highest user rate defined by the CCHS was children 4-8 y (38%), and the lowest rate of supplement use occurred among males age 14-18 y (13%). The CHMS also found high rates among children < 8 y (29%) as well as females 70 y + (38%). 23% of males and 28% of females reported use of a supplement containing FA in the CCHS and 19% and 25% reporting use in the CHMS, respectively. With the exception of males 9-13 y, females at every other age group had higher rates of FA supplement use using CCHS 2.2 data. Similar patterns were found using CHMS pooled cycles, with females reporting higher supplement use in every age group with the exception of those under 8 y. Females > 31 y reported higher supplement use proportion than the national average. CHMS data observed the same trend (data not shown). Overall, comparable proportions were reported by WCBA (24% in the CCHS and almost 23% in the CHMS) and in the general population (26% in the CCHS and 22% in the CHMS).

Table 5.2. Proportion of FA supplement users[a] from CCHS 2.2 and CHMS:

Life Stage Group	CCHS 2.2		CHMS combined cycles	
	Males (N=2612)	**Females (N=3932)**	**Males (N=1556)**	**Females (N=1978)**
1 to 3	28.4%		--	
4 to 8	38.4%		28.5%	
9 to 13	22.2%	21.5%	17.5%	20.1%
14 to 18	12.8%	15.1%	11.5%	14.5%
19 to 30	17.4%	24.4%	13.1%	18.6%
31 to 50	20.8%	30.2%	18.4%	27.0%
51 to 70	24.7%	33.3%	22.8%	28.7%
71 and over	24.5%	32.1%	26.0%	38.1%
Overall*	24.7%		22.1%	

[a] Reported vitamin or supplements in the 30 days prior to interview

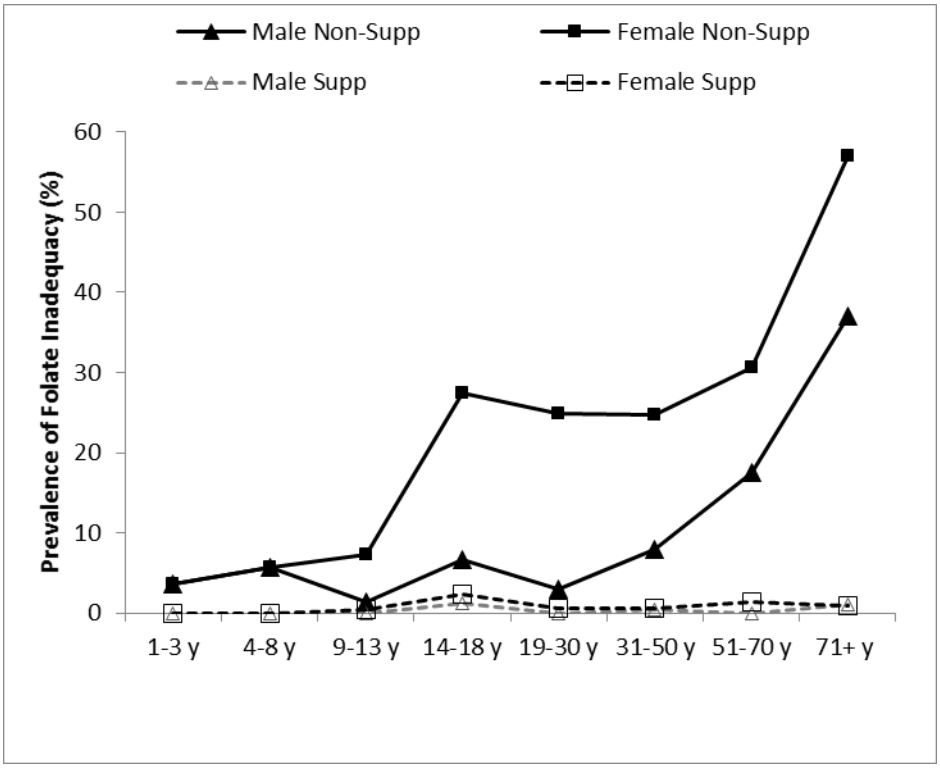
Assessment of the Prevalence of Folate Inadequacy: Dietary Folate

Folate inadequacy among Canadians was calculated by measuring the proportion of individuals in each DRI life stage group who did not meet the EAR criteria for folate (Fig 5.1). Overall, the prevalence of folate inadequacy (POFI) was low (< 6%) among respondents < 13 y, regardless of the calculation method or supplement use status. Among supplement users, the POFI was also very low (<5%) for all adolescent and adult DRI life stage groups, regardless of calculation method. Among non-supplement users, however, the POFI increased with age and was highest among older adults > 50 y, with the highest proportion occurring in females >70 y. When POFI was estimated using only folate from unfortified food sources, inadequacy levels were very high, ranging from 38% to a staggering 94% among females 14-18 y (results not shown). When

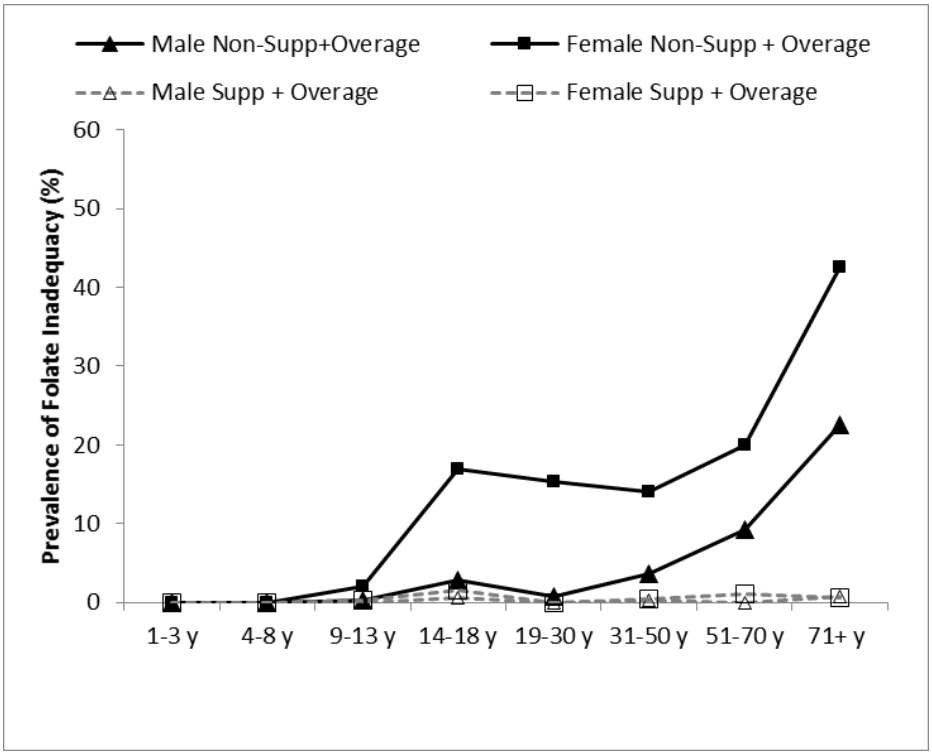
estimated with overage factors, POFI was low among supplement users (<2%), but rose to 43% in older females who did not report supplement use. Additionally, almost one quarter (23%) of older males 70 y and above had intakes below the EAR (Fig 5.1b).

Fig. 5.1. Prevalence of Folate Inadequacy (POFI) by DRI Life Stage Group based on intake levels below the Estimated Average Requirement (EAR) by gender and FA supplement use[a] based on general fortification (1a) and based on general fortification plus overage[b] (1b).

5.1a.



5.1b.



Estimated Average Requirement values (μg): 1-3 y (120), 4-8 y (160), 9-13 y (250), 14-18 y (330), 19+ y (320) [Source: Institue of Medicine]

[a] Supp: supplement user

[b] Overage is defined as the potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage

Assessment of the Prevalence of Folate Inadequacy: Red Blood Cell Folate

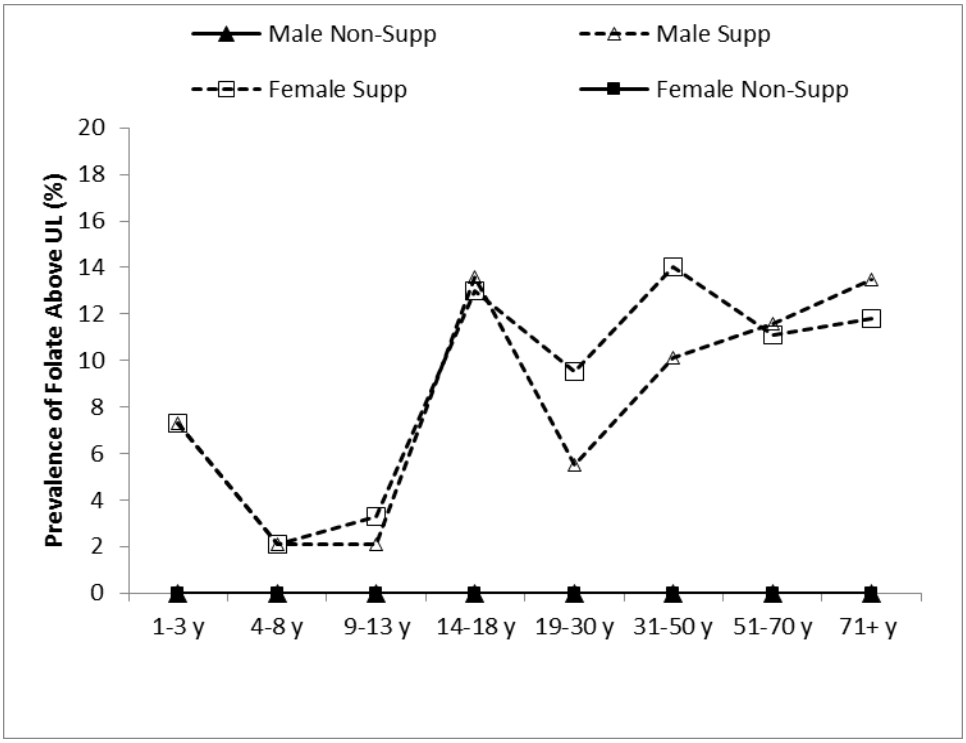
Results from the observed RBC folate concentrations showed that folate deficiency among Canadians (RBC folate < 305 nmol/L) was virtually non-existent (<1%), regardless of whether or not the conversion method was used. This level (305 nmol/L) has been deemed by the IOM to denote a deficiency state, based on its association with megaloblastic anemia[49,50]. Among WCBA, approximately 23% showed red blood cell folate concentrations below those deemed "optimal" for maximal neural tube defect-risk reduction (< 906 nmol/L).

Assessment of the Prevalence of Folate Overconsumption: Dietary Folate

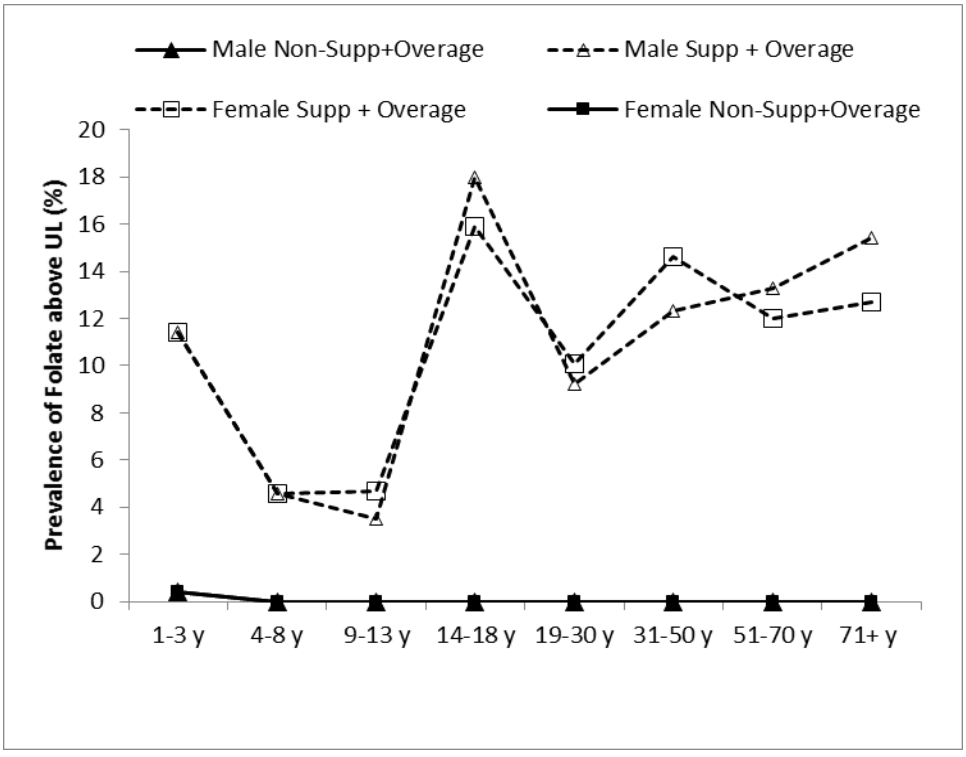
The ULs for folate established by the IOM are based the DFE intake of synthetic forms of folate (i.e., FA). Intake estimates to be compared against the UL do not include natural sources of folate because high intakes from food have not been linked to adverse health effects[15]. Among non-supplement users, intake above the UL was virtually non-existent, even with overage additions taken into account (Figs. 5.2a,b). Among supplement users, however, the percentage with FA intakes above the UL was highest in respondents > 13 y, regardless of gender; and the prevalence rate of folate above UL reached 18% among male supplement users 14-18 y when overage factors were considered.

Fig. 5.2. Prevalence of Folic Acid Overconsumption by DRI Life Stage Group based on intake levels above the Tolerable Upper Intake Level (UL)[*] by gender and FA supplement use[a] based on general fortification (2a) and based on general fortification plus overage[b] (2b).

5.2a.



5.2b.



Tolerable Upper Intake Level values (μg): 1-3 y (300), 4-8 y (400), 9-13 y (600), 14 to 18 y (800), 19+ y (1000) [Source: Institute of Medicine]

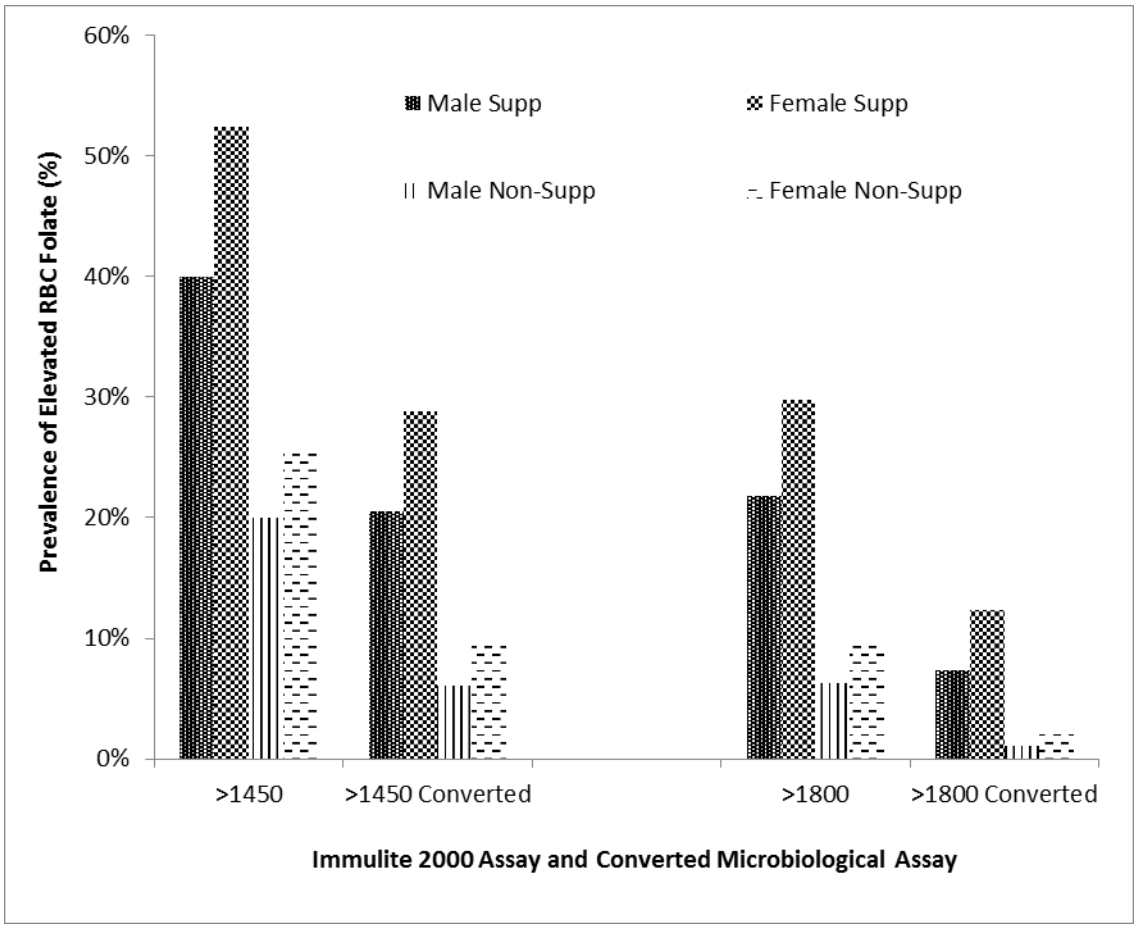
[a] Supp: supplement user

[b] Overage is defined as the potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage

Assessment of the Prevalence of Folate Over-Concentration: Red Blood Cell Folate

High red blood cell folate concentrations do exist among certain sub-groups of the Canadian population (Fig 5.3). Using the previously mentioned lower cut-offs determined by Colapinto et al. (49) in conjunction with their proposed conversion method, almost 30% of female supplement users and 20% of male supplement users had blood folate concentrations above 1450 nmol/L compared with 10% and 6% of non-supplement using females and males, respectively. Using the middle level cut-off, 12 and 7% of female and male supplement users had blood folate concentrations above 1800 nmol/L, compared to 2.5 and 1% of non-supplement users of the corresponding gender. Using the highest cut-offs, 4% of supplement using Canadians had blood folate levels above 2150 nmol/L, compared with <1% of their non-supplement using counterparts (Table S8 of Appendix D).

Fig. 5.3. Prevalence of Elevated Red Blood Cell Folate Concentrations by Gender and Supplement Use Status based on proposed cut-offs, as well as by conversion factor*.



[a] Supp: supplement user
*Converted microbiological assay concentration = -22.95 x (0.81) x Immulite 2000 assay concentrations (Colapinto et al, 2015).

5.5 Discussion

Folate Inadequacy

The current results demonstrate that mandatory folate fortification has led to significant improvements in the overall intake of folate in the Canadian population as the overall prevalence of folate inadequacy (dietary and clinical) was found to be low. However, non-supplement users, in particular adults > 70 y may still be at risk for folate deficiency. Rates of POFI were comparable with previous work by Bailey and Shakur, with the highest POFI occurring among

females > 70 y, and may reflect the fact that older adults tend to consume less food overall[34,51]. These results were similar to recent findings that showed up to one-third of elderly Irish men and women (>64 y) did not meet the requirements for optimal intake(52). Overall, estimates of total DFE intake were comparable to those previously published by Shakur and colleagues using results from the CCHS 2.2[34]. Any small differences may be attributed to disparities in inclusion criteria as well as the fact that their study employed the CCHS 2.2 Master File (n=35,107), while this study utilized the Share File (n=33,469) which is a slightly smaller subset of the Master file containing data on respondents willing to share responses with the survey Share partners[53]. Similarly, overall prevalence of red blood cell folate concentrations < 305 nmol/L (used to estimate deficiency) as well as that of WCBA were similar to results from Colapinto and colleagues using cycle 1 of the CHMS[47].The unique contribution of this study is that it utilized both the 24 hr dietary recall and blood sample estimates to observe folate inadequacy, which is pertinent in light of our objective and strengthens our findings by providing a more in depth assessment of not only dietary intake but also biomarker levels and long term folate status. POFI based on 24 hr dietary recall data were shown to be comparable to the estimates based on RBC Folate data. Results from both surveys showed 24.5% and 23% of WCBA below the optimal level of folate for maximal neural tube defect-risk reduction, indirectly validating the 24 hr recall method on estimating nutrient intake and status.

Supplement Use and Folate Intake

Results from both the CCHS and CHMS showed similar proportions of FA supplement users. Although the highest user rate appeared among respondents age 4-8 y in the CCHS, the CHMS results showed that females > 70 had the highest reported rates of FA supplement use, followed by females 51-70 and children under 8 y. In our study, the prevalence of supplement use among

children 4-8 y (38%), was comparable to the 36% supplement use prevalence observed in US children ≤ 13 y based on NHANES data[51], although slightly lower rates (29%) were observed among children in the CHMS. This difference could be due to the smaller sampling size of children in the CHMS. Our FA supplement use percentages also corresponded with previous CCHS 2.2 analyses[34].

CCHS supplement users were unlikely to be folate deficient. However, as demonstrated by our analysis, it is critical that supplement users should be observed separately from non-supplement users, as this particular group is at an increased risk of possible overconsumption[54]. Intakes of folate in non-supplement using adults in this study were higher than those generated from the NHANES 1999-2000 data (≥ 20 y, n=2121), perhaps due to demographic differences (such as including a younger age range of 2-18 y) or variations in food sources consumed by both populations[55]. Conversely, mean folate intakes from this study were somewhat lower than those previously reported in a study which used data from NHANES 2003-2006 to examine folate intake in Americans ≤ 13 y[7,51]. Pediatric intakes from this study are similar to intakes in Ontario preschool children age 3-5 y (n=254), whose average intake of DFE was calculated to be 336 μg [56]. Results from both surveys showed that <25% of WCBA consumed FA containing supplements, similar to the findings of Shuaibi et al who observed that only 26% of females 18-25 used supplements[14]. However, although supplement use was found to ameliorate folate inadequacy, it should be noted that CCHS 2.2 data also provides evidence that certain subgroups are consuming levels of FA above the UL.

Folate Consumption above the Tolerable Upper Limit and Elevated RBC Folate Concentration

The pooled CHMS data shows that certain Canadians have elevated red blood cell folate concentrations. Rates of intake above the UL for FA reached 18% in male supplement users age 14 to 18 y (when potential overages were accounted for), and as many as 25% of supplement users have elevated blood folate levels. In light of recent speculation that high levels of FA may be potentially linked to increased cancer risk and cognitive decline, this may be cause for concern. Although there is little data on the implications of FA toxicity among growing children and adolescents, some researchers have suggested consideration be given to removing FA from supplements designed for children and men[36].

Previous work has suggested that FA intakes greater than the UL are considered safe and that care must be taken when interpreting risks of intakes greater than the UL[54] because the body of literature used to derive UL for most nutrients is limited[54,57]. However, recent observations from the Framingham study demonstrate that regular users of vitamin supplements have a mean concentration of unmetabolized FA in fasting plasma that is ~40% higher than non-users[17]. Moreover, > 80% of regular vitamin users have detectable levels of unmetabolized FA in their plasma[17,32], which may decrease the cytotoxicity of lymphocytes thought to play a role in the destruction of neoplastic cells in older women[32], a group which this study shows are consuming FA above the UL. This same population is also more susceptible to vitamin B_{12} deficiency[9], and potentially masking this problem with adequate FA intake[9,33,58]. A unique contribution of this study is the estimation and comparison of the prevalence of folate overconsumption and elevated folate status. The proportion of folate consumption above the UL based on dietary intake data were higher than the estimates based on elevated RBC folate concentrations and using the middle

or higher cut offs, but lower than the estimates using the lower cut off. Nevertheless, both estimates (24 hr dietary recall and RBC folate concentration) revealed that higher than optimal folate intakes and elevated blood concentrations exist in the Canadian population.

The data presented in this report is subject to a few limitations. In the CCHS 2.2, only a subset of respondents provided a second day of dietary intake in which to base usual intake estimates, although the use of SIDE software aids in remedying this by using the subset of second day recalls to adjust variance estimates and generate stable usual intake estimates for groups. Although the use of overage factors have been based on a rather limited amount of fortified foods (n=92), the resulting overage percentages reported are comparable to those determined previously by other authors[34-36]. Additionally, the CCHS 2.2 is a self-reported survey and non-sampling errors such as non-response, recall bias and social desirability may affect the validity of results. Although the five step multiple pass method utilized during the 24 hr. recall has been shown to enhance accuracy and assist the respondent in remembering what and how much food they consumed[59], it has been reported that the average under-reporting of energy intake in the CCHS 2.2 is estimated at 10%, with a greater under-reporting rate among respondents who were overweight or obese, adults compared with teenagers, and women compared with men[60]. Finally, a limitation of this study is that there does not exist a universal cut-off for elevated RBC folate, and it must be noted that using higher cut-offs may discount individuals with potentially high folate statuses. Consequently, using slightly higher cut-offs will also provide information on those sub-groups who have a particularly greater prevalence of high folate status.

The reference range of RBC folate varies by age, but generally falls between 317-1422 nmol/L[61]. Researchers have used quantiles of study populations to postulate cut-offs for RBC

folate[47,62,63]. Macfarlane and colleagues proposed an RBC folate cut-off of 1090 nmol/L[62] based on data from Quinlivan and Gregory, who determined the relationship between dietary folate intake and RBC folate to be 2.1 mg DFE per 1090 nmol/L[63]. This reflects a combined intake of 0.4 mg DFE (based on Recommended Dietary Allowance) and a 1 mg FA supplement (bioavailability 1.7 mg). Using results from the Canadian Health Measures Survey (CHMS) 2007-2009, they observed that 63.5% of Canadians had RBC folate levels above the 1090 nmol/L cut-off [62]. However, a more stringent cut-off of 1360 nmol/L, which was based on the concentration at the 97th percentile of the National Health and Nutrition Examination Survey (NHANES) 1999-2004 suggested by Colapinto, reduced this proportion to 40%[47]. Pfeiffer and colleagues suggested 3 cut-offs using NHANES data (RBC folate concentrations obtained via microbiological assay) using upper RBC folate concentrations at the 90th, 95th and 97.5th percentiles of 1820, 2140 and 2490 nmol/L[64]. Colapinto mimicked these percentiles using converted CHMS RBC folate data, obtaining slightly lower cut-offs than Pfeiffer. For the purpose of practicality, this study uses the cut-offs established by Colapinto and colleagues[49] which were based on post-fortification NHANES data, of 1450, 1800 and 2150 nmol/L. In comparison to Macfarlane's results, using the conversion factor and more stringent high RBC folate cut-offs decreased the proportion of Canadians with high RBC folate status to 16.4%[49]. It is evident that both fortified foods and FA supplements are increasing the RBC folate status of Canadians. With this in mind, it is possible that a future RBC folate cut-off based on these higher population intakes may be established. However, any cut-offs established in the future must be treated carefully and be paired with extensive research and long term studies, particularly in light of recent data showing cognitive impairment associated with high plasma folate[18]. It will be

necessary to strike a balance between establishing a cut-off that reflects the dietary changes of the population at hand, but also protects this same population from adverse health effects.

A strength of this project is that the prevalence of folate inadequacy as well as toxicity was based on a combination of dietary, supplemental and clinical measures of status. The results of both survey data showed comparable results on folate intakes below EAR and above UL as well as those above the recommended cut-offs. Therefore, the results of this study are generalizable to other similar populations with a similar food supply environment.

5.6 Conclusions

Results of several studies leave little doubt that mandated folate fortification has led to significant improvements in the overall intake of folate in North America and to impressive decreases in the incidence of NTDs. Ideally, one's diet should provide sufficient intake of folate, and data provides evidence that although folate intake of Canadians has increased post-fortification, consumption of folate from naturally occurring and fortified food sources may not be enough to achieve the desired levels of folate as recommended by the IOM, particularly for WCBA. In light of our results that 23% of WCBA had RBC folate concentration below those deemed "optimal" for maximal neural tube defect-risk reduction, it should be suggested that females 15-45 y who do not consume a FA containing supplement on a daily basis should start to do so if a pregnancy is expected or planned.

Although previous work has suggested that FA intakes above the UL are considered safe, a need for more careful evaluation of the risks and benefits of food fortification, particularly over

mandated levels, is necessary. Recent research shows cognitive impairment associated with high RBC folate, and emerging animal studies highlight other adverse outcomes linked with excessive FA. In light of increasing evidence suggesting that certain subgroups of the population may be at risk by being subjected to high levels of FA , it seems prudent that persons of all ages who may be considering a FA containing supplement should be cautioned about the potential risk involved with FA consumption above the UL. In particular, it may be suggested that vitamins targeted toward children, men, women past child-bearing age and older adults not include FA. Future research and studies monitoring excessive folate intake are imperative in order to find a healthy balance to achieve optimal intake and well-designed longitudinal studies are needed to draw definitive conclusions regarding adverse effects of consuming excess FA. Consequently, it is important that universal guidelines be put in place to define high blood folate status, in order to better inform health professionals and the public about FA guidelines and status.

5.7 Chapter 5.0 References

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CHAPTER 6.0: How to use population-based food frequency data to estimate nutrient intakes: Methodology exploration and recommendations

6.1 Abstract

The food frequency questionnaire has demonstrated good relative validity in the estimation intake of some of the major nutrients in dietary intervention trials and has been shown to be a reasonably valid tool in assessing overall food intake. At the individual level, moderate to high degrees of correlation exist between the food frequency questionnaire and the reference method for most of the food and food groups. However, most correlations between dietary intake and biomarker levels of vitamins such as folate have been weak. The objectives of this study were to 1) explore new methods in estimating nutrient intakes based on food frequency questionnaire data and parameters generated from the 24 hour dietary recall data, 2) explore two other methods using portion sizes (predetermined and fitted) to estimate nutrient intakes based on food frequency data, 3) to assess the correlations of estimated nutrient intakes (folate and vitamin B_{12}) based on the three methods as well as red blood cell folate and serum B_{12} status, and 4) make recommendations of an applicable method to estimate nutrient intakes based on food frequency data. Secondary data analysis was performed using data from the Canadian Community Health Survey, Cycle 2.2's 24 hour dietary recall component (N=32,776). Data from the CCHS 2.2 was used to generate parameters for folate intake from foods as well as B_{12} intake given the complex relationship of folate and B_{12} status to various health outcomes. The method for estimating relative folate and B_{12} intake took into account the food consumed, age and gender, and varying portion sizes. The parameters were then applied to estimate intakes based on food frequency questionnaire data from 3 cycles of the Canadian Health Measures Survey (N=15,754) and

compared with biomarkers of each nutrient. Although estimates of folate and B_{12} intakes based on applying the parameters and from 24hr recall data to the FFQ data showed mostly lower intakes than estimates previously found using 24 hour dietary recall data, these differences were not significantly different, showing that this may be an acceptable method in estimating nutrient intake data from FFQ responses. Similar results were found when using fitted portion sizes from the CCHS 2.2. Overall low correlations between dietary intake and blood levels, particularly for vitamin B_{12} were found regardless of method used. It is possible that other factors such as bioavailability, malabsorption and genetic interactions play an important role in the relationship between folate and vitamin B_{12} intake and status, or perhaps a limitation of the surveys themselves. Further work on folate and vitamin B_{12} intake patterns are necessary to clarify the relationship between nutrient intake and nutrient status. Additionally, it is recommended that other nutrient intakes be calculated using these methods to further test the appropriateness of these novel methods.

6.2 Introduction

Folate (vitamin B_9) is a water soluble vitamin necessary for cell division and growth, which occurs naturally in foods such as leafy green vegetables, fruits and legumes. Its synthetic form, folic acid (FA), is found in supplements and fortified foods [1]. Folate deficiency during the peri-conceptional period (3-4 weeks after conception) has been associated with an increased risk for Neural Tube Defects (NTDs) such as spina bifida and anencephaly [2-4] and several studies have corroborated the effect of folic acid supplementation on lowering the incidence of NTDs and other birth defects[5-7]. As such, adequate folate intake is essential for women of childbearing age (WCBA) and the Institute of Medicine (IOM) recommends that WCBA consume 400 μg (0.4 mg) a day of folic acid in addition to consuming foods naturally rich in folate [8,9]. In 1996,

Canada mandated the addition of FA to white flour and enriched pasta and cornmeal at 0.15 mg FA per 100 g of flour and 0.20 mg FA per 100 g of pasta [9] predicting an overall intake increase of 100 μg/d [10]. Post fortification results showed a dramatic decrease (~40%) in national rates of NTDs [11,12] and an improvement in folate status [13,14]. However, despite this success, there exists some controversy regarding possible health concerns associated with FA overconsumption [15-17]. Vitamin B_{12} (cobalamin) is also an essential water soluble vitamin found in animal foods that has been shown to increase the risk of NTDs when consumed in insufficient amounts [18,19] as well as interacts with folate in terms of metabolic interrelationships[20,21]

Assessment of Dietary Intake of Folate and Vitamin B_{12}

There have been numerous methods used to estimate folate intake, ranging from extensive dietary records, weighed food records (where the participant measures the portion sizes of food consumed as well as food waste) and in-depth assessment of the intake of folate rich foods to short folate screeners and standard food frequency questionnaires (FFQ) to capture dietary patterns. Regardless of the method used, most correlations of estimated intake with RBC folate (or serum folate) have been within the low to moderate range of r=0.05 to r=0.54 [22-25], with slightly higher correlations associated with supplementation [26]. The majority of these studies utilized semi-quantitative FFQ as a test method to assess intake of folate rich foods, with respondents providing details on frequency consumed as well as portions in comparison to a standard. Other methods such as the Food Choice Map and focused recalls have also been used in comparison to blood folate concentration, with similar results [26].

However, the FFQ alone may not provide accurate estimate of folate intake, and evidence involving the association of detailed dietary intake of folate (e.g., 24-hr dietary recall) and RBC folate is lacking. Additionally, some studies have suggested that there are certain genetic variations in folate absorption (hyper and hypo) [27,28]. Therefore, it is essential to assess the association of folate intake from different dietary sources as well as intake measurements with RBC folate as well as in different demographic characteristic groups in order to explore the relationship between these factors and blood folate status. Current literature indicates that the validation of dietary folate intake and blood folate concentrations have been moderate and have been limited mostly to single dietary intake measurement. The rationale behind this study was to introduce a novel approach which will provide information on how nutrient consumption from 24-hr dietary recall and the frequency of consuming foods from key food groups (which were selected because they were high in folate and B_{12}) may contribute to estimating respondents' total folate and vitamin B_{12} intake. Although previous studies have shown strong correlations between B vitamin intake assessed from FFQs and dietary recalls, these studies have used the same respondents [29] while our study seeks to improve the predictive power of the FFQ using 24 hr recall patterns for differing population groups.

Currently there are no Canadian population nutrition surveys which measure both detailed nutrient intake (e.g., 24-hr dietary recall) and blood biomarkers of nutrient status. Our approach consisted of applying the parameters for nutrient intakes developed from population-based 24-hr dietary recall data to estimate the nutrient intakes based on FFQ data of another population-based survey conducted in the similar time frame (within 5-10 y) of the same population. We also estimated the folate and B_{12} intakes by applying the portion size 1) recommended by Canada's

Food Guide to Healthy Eating and 2) calculated using the 24 hour dietary recall to the FFQ data using daily frequency of consumption. The correlations of folate and B_{12} intakes based on these estimates withblood RBC folate and B_{12} will be described. Therefore, the objectives of this study were to 1) explore new methods in estimating nutrient intakes based on food frequency questionnaire data and parameters generated from the 24 hour dietary recall data, 2) explore two other methods using portion sizes (predetermined and fitted) to estimate nutrient intakes based on food frequency data, 3) to assess the correlations of estimated nutrient intakes (folate and vitamin B_{12}) based on the three methods with red blood cell folate and serum B_{12} status, and 4) provide recommendations of an applicable method to estimate nutrients intakes based on food frequency.

6.3 Methodology

Setting and Participants

This study used data from the 2004 Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2) as well as data from 3 cycles of the Canadian Health Measures Survey (CHMS). The CCHS 2.2 utilized a complex cross sectional sample design to collect a representative sample of the Canadian population, living in private dwellings in all ten provinces, excluding those living on reserves and certain remote areas, institutional residents and full-time members of the Canadian Forces [30]. In addition to a comprehensive set of socio-demographic variables, this survey collected data on food intake, supplement use and eating patterns [31]. In total, the CCHS 2.2 surveyed 35,107 respondents, each of whom completed a general health questionnaire as well as a 24 hour dietary recall, administered by trained interviewers. A sub-sample of 10,786 respondents completed a second 24 hour dietary recall three to ten days later[30]. This study

utilized the CCHS 2.2 Share File, restricting the analysis to the 95.3% respondents who agreed to share their information for research purposes [30,31]. In addition to demographic details, the general health component of the CCHS 2.2 captured information on vitamin and mineral supplement use during the 30 days prior to the interview [32]. Exclusion criteria for this study included women who were currently pregnant or breastfeeding, those who reported consuming only breast milk, those who did not report any food items consumed during the 24 hour recall period, and those who had an invalid or missing dietary recall, resulting in a final sample size of N = 32,776. Invalid or missing dietary recalls were defined by Statistics Canada as those with extreme portion sizes and/or nutrient amounts or with incomplete meals and interviews[31,32.] Although some studies have observed that non-consideration of misreporting in statistical models revealed insignificant or even reversed associations and suggest that misreporting should be addressed in the model building process, only 62 individuals out of 35,107 were excluded due to their "implausible" recalls, which would not significantly alter results.

The CHMS is a comprehensive direct health measures survey which includes blood, urine and anthropometric measures and banks specimens for future measurements and genetic research. The CHMS collects data in cycles (2007-2009, 2009-2011 and 2012-2013) with a minimum of 500 respondents for each gender from ten age groups on Canadians 6-79 y (Cycle 1) and 3-79 y (Cycles 2 and 3). Cycles 1,2 and 3 surveyed 5604, 6395 and 5785 respondents, respectively. The CHMS also included a targeted food frequency questionnaire (FFQ) intended to provide estimates of relative nutrient intake linked to specific CHMS nutrient biomarkers, including red blood cell folate (which has been shown to be the best indicator of long term status) and serum B_{12}. The CHMS also captured the intake of vitamin and mineral supplements [33,34]. Cycles of the

CHMS were pooled to provide sufficient numbers for analysis. Biomarker analysis performed from blood samples taken by a certified phlebotomist (and measured using the Immulite 2000 assay) is described briefly here and in detail elsewhere [35]. Participants who did not give blood or had unusable samples were excluded from the analysis, as were respondents under 6 y, to ensure compatibility between cycles (only cycles 2 and 3 had participants age 3 y and above), resulting in a final sample size of 15,754.

Methods

Method 1: Generating Regression Models

Foods represented in the CHMS FFQ were those with the greatest contribution to nutrient intake in the Canadian population, according to the Canadian provincial nutrition surveys conducted throughout the 1990s [34]. Based on these 38 foods, 19 were selected as high sources of folate, and 13 selected as high sources of vitamin B_{12}. High folate foods included: eggs, liver, dried beans, nuts/seeds, hot or cold cereals, brown bread, white bread, pasta, rice, fruit, fruit juice, tomatoes, leafy green vegetables, potatoes (regular and fried) spinach and collards, other vegetables and vegetable juices. High B_{12} foods included liver, red meat, organ meats, beef or pork hot dogs, sausage, bacon, fish (saltwater and freshwater), eggs, milk, cottage cheese, ice cream and yogurt [Table 6.1**]**. The objective of this part of our study was to use data from the CCHS 2.2's dietary recall, obtaining nutrient intakes from the pre-selected folate and B_{12} foods and to create regression models which would provide information on how consuming foods from these preselected food groups contributed to Canadian's total folate and vitamin B_{12} intake. Even though they are both representative of the Canadian population, this study used two separate datasets to compare folate and vitamin B_{12} intake with blood status (CCHS 2.2 and CHMS).

Thusly, we calculated regression models based on age, gender and cultural background to closely match respondents from both surveys.

This novel method took into consideration that intake may vary by person's food culture, age, and sex. Regression models were then created separately for males and females of each of the 14 cultural groups for each of the aforementioned food groups as well as by age to minimize confounding factors linked to these characteristics [Table 6.1, Fig. 6.1**]**. The example below shows an example of a regression model for the amount of Dietary Folate Equivalent (DFE) from pasta. The term 'DFE' was previously calculated as the amount of food folate (μg) plus 1.7 times the amount of synthetic folic acid (μg) from the 24 hour dietary recall thus taking into account both natural folate and synthetic folic acid, as put forth by the Institute of Medicine (IOM) [36].

Table 6.1. Food Group Details for Food Sources used in Regression Equations

Food Item	Details from CHMS FFQ
Folate food sources	
Liver	All types of liver, including beef, veal, pork and chicken, but excluding liverwurst and liver pate
Eggs	Eggs and egg dishes such as omelets, frittatas or quiche, including the yolk (excluding all egg dishes made from egg white)
Beans	Cooked dried beans, such as refried beans, baked beans, pea soup or kidney beans and excluding green or yellow beans
Tomatoes	Tomatoes, tomato sauce, including salsa, tomato soup, spaghetti sauce but excluding pasta, ketchup and pizza sauce
Lettuce	Lettuce or leafy green salads with or without other vegetables
Spinach	Includes spinach, cabbage, mustard greens or collards, excluding kale
Pasta	Any kind of pasta, including spaghetti, noodles, macaroni and cheese or pasta salad
Rice	Instant, seasoned or wild rice
Nuts and Seeds	Peanuts, walnuts, seeds, other nuts, excluding nut butters
Fruits	Fresh, frozen or canned
Fruit Juices	100% pure fruit juices such as apple, orange or grapefruit, regardless of whether or not they are made from concentrate
Potatoes	Baked, boiled, mashed or in potato salad, but excluding sweet potatoes
Potatoes-Fried	French fires, home fries, or hash brown potatoes
Vegetables	All other vegetables not mentioned
Vegetable Juices	No description
White Bread	White breads, including bagels, rolls, pita breads and tortillas
Brown Bread	Brown breads, including bagels, rolls, pita breads and tortillas
Cereal: Hot and Cold	Split into 2 due to differing folic acid levels
B12 food sources	
Hot Dogs	Beef or pork hot dogs
Bacon	Sausage or bacon, including all types of sausages such as breakfast, pepperoni and Kielbassa but excluding light, low fat or turkey varieties
Red Meat	Beef, hamburger, pork or lamb
Liver	All types of liver, including beef, veal, pork and chicken, but excluding liverwurst and liver pate
Offal	Organ meats such as kidneys, heart or giblets
Eggs	Eggs and egg dishes such as omelets, frittatas or quiche, including the yolk (excluding all egg dishes made from egg white)
Freshwater Fish	Trout, walleye, pickerel or other freshwater varieties
Saltwater Fish	Salmon, tuna, fish sticks or other saltwater varieties
Shellfish	Shrimp, mussels, scallops, lobster, clams, oyster or crab
Cottage Cheese	Cottage cheese
Yogurt	Yogurt (excluding ice creams or frozen yogurts)
Ice cream or frozen	Ice cream or frozen dairy desserts such as frozen yogurt

yogurt	
Milk	Milk or enriched milk substitutes

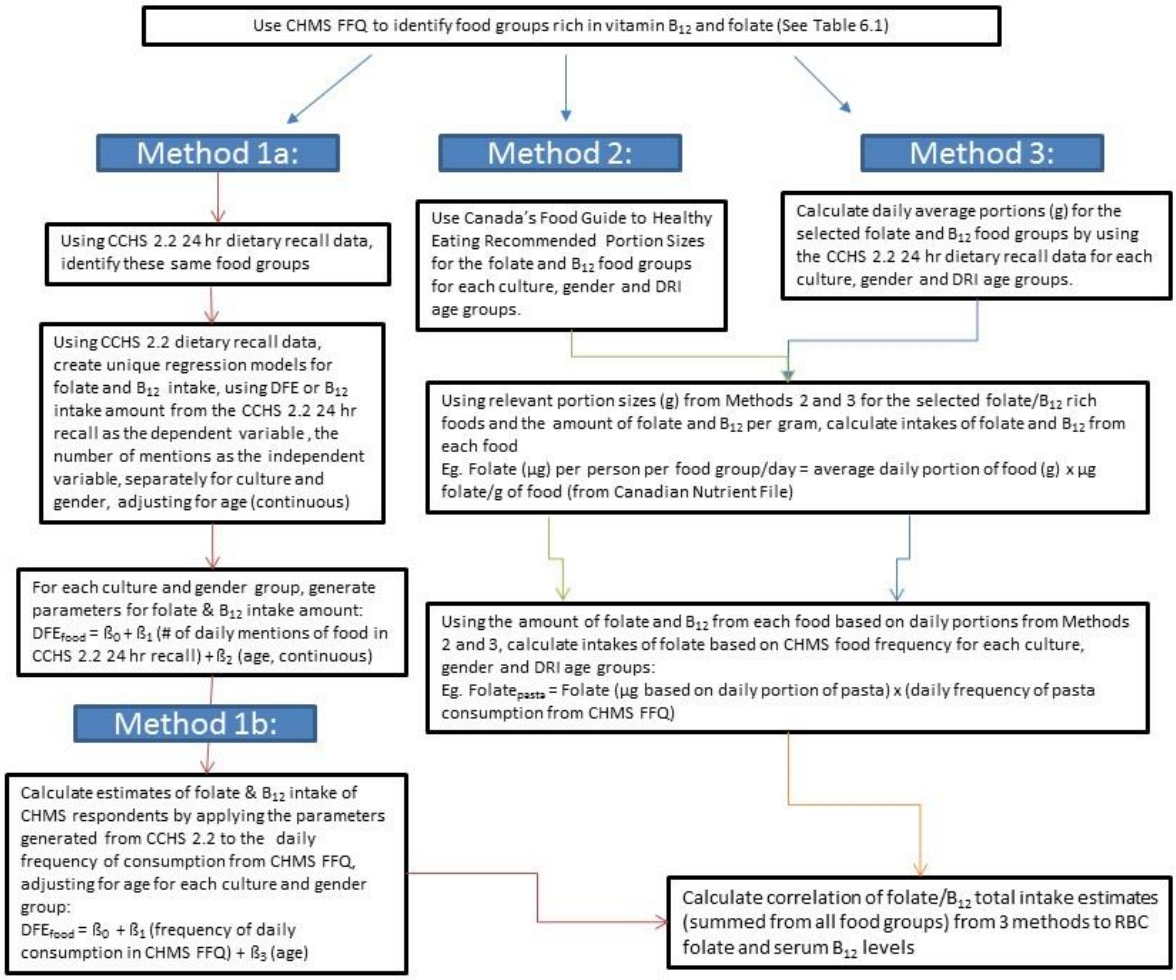

Fig. 6.1. Overview of Methodology

Model 1a: Sample Regression Equation

Total DFE intake from pasta = $\beta_0 + \beta_1$ (times pasta was consumed from CCHS 2.2 24 hour dietary recall) + β_2 (age, continuous)

In this equation, β_0 is the intercept parameter, and β_1 and β_2 are the parameters which will be used to estimate intake using the CHMS FFQ (frequency of daily consumption and age, respectively).

Generating Intakes Using CCHS 2.2 Developed Parameters

For example: using data from the CCHS 2.2 specific to females of Caucasian culture, the equation is shown as follows:

Total DFE intake from pasta = 3.9 + 162 (x frequency of daily consumption from the CHMS FFQ) -0.1 (age of respondent).

In this equation, β_0 is the intercept parameter, β_1 is the parameter that will be multiplied by the daily frequency of consumption of each food using the CHMS FFQ and β_2 is the age of the CHMS respondent.

Method 1b: Applying parameters to FFQ data

The parameters produced using method 1a were then used in the CHMS dataset, where units of consumption (calculated per day for each predetermined food group based on the FFQ response portion of the CHMS) and age was substituted into the formula to obtain estimates of folate and vitamin B_{12} intake from each food group. This was done separately for each specific culture and for males and females separately. These regression models were then used in conjunction with FFQ results (units of consumption per day based on "how often" the categorized food item was consumed by the respondent) from the CHMS to obtain the amount of dietary folate and vitamin B_{12} per day per food item. The total intakes of these nutrients were then calculated by summing

the amount of folate and vitamin B_{12} from each of the applicable food groups and comparing them with the blood levels of these nutrients.

Method 2: Predetermined Portion Sizes

Considering that the frequency (or how often) a food is consumed does not define the *amount* of food consumed, we further tested another model by applying the amount of food using the recommended portion size according to Canada's Food Guide to Healthy Eating (CFGHE) on each of the predetermined foods (one serving). It is commonly understood that people usually consume more food than the recommended servings [37]. Therefore it was hypothesized that the generated folate and B_{12} amounts estimated based on this model would be underestimated. Using CFGHE, portion sizes were obtained for the folate and B_{12} foods from Table 6.1 by life stage groups. Using these corresponding portion sizes (g) from for the selected folate/B_{12} rich foods and the amount of folate and B_{12} per gram of these foods (determined from the Canadian Nutrient File) the total intakes of folate and B_{12} from each food was calculated per respondent and summed for a total intake of folate and vitamin B_{12}.

Eg. Folate (µg) per person per food group/day = average daily portion of food (g) x µg folate/g of food (from Canadian Nutrient File)

$Folate_{pasta}$ = Folate (µg based on daily portion of pasta) x (daily frequency of pasta consumption from CHMS FFQ)

Method 3: "Fitted" Portion Sizes

The third method created to compare intakes and status relied on portion size data from both the CCHS 2.2, again using concepts put forth by Nothlings [38] and Hjartaker [39]. The objective was to obtain mean portion size data (g) from the 24 hour dietary recall component of the CCHS 2.2 by life stage groups to obtain *daily* intakes (g) of the aforementioned foods reported in the CHMS FFQ. These intakes will be referred to henceforth as "fitted" intakes. Similarly, intakes from CFGHE (or "predetermined" intakes) were also obtained for the same foods (Method 2). Using these g/day values and the Canadian Nutrient File (CNF) [40], the amount of folate (DFE) and B_{12} - was calculated for each food group by its unique fitted portion size (which was specific to a life stage group) as well as the predetermined portion size recommended by CFGHE.

- For example: if the mean fitted portion size of nuts or seeds for a 13 y old male determined from the CCHS 2.2 was *40 g*, and the average amount of folate per 100 g serving was *31 µg* (calculated from the CNF), it is assumed that this respondent obtained 12.4 µg folate from these foods.

These values were then multiplied by the frequency per day of consumption of the corresponding food group (from the CHMS FFQ portion of the survey).

In this scenario, if the frequency of consumption of nuts or seeds was 0.5 times per day, then the respondent was assumed to have consumed 6.2 µg of folate from this individual food group. These individual intakes were summed to obtain total folate and B_{12} intakes from each of the preselected food groups shown in Table 6.1. Additionally, supplemental FA and B_{12} was added

to the intake of those respondents who reported taking a supplement or multivitamin containing either nutrient, with the amount corresponding to the Anatomical Therapeutic Chemical (ATC) Classification System.

These calculated intakes were then compared to the blood levels of the nutrients using weighted correlation analysis and SPSS and Pearson Product Moment Correlation. SPSS, version 22 and STATA software version 13 were used along with bootstrapping weights for this analysis. Additionally, mean intakes generated from each of the three aforementioned method were compared to intakes from the CCHS 2.2 24 hr dietary recall using independent sample t-tests and correction (Bonferroni) for multiple comparisons.

6.4 Results

Folate and B_{12} Intakes Calculated from Regression Models (age adjusted culture and gender specific parameters) (Method 1)

Folate

When folate intake was generated using parameters from the CCHS 2.2 among CHMS respondents, overall intake was 415 μg. Among men, folate intake was approximately 451 μg, and among women, 379 μg. The highest intakes calculated were among adults > 70 y as well as males ≤ 8 y. Among non-supplement users, highest intakes were calculated in males 19-30 y and females 31-50 [Table 6.2a].

Vitamin B_{12}

When vitamin B_{12} intake was calculated from the regression method, overall intake was 10.3 μg. Among men, B_{12} intake was 14.1 μg and among women, 6.4 μg. Supplement users had more than triple the intake of non-supplement users (21.1 μg vs 6.6 μg, respectively) (Data not shown). The highest overall intakes of B_{12} were also among older adults, in particular, men > 50 y. Among non-supplement users, intakes were highest among older men > 50 y and women 14-18 and 70 + y [Table 6.2 b].

Folate and B_{12} intakes based on Predetermined Portions (Method 2) and Portions Fitted Model (Method 3)

Folate

Intakes generated by using predetermined intakes were 4 percent lower than those calculated when using the regression methods, while fitted intakes were 11 percent higher than those

observed using regression models. The highest intakes of folate were observed among females >70 y using both portion methods, and the lowest was among males 14 to 18 y when predetermined portions were used, and males 19 to 30 y when fitted portion sizes were used. Among non-supplement users, intake was highest among young males 9-13 y and females 51-70 using predetermined portions, and among males 19-30 y and females 31-50 y using fitted intakes [Table 6.2a].

Vitamin B_{12}

Intake of vitamin B_{12} was 4 percent higher when fitted portions were used to calculate intake compared to predetermined portions. Intakes using both predetermined and fitted portions were approximately 3.5 times higher than intake calculated from Method 1. The highest intakes of B_{12} were also observed among older adults > 70 y, both overall and by supplement use, with low intakes seen among children ≤ 8 y, regardless of portion size used [Table 6.2b].

Comparing to the estimates based on CCHS 2.2 dietary recall

Folate

It is interesting to note that when calculated intakes from the three methods were compared to intakes from the CCHS 2.2's dietary recall, in particular, both regression model (age adjusted culture and gender specific parameters) and fitted portions produced comparable (i.e., $P > 0.05$) folate intake estimates as the intakes from 24 hr dietary recall. In particular, folate intakes generated for males 9-13 y, and females 19-30 y and 51-70 y were comparable (< 50 µg difference). Similarly, the intake of females 19-30 y generated by the predetermined portion method was almost identical to that obtained from the CCHS 2.2 dietary recall (32 µg

difference). Folate intake was generally underestimated when both regression and predetermined portion methods were used, with the exception of males and females > 70 y. Statistically, when compared with 24 hr recall intakes using independent t-tests, only overall predetermined intakes for males were found significantly different ($p < 0.016$). However, among non-supplement users, intakes were significantly different using all 3 methods [Table 6.2a].

Vitamin B_{12}

Predetermined and fitted portions produced comparable B_{12} intake estimates as the estimates from 24 hr dietary recall for males, and regression models produced comparable B_{12} intakes for females. B_{12} intake calculated for females ≤ 30 y generated by regression models (Method 1) provided close estimates to those reported from the 24 hr dietary recall (< 1 µg). In particular among respondents ≤ 30 y, vitamin B_{12} calculated from the regression methods was fairly similar to CCHS 2.2 intakes (between 0-4 µg differences). However, overall intakes for males generated from regression models were statistically different than those reported from the 24 hour dietary recall when adjusted for multiple comparisons, as were intakes from non-supplement using males when using predetermined portions ($p<0.016$). Both fitted and predetermined portions overestimated B_{12} intake among all ages and gender by as much as 82.5 µg. Significant differences were found between overall intakes for females using both predetermined and fitted portions when compared to 24 hr recall intakes ($p<0.016$) [Table 6.2b]. It is noted that when supplement users and non-supplement user's B_{12} intake were estimated separately, both regression models (method 1) and portion size (method 2) produced comparable B_{12} intakes for both males and females [Table 6.2b].

Table 6.2a Calculated Folate Intake of Canadians based on 24 hr recall data (CCHS2.2) and estimates based on FFQ data (CHMS) by using three methods in the Canadian adult population

Gender	Age Group (y)	DFE Folate (CCHS 2.2)	Folate Intake from Regression Models (µg)[1]	Difference[c] between Regression and CCHS 2.2 intake (µg)	Folate Intake using Predetermined Portions[1,a] (µg)	Difference[c] between Predetermined Portions and CCHS 2.2 intake (µg)	Folate Intake using Fitted Portions[1,b] (µg)	Difference[c] between Fitted Portions and CCHS 2.2 intake (µg)
Both supplement and non-supplement users								
Men	≤ 8*	NA	525.7 ± 27.2	NA	390.3 ± 16.0	NA	417.2 ± 15.1	NA
	9 to 13	466 ± 123	432.2 ± 12.8	+33.8	331.6 ± 9.5**	+134.4	398.3 ± 9.4	+67.7
	14 to 18	563 ± 182	388.0 ± 15.9	+175.0	313.5 ± 11.8**	+249.5	446.5 ± 13.7	+116.5
	19 to 30	576 ± 171	402.9 ± 19.2	+173.1	319.5 ± 15.9**	+257.5	342.2 ± 20.2	+233.8
	31 to 50	521 ± 155	438.9 ± 13.8	+82.1	349.0 ± 17.2**	+172.0	470.1 ± 24.8	+50.9
	51 to 70	463 ± 155	492.9 ± 19.3	-29.9	372.9 ± 18.4**	+90.1	451.2 ± 19.5	+11.8
	70 +	394 ± 123	543.9 ± 36.3	-149.9	433.6 ± 46.2**	-39.4	468.8 ± 47.1	-74.8
Women	≤ 8*	NA	324.3 ± 19.8	NA	348.3 ± 10.7	NA	397.6 ± 15.0	NA
	9 to 13	403 ± 108	260.5 ± 21.2	+142.5	348.3 ± 10.7	+83.7	368.3 ± 11.2	+63.7
	14 to 18	432 ± 150	298.4 ± 23.1	+133.6	322.3 ± 15.0	+109.7	408.1 ± 16.0	+23.9
	19 to 30	410 ± 124	399.3 ± 21.8	+11.7	442.1 ± 40.6	-32.1	523.4 ± 40.7	-113.4
	31 to 50	406 ± 137	324.3 ±	+78.7	463.9 ± 19.8	-60.9	523.3 ±	-84.3

Gender	Age Group (y)	DFE Folate (CCHS 2.2)	Folate Intake from Regression Models (µg)[1]	Difference[c] between Regression and CCHS 2.2 intake (µg)	Folate Intake using Predetermined Portions[1,a] (µg)	Difference[c] between Predetermined Portions and CCHS 2.2 intake (µg)	Folate Intake using Fitted Portions[1,b] (µg)	Difference[c] between Fitted Portions and CCHS 2.2 intake (µg)
			19.8				22.1	
	51 to 70	381 ± 113	415.5 ± 19.2	-34.5	448.7 ± 22.3	67.7	465.3 ± 23.0	+47.3
	70 +	327 ± 102	535.8 ± 44.8	-208.8	529.6 ± 41.3	202.6	525.7 ± 40.8	+198.7
				Supplement Use				
Non Supplement Users								
Men	≤ 8*	NA	112.4 ± 20.8	NA	206.3 ± 90.8	NA	251.8 ± 87.6	NA
	9 to 13	470 ± 127	287.3 ± 94.4**	+182.7	291.5 ±79.9**	+218.5	286.1 ± 93.3**	+183.9
	14 to 18	565 ± 180	249.5 ± 130.4**	+315.5	240.5 ± 178.3**	+324.5	284.9 ± 160.4**	+280.1
	19 to 30	568 ± 159	289.9 ± 150.9**	+278.1	250.5 ± 187.1**	+317.5	384.9 ± 190.4**	+183.1
	31-50	527 ± 163	284.5 ± 116.0**	+242.5	236.2 ± 95.9**	+290.8	254.4 ± 162.1**	+272.6
	51-70	445 ± 139	266.2 ± 117.7**	+178.8	227.1 ± 146.7**	+217.9	247.8 ± 269.2**	+197.2
	70 +	371 ± 116	262.2 ± 85.8**	+108.8	232.1 ± 78.0**	+138.9	262.2 ± 98.3**	+108.8
Women	≤ 8*	NA	91.3 ± 12.2	NA	240.8 ± 98.3	NA	262.5 ± 95.3	NA
	9 to 13	396 ± 11	102.1 ± 23.2**	+293.9	247.9 ± 83.7**	+148.1	270.8 ± 92.0**	+125.2
	14 to 18	426 ± 14	88.2 ±	+337.8	215.2 ± 90.0**	+210.8	318.1 ±	+107.9

Gender	Age Group (y)	DFE Folate (CCHS 2.2)	Folate Intake from Regression Models (µg)[1]	Difference[c] between Regression and CCHS 2.2 intake (µg)	Folate Intake using Predetermined Portions[1,a] (µg)	Difference[c] between Predetermined Portions and CCHS 2.2 intake (µg)	Folate Intake using Fitted Portions[1,b] (µg)	Difference[c] between Fitted Portions and CCHS 2.2 intake (µg)
			28.5**				143.6**	
	19 to 30	417 ± 136	92.2 ± 20.5**	+324.8	235.2 ± 102.0**	+181.8	317.1 ± 133.7**	+99.9
	31-50	419 ± 138	99.1 ± 32.2**	+319.9	240.0 ± 94.6**	+179.0	323.1 ± 132.9**	+95.9
	51-70	378 ± 103	96.5 ± 28.8**	+281.5	252.5 ± 62.3**	+125.5	322.2 ± 109.5**	+55.8
	70 +	317 ± 97	81.0 ± 29.7**	+236.0	236.5 ± 91.4**	+80.5	234.6 ± 97.9**	+82.4
Supplement Users		1344 ± 824	1240.5 ± 4.7	+103.5	938.8 ± 25.6	+405.2	1001.3 ± 26.1	+342.7
Overall		230 ± 96	414.6 ± 8.4	-184.6	397.0 ± 8.4	-167.0	460.0 ± 9.2	-230.0

[1] Values are mean ± SD

[a] Calculated from the CCHS 2.2 24 hour dietary recall

[b] Recommended by Canada's Food Guide to Healthy Eating

[c] Difference in mean intake from CCHS and calculated intake using appropriate method

** Statistically different from intakes calculated from CCHS 2.2 dietary recall ($p<0.016$)

Note: Folate estimates for younger children by age and sex are not available due to small sample sizes

Table 6.2b. Calculated B_{12} intake of Canadians by method

Gender	Age Group (y)	B_{12} (CCHS 2.2)	B_{12} Intake from Regression Models (µg)[1]	Difference[c] between Regression and CCHS 2.2 intake (µg)	B_{12} Intake using Predetermined Portions[1,a] (µg)	Difference[c] between Predetermined Portions and CCHS 2.2 intake (µg)	B_{12} Intake using Fitted Portions[1,b] (µg)	Difference[c] between Fitted Portions and CCHS 2.2 intake (µg)
Both Supplement and Non Supplement Users								
Men	≤ 8*	NA	6.6 ± 0.3	NA	6.3 ± 0.4	NA	8.2 ± 0.5	NA
	9 to 13	4.6 ± 1.7	6.6 ± 0.2**	-2.0	8.7 ± 2.4	-4.1	11.6 ± 2.5	-7.0
	14 to 18	5.7 ± 2.6	6.8 ± 0.3**	-1.1	9.5 ± 1.4	-3.4	13.6 ± 10.2	-7.9
	19 to 30	5.4 ± 2.8	9.6 ± 0.6**	-4.1	23.8 ± 10.1	-18.4	27.4 ± 10.4	-22.0
	31 to 50	5.4 ± 2.9	14.4 ± 0.5**	-9.0	19.8 ± 3.6	-14.4	22.0 ± 3.8	-16.6
	51 to 70	4.9 ± 2.5	20.3 ± 0.9**	-17.4	27.8 ± 4.6	-22.9	30.8 ± 4.6	-25.9
	70 +	4.6 ± 3.3	23.5 ± 1.5**	-18.9	83.0 ± 21.3	-78.4	86.8 ± 21.8	-82.2
Women	≤ 8*	NA	4.2 ± 0.2	NA	6.5 ± 0.4	NA	5.8 ± 0.5	NA
	9 to 13	3.4 ± 1.3	3.5 ± 0.2	-0.1	7.4 ± 1.4**	-4.9	6.6 ± 1.5**	-3.2
	14 to 18	3.3 ± 1.5	3.3 ± 0.2	0	21.2 ± 8.8**	-17.9	21.3 ± 9.1**	-18.0
	19 to 30	3.5 ± 1.5	4.2 ± 0.3	-0.7	32.4 ± 14.4**	-28.9	32.1 ± 14.8**	-28.6
	31 to 50	3.9 ± 2.5	8.4 ± 0.4	-4.5	72.4 ± 13.3**	-68.5	72.6 ± 13.6**	-68.7
	51 to 70	3.9 ± 2.2	8.4 ± 0.4	-4.5	76.4 ± 13.3**	-72.5	74.6 ± 13.7**	-70.7
	70 +	3.6 ± 2.3	11.4 ± 0.8	-7.8	85.9 ± 17.9**	-82.3	86.1 ± 18.1**	-82.5
Supplement Use								
Non Supplement Users								
Men	≤ 8*	NA	3.8 ± 1.2	NA	3.5 ± 0.4	NA	3.8 ± 0.5	NA
	9 to 13	4.6 ± 3.8	5.3 ± 3.9	-1.3	6.8 ± 6.4	-2.2	10.3 ± 8.4**	-5.7
	14 to 18	5.7 ± 2.5	5.2 ± 3.7	+0.5	5.8 ± 4.3	-0.1	6.7 ± 5.0**	-1.0

Gender	Age Group (y)	B_{12} (CCHS 2.2)	B_{12} Intake from Regression Models (µg)[1]	Difference[c] between Regression and CCHS 2.2 intake (µg)	B_{12} Intake using Predetermined Portions[1,a] (µg)	Difference[c] between Predetermined Portions and CCHS 2.2 intake (µg)	B_{12} Intake using Fitted Portions[1,b] (µg)	Difference[c] between Fitted Portions and CCHS 2.2 intake (µg)
	19 to 30	5.2 ± 2.5	6.1 ± 5.2	-0.9	8.2 ±6.5	3.0	13.7 ± 6.8**	-8.5
	31 to 50	5.3 ± 2.9	8.4 ±7.8	-3.1	7.9 ± 55.1	2.6	12.8 ± 6.3**	-7.5
	51 to 70	4.9 ± 2.5	12.3 ± 10.2	-7.4	18.4 ± 15.2	13.5	21.6 ± 13.6**	-16.7
	70 +	4.4 ± 3.6	21.0 ± 15.5	16.6	28.7 ± 18.7	24.3	24.9 ± 21.8**	-20.5
Women	≤ 8*	NA	2.2 ± 2.4	NA	3.6 ± 3.7	NA	4.3 ± 3.5	NA
	9 to 13	3.3 ± 1.1	2.0 ± 1.9	+0.7	4.5 ± 3.5	-1.2	5.2 ± 33.9	-1.9
	14 to 18	3.4 ± 1.5	4.2 ± 2.1	-0.8	4.3 ± 2.4	-0.9	4.9 ± 2.8	-1.5
	19 to 30	3.4 ± 1.6	2.3 ±1.4	+1.1	4.4 ± 2.1	-1.0	6.7 ± 4.2	-3.3
	31 to 50	4.0 ± 2.7	2.5 ± 2.0	+1.5	5.2 ± 2.8	-1.2	5.4 ± 2.1	-1.4
	51 to 70	4.0 ± 2.1	2.7 ± 1.7	+1.3	19.6 ± 12.5	-15.6	19.8 ± 12.3	-15.8
	70 +	3.4 ± 1.7	4.0 ± 3.6	-0.6	16.6 ± 13.9	-13.2	16.7 ± 12.4	-13.3
Supplement Users		55.5 ±157.2	21.1 ± 0.3	-34.4	125.1 ± 12.6	-69.6	124.4 ± 12.8	-68.9
Overall		4.5 ± 2.7	10.3 ± 0.2	-5.8	35.1 ± 3.4	-30.6	36.4 ± 3.4	-31.9

[1] Values are mean ± SD

[a] Calculated from the CCHS 2.2 24 hour dietary recall

[b] Recommended by Canada's Food Guide to Healthy Eating

[c] Difference in mean intake from CCHS and calculated intake using appropriate method

** Statistically different from intakes calculated from CCHS 2.2 dietary recall ($p<0.016$)

Note: B_{12} estimates for younger children by age and sex are not available due to small sample sizes

Correlations between Dietary Intakes and Blood Levels of Folate

Folate

Overall, poor correlations (< 0.1) were shown between the generated intakes of folate by all three models and RBC folate in younger populations (age ≤ 30 y), regardless of gender. For older age groups (age > 30 y), the regression model produced relatively greater correlation coefficients (r = 0.12 – 0.23, $p<0.01$) for both men and women. Predetermined and fitted portions produced greater correlation coefficients only for older age groups in men (age > 50 years, r = 0.12 – 0.16, $p<0.01$) and adults women (age > 19, r = 0.10 – 0.30, $P<0.01$).

By life stage, correlations between levels of RBC folate and calculated regression intakes of ranged the strongest correlations observed among males and females > 70 y (Regression model r = 0.17 and 0.23; Predetermined & fitted portions 0.10 – 0.3). Negative correlations were observed when respondents were separated by supplement use [Table 6.3a].

Slightly stronger correlations were observed using predetermined and fitted portions (0.16 and 0.17, respectively). Although they were still low, gender differences were observed when using the portion size method to calculate folate intake, with females having higher correlations than males, using both fitted and predetermined methods (0.14 and 0.13 vs 0.07 and 0.05). When correlations were observed by life stage group, females > 70 y showed the highest correlation of folate intake and status (0.3), regardless of portion size method used [Table 6.3a].

However, when we utilized the adjustment method proposed by Colapinto [41] to convert RBC folate Immulite 2000 assay results to those on par with the microbiological assay used in the

National Health and Nutrition Examination Survey (NHANES), which is the "gold standard", correlations were improved among all life stage groups, with correlations as high as 0.38 among women > 70 y. However, when we observed non-supplement users only, generally correlations below 0.1 were observed, with the exception of males 70 + and females 9-13 y using the regression method (0.20), and males and females 19-30 and 51-70 y using predetermined portions. As well, males 19-30 y (0.14) and males and females 51-70 y had correlations > 0.1 using fitted portions [Table 6.3a].

Vitamin B_{12}

Poor, negative correlations of B_{12} were seen among children and adolescents, with stronger negative correlations found using predetermined as well as fitted portions, in particular, among men 70 + y (0.12) [Table 6.3b]. A similar pattern was observed for B_{12}, with even weaker correlations observed among regression generated B_{12} intakes and serum B_{12}, ranging from -0.001 (males ≤ 8 y) to 0.18 (males > 70 y). Among non-supplement users, the majority of correlations were < 0.1, with the exception of females 9-13 y (-0.31) using fitted portions and males 4-8 (0.21) using regression intakes. Among supplement users, no correlation was observed (0.01) [Table 6.3a].

Table 6.3a. Pearson correlation coefficient between generated folate intake and RBC[a] folate-status

Gender	Age Group (y)	Correlation with RBC Folate			Correlation with Converted RBC Folate		
		Regression Models	Predetermined Portions	Fitted Portions	Regression Models	Predetermined Portions	Fitted Portions
Both supplement and non-supplement users							
Men	≤ 8*	-0.08**	-0.08**	-0.07**	0.002**	0.04**	0.03**
	9 to 13	0.04**	0.08**	0.08**	0.17**	0.14**	0.13**
	14 to 18	-0.06**	-0.05**	-0.05**	0.12**	0.12**	0.01**
	19 to 30	-0.01**	-0.01**	-0.03**	0.06**	0.08**	0.08**
	31 to 50	0.12**	0.09**	0.05**	0.18**	0.14**	0.08**
	51 to 70	0.19**	0.12**	0.12**	0.33**	0.26**	0.26**
	70 +	0.17**	0.16**	0.16**	0.36**	0.25**	0.24**
Women	≤ 8*	0.08**	0.07**	0.08**	0.16**	0.19**	0.19**
	9 to 13	0.01**	0.05**	0.06**	0.22**	0.12**	0.12**
	14 to 18	0.03**	0**	0**	0.24**	0.25**	0.24**
	19 to 30	0**	0.12**	0.12**	0.21**	0.13**	0.12**
	31 to 50	0.12**	0.11**	0.10**	0.26**	0.23**	0.20**
	51 to 70	0.13**	0.26**	0.26**	0.34**	0.32**	0.32**
	70 +	0.23**	0.3**	0.3**	0.38**	0.25**	0.25**
Supplement Use							
Non Supplement Users							
Men	≤ 8*	0.05**	0.05**	0.07**	-0.10**	-0.05**	-0.06**
	9 to 13	-0.01**	0.10**	0.09**	0.10**	0.10**	0.08**
	14 to 18	-0.07**	-0.48**	-0.03**	0.08**	0.08**	0.05**
	19 to 30	0.07**	0.04**	-0.01**	0**	0.20**	0.14**
	31 to 50	-0.02**	-0.04**	-0.04**	0.04**	-0.08**	-0.08**
	51 to 70	0.01**	0.05**	0.04**	0.05**	0.13**	0.11**
	70 +	0**	0**	0**	0.20**	0.20**	0.09**
Women	≤ 8*	-0.15**	0.03**	0.06**	0**	0.06**	0.08**
	9 to 13	-0.10**	0.02**	0.04**	0.05**	0.04**	0.04**
	14 to 18	-0.14**	-0.18**	-0.07**	0.20**	0.01**	0**
	19 to 30	-0.14**	-0.01**	-0.04**	-0.04**	0.12**	0.06**
	31 to 50	-0.03**	0**	0**	-0.06**	0.05**	0.03**
	51 to 70	-0.04**	0.04**	0.03**	0.08**	0.15**	0.14**
	70 +	-0.08**	0**	0.02**	0**	0.03**	0.02**
Supplement Users		-0.003**	0.16**	0.17**	0.16**	0.14**	0.14**
Overall		0.08**	0.16**	0.17**	0.26**	0.2**	0.23**

**Correlation is significant at the 0.01 level (2-tailed)

[a]*Converted microbiological assay concentration = -22.95 x (0.81) x Immulite 2000 assay concentrations (Colapinto et al, 2015)

Table 6.3b. Pearson correlation coefficient between generated B_{12} intake and serum B_{12} status

Gender	Age Group (y)	Regression Models	Predetermined Portions	Fitted Portions
		Gender		
Men	≤ 8*	0**	-0.02**	-0.02**
	9 to 13	-0.01**	0.02**	0.01**
	14 to 18	-0.07**	-0.02**	-0.01**
	19 to 30	-0.04**	0**	0.001**
	31 to 50	0**	0**	0.002**
	51 to 70	0.01**	0.12**	0.12**
	70 +	0.18**	-0.03**	-0.03**
Women	≤ 8*	-0.03**	0.03**	0.02**
	9 to 13	-0.05**	-0.01**	-0.01**
	14 to 18	0.03**	-0.01**	-0.01**
	19 to 30	-0.01**	-0.03**	-0.03**
	31 to 50	-0.04**	0.02**	0.02**
	51 to 70	-0.05**	-0.01**	-0.01**
	70 +	-0.08**	-0.03**	-0.03**
		Supplement Use		
Non Supplement User				
Men	≤ 8*	0.21**	-0.05**	-0.02**
	9 to 13	-0.10**	-0.09**	-0.01**
	14 to 18	-0.07**	-0.06**	0.02**
	19 to 30	0.06**	-0.06**	-0.02**
	31 to 50	0.01**	0.01**	0.07**
	51 to 70	0.01**	0.05**	-0.02**
	70 +	0.06**	-0.01**	0.02**
Women	≤ 8*	-0.13**	-0.09**	-0.08**
	9 to 13	-0.06**	-0.06**	-0.31**
	14 to 18	-0.03**	-0.07**	-0.02**
	19 to 30	-0.07**	-0.15**	-0.08**
	31 to 50	-0.05**	-0.07**	-0.06**
	51 to 70	-0.01	-0.03**	-0.02**
	70 +	-0.09**	-0.14**	-0.10**
Supplement User		0.01**	0.08**	0.01**
Overall		-0.05**	0.001**	0.001**

**Correlation is significant at the 0.01 level (2-tailed)

6.5 Discussion

Estimating Folate and B_{12} Intakes Based on Regression Estimates with FFQ Data (Method 1)

Using the parameters generated from the adjusted regression models (Method 1), the estimated intakes of folate and B_{12} produced similar estimates that were not statistically different from intakes using CCHS 2.2's dietary recall. The predetermined and fitted portions methods (2 and 3, respectively) generated relatively higher estimates for both folate and $B_{12.}$ The largest differences in folate intake (> 100 µg) were among young Canadians (< 30 y). It is possible that these differing estimates, in particular among those 14-18 y are perhaps due to irregular eating habits and meal skipping that has been reported by youth[42,43], which can result in unpredictable nutrient intake[44]. Large differences were also observed when comparing intakes among supplement users, particularly for folate. Overall intakes of vitamin B_{12} and intakes among males of all ages were higher than those calculated by the authors using CCHS 2.2 nutrient intake data, although female youth and adolescent intakes were quite similar. These lack of differences are important when trying to determine if this new method improves estimates of nutrient intakes using regression parameters in conjunction with FFQ data by producing comparable estimates to 24 hr dietary recall data.

Although previous work has shown that using fitted portion sizes (Method 3) provided better quantitative estimates of nutrient intake compared to using recommended portions (Method 2) to obtain nutrient consumption [38], folate and B_{12} intakes were not improved by using fitted portion sizes, and only marginal differences were found between the two methods. Another result was that for certain age/sex groups, the intakes generated by regression and predetermined methods were similar to intakes reported in the CCHS 2.2 dietary recall, producing close estimates that

may be used in the future as acceptable methods of improving estimates of dietary intakes from FFQ data.

Correlations between estimated Folate/B_{12} intakes and RBC Folate and Serum B_{12} levels

Overall, the weak correlations between the estimated dietary folate and B_{12} intake and status of these nutrients in the blood may be due to nutrient bioavailability[45], or other confounding factors that may affect nutrient status, such as genetic effects which affect individual's ability to metabolize these nutrients[46]. The bioavailability of non-fortified natural folates is influenced by factors such as cooking as well as the stability of natural folate in foods (green vegetables, which are good sources of folate) [47,48]. It is interesting to note that when non-supplement users were isolated, their correlations were sometimes weaker than the overall combined correlation, in particular among males 31-50 y and females 70 + regardless of the estimation method used. This may be explained by the higher bioavailability of folic acid found in supplements[51,] or perhaps the fact that the recall of supplement use may be more reliable than ones dietary recall. Similarly, the bioavailability of B_{12} is also dependent of food type. For example, eggs have been shown to have a lower absorption rate when compared with meat [49]. The very low correlations between B_{12} intake and status among Canadian women > 70 y may be due to the fact that vitamin B_{12} malabsorption is most commonly found among the elderly population, perhaps due to their low gastric hydrochloric acid secretion, which in turn may impact the ability of vitamin B_{12} to be released from food and absorbed [50,51]. Additionally, considering that "peak" meat consumption in American adults is among those 20-49 y, it is possible that lower consumption of red meat among the elderly as well as among younger people may be associated with these lower absorptions and thus lower correlations due to lower serum B_{12} levels[52].

While using Colapinto's conversion factor increased the correlations between overall calculated intake and nutrient status [Table 6.3a], this is likely due to the fact that converting results from the CHMS's Immulite 2000 assay to microbiologic assay values *lowered* RBC folate concentrations. The authors are quick to point out that this conversion method was based on a small set of blood samples and not carried out in an NHANES laboratory, and as such, these results must be interpreted with caution. However, our results are quite similar to those previously published regarding folate. As mentioned previously, regardless of methodology, the majority of correlations of estimated folate intake in the literature with RBC folate have been within the range of r=0.20 to r=0.35, and a recent pooled estimate observing the relationship between folate intake and RBC folate found a correlation coefficient of 0.30 [53]. It is also important to note that the majority of previous studies used the same sample pool to observe nutrient intake and status, while this study utilized two independent Canadian population-based databases on nutrition and health. Additionally, the high level of significance from this study may be due to the large sample sizes of these databases.

The correlations between serum B_{12} and dietary intakes using both portion methods were identical for Canadian adults. This was an unexpected result, as literature has shown that the most commonly available food portions have been found to exceed the US Department of Agriculture and Food and Drug Administration standard portion sizes [54], particularly among North Americans [55,56]. It was hypothesized that using fitted portion sizes would provide better correlations with nutrient status because of the theorized differences between recommended serving sizes and what people are actually eating. However, our results show that there were no differences between both methods.

From a methodological perspective, the work in this study aimed to create new approaches to study the relationship between nutrient intake and nutrient status, employing results from different types of dietary recalls to elucidate the topic of nutrient intake and exposure and status. Although two separate datasets were used in this study, both were representative of the Canadian population, and both had data on culture, sex and age of the respondents. The significant contributions of this study is the exploration of a novel approach to estimate population level nutrient intakes based on FFQ data via the parameters generated from 24 hr dietary recall data from another cross-sectional nutritional survey of the same population. To our knowledge, there is no available methodology on estimation of nutrient intakes based on FFQ data that does not contain portions or amounts of the foods consumed. One advantage of the FFQ is that it covers a broad spectrum of the frequency of food intake during a longer period of time, compared to a 24 hr dietary recall, which represents food consumed on any given day. The advantage of using both components is that results from the 24 hr recall "any given day" can be used in tandem with the FFQ "frequency per day". Another strength of this project is that this study adjusted intake by culture, sex and age, which to our knowledge has not been done before and contributes greatly to this area of research. Our results show that our new method of estimation of nutrient intake provides comparable estimates when compared with 24 hr dietary recall intakes. These results validated this novel method as a way to estimate nutrient intakes using regression parameters in conjunction with FFQ data to produce comparable estimates to 24 hr dietary recall data.

The main limitation of this project is that two separate datasets were used to compare folate and vitamin B_{12} intake with blood status. However, this was mitigated by using parameters based on age, gender and cultural background to closely match respondents from both surveys.

Additionally, the CCHS 2.2 is a self-reported survey and non-sampling errors such as non-response, recall bias and social desirability may affect the validity of results, especially during the 24 hr dietary recall component. Although the five step multiple pass method utilized during the 24 hr recall has been shown to enhance accuracy and assist the respondent in remembering what and how much food they consumed [57], it has been reported that the average under-reporting of energy intake in the CCHS 2.2 is estimated at 10%, with a greater under-reporting rate among respondents who were overweight or obese, adults compared with teenagers, and women compared with men [58]. Similarly, the restricted amount of foods in the CHMS FFQ likely influenced the nutrient intakes calculated in this study, as does the fact that the FFQ did not detail specifics about the food consumed. For example, asking about frequency of consumption of beans does not take into account the variety (eg. black beans vs lentils), which may contain differing amounts of folate, and thus contribute differently to total intake. It is also possible that the high-folate food groups covered in the CHMS FFQ [Table 6.1] did not adequately account for the majority of foods that Canadian consume folate from, although this is unlikely due to the fact that the majority of high-folate foods were included in the list. Previous work by Hjartaker and colleagues found that, while the ability of the FFQ was good for estimating macronutrient intake (carbohydrates, fat, protein), it was less successful for foods eaten less seldom and for micronutrients[39]. Additionally, reported estimates of relative bioavailability of folate in foods have shown great variation, ranging in value depending on the methodology used and response index (increase in serum folate, decrease in plasma homocysteine, etc)[59]. Furthermore, the established 1.7 multiplier for converting folic acid to DFE is based on underlying assumptions: namely that added FA is 85% available, compared to food folate's 50%, yielding an 85/50 ratio (resulting in 1.7). However, there exists a degree of uncertainty with the bioavailability inequities

of food folate in particular. The 50% estimate of food folate used in the DFE equation was based on a single study. Since then, it has been reported that the bioavailability of food folate (in comparison to FA) may range from 60% to 98%[60]. Although recent studies have yielded results supporting the validity of using the 1.7 multiplier[61], confidence in its precision still remains an issue. Another issue pertaining to precision involves the potency of FA or vitamin B_{12} found in vitamins or supplements: a recent analysis of vitamin D over-the-counter supplements found that 30% of the products analyzed had either lower or higher amounts than those deemed acceptable by the U.S. Pharmacopeial Convention (USP) which require that compounded pills contain 90 to 110% of the active ingredient[62]. With this in mind, it seems entirely plausible that these discrepancies are also seen among other vitamins sold over-the counter.

Chronic antacid use as well as proton pump inhibitors (PPI) (which were not accounted for in this study) have also been shown to influence folate absorption. One study showed that antacid use did in fact decrease folate absorption, although not significantly[63] while others have shown patients treated with omezaprole (a medication used in the treatment of gastroesophageal reflux disease, peptic ulcer disease, and Zollinger–Ellison syndrome) sustained significantly lower serum B_{12} levels than those treated with a histamine H2 receptor antagonist [64]. However, consistent data evaluating the relationship between absorption of folate and vitamin B_{12} and use of antacids and PPIs is not conlcusive enough to create therapeutic guidelines or make suggestions for supplementation[65]. It is also conceivable that there is an underlying interaction in the relationship between folate and vitamin B_{12} intake and status. Additionally, a number of genes have been shown to potentially interact with nutrient intake levels or act as modifiers of nutritional status independent of dietary intake. Circulating levels of B vitamins have been

shown to be genetically determined, as polymorphisms in the FUT2 gene have been shown to interfere with B_{12} status by inhibiting absorption by interfering with gastric mucosa. Similarly, the TCN2 gene encodes a protein responsible for transporting B_{12} to cells from the blood. Hereditary folate malabsorption also exists, with a mutation in the SLC46A1 gene resulting in a malfunctioning protein that impairs the body's ability to absorb folate [46,66-68]. It is also possible that these methods may only produce valid results for certain nutrients, given that the majority of correlation tests involving B_{12} intake and serum B_{12} yielded negative correlations, while folate intake correlations with RBC folate were predominantly positive, warranting future research in this area.

6.6 Conclusions

Based on the parameters generated from 24 hour food recall data which were adjusted by gender, age and culture, this study demonstrated that the estimated nutrients intakes based on FFQ data are within close range of the estimates based on 24 hr dietary recall data. Results from our study validated a new methodological approach to estimate nutrient intakes using regression parameters in conjunction with FFQ data to produce comparable estimates to 24 hr dietary recall data. However, further investigations should be explored to estimate other nutrient intakes using the same methodology, in particular it would be interesting to explore intake of micronutrients (other B vitamins such as niacin or vitamin B_6) compared to macronutrients (fibre, carbohydrate, various fatty acids), as well as nutrient intakes required in mg vs µg. Our results gleaned from this study showed low correlations between generated intakes and blood levels of these nutrients, similar to previously published results and corroborating past research. Similarly, intakes generated using unique portion sizes, as well as those recommended by Canada's Food Guide

also showed low correlations when compared with nutrient status. Overall, the poor correlation of estimated folate intake and RBC folate level leads to the conclusion that this method cannot be used as an evaluation standard for estimating folate intake. Similarly, intakes generated using both portion sizes also provided close estimates to intakes from the CCHS 2.2 dietary recall. Females > 70 y showed the highest correlation of folate intake and status (0.3), regardless of portion size method used, while poor correlations of B_{12} were seen among respondents among children and adolescents, with stronger correlations found using predetermined as well as fitted portions, but weaker correlations among non-supplement users.

In conclusion, further work on nutrient intake patterns, especially vitamin $B_{12,}$ are necessary to clarify the relationship between nutrient intake and nutrient status. Additionally, the uncertainty of folate bioavailability needs to be further addressed. It should be suggested that future work using a wider variety of nutrients be used to explore these methods further and to clarify whether or not they are more acceptable for certain vitamins as well as for certain sub-groups of the population. Additionally, aspects of dietary surveys need to be examined closely, in particular, methods chosen to evaluate nutrient status, their efficacy and their limitations.

6.7 Chapter 6.0 References

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CHAPTER 7.0 Discussion, Conclusions and Implications

7.1 Discussion and Conclusions

This dissertation was comprised of four chapters which focused on using secondary analysis to observe food and nutrient intake to shed light on groups in the Canadian population who may be at risk for adverse health effects linked to their dietary intakes and eating patterns, as well as to investigate the impact of food fortification on the Canadian population. This thesis used data from both the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2) as well as the Canadian Health Measures Survey (CHMS); comprehensive, cross-sectional surveys which provide data on food and nutrient intake as well as biomarkers which can be used to assess nutrient status.

Our first study used target foods (pulses and soy) as an indicator to evaluate the eating profile of Canadians as a whole (Chapter 3), as well as a sub-population of young Manitobans 2-18 y (Chapter 4), who indeed are at an increased risk of childhood overweight and obesity, exceeding the national average and leading to a variety of negative health outcomes (Shields, Tjepkema 2006). Both pulses and soy are grown in Canada and are rich in many nutrients (Venter, Ochse & Swart 2013). Chapter 3, which examined both the prevalence and association of soy food consumption with nutrient intakes and dietary patterns of Canadians observed higher intakes of fibre and lower intakes of saturated fat among consumers, potentially reducing the risk of disease outcomes such as CVD. Our results also showed that consuming at least one soy food during a dietary recall was linked with higher intakes of calcium, magnesium, phosphorus and potassium, which are crucial in preventing bone loss and maintaining bone health (Tranquilli et al. 1994). However, our results also showed an increase in energy intake among soy consumers, which

needs to be further explained using long term studies to determine what dietary habits other than soy food consumption may be contributing to this trend. Although the reasons for these effects on potential dietary improvements need to be clarified, this in an important finding as many Canadian adults struggle to meet dietary recommendations and fall below the recommended values of nutrient intakes.

Although recent data has shown an enhanced nutrient intake profile associated with pulse consumption (Mudryj et al. 2012, Mitchell et al. 2009), and results from our soy study indicated that soy food consumption was associated with an improved diet quality of Canadians (Mudryj, Aukema & Yu 2015), pulse or soy consumption did not appear to be related to enhanced nutrient intake profile of young Manitobans. Regardless, results from this study highlighted the poor *overall* eating habits of Manitoba's children and youth. The high intakes of sodium and low intakes of vegetables and fruit, fibre, and vitamin D signal a potential public health issue for their future cardiovascular and bone health. Children who are at an unhealthy weight or practice poor dietary habits are also more likely to continue these habits throughout their life, suggesting that there exist opportunities to specifically target 'at risk' groups. Therefore, these data are relevant to the topic of childhood obesity and dietary intake patterns of children and youth. Efforts targeting the dietary habits of young Canadians should be paired with others aimed at improving the quality of the food supply (e.g. reducing sodium, increasing vitamin D and vegetable and fruit intake) and nutrition education in children.

Our second study (Chapter 5) assessed folate and folic acid (FA) exposure from diet, fortified foods and supplements in Canadians in a post-fortification era using both the CCHS 2.2 as well as the CHMS (Chapters 5, 6) to observe red blood cell folate (RBC) status and estimate and

investigate parameters associated with nutrient intake in relation to nutrient status. Our results showed that based on unfortified food sources alone, Canadians struggled to consume adequate amounts of folate. However, when folate intakes from both fortified and natural sources were considered, the overall prevalence of folate inadequacy was low across all age and gender groups, with the exception of elderly women > 70 y, mimicking results from previous work using dietary intake from the CCHS 2.2 (Shakur et al. 2010) and biomarker data from the CHMS (Colapinto, O'Connor & Tremblay 2011). However, food fortification, while successful in combating deficiencies (as evidenced by the low prevalence of inadequacy), can also become a health risk if it results in usual nutrient intakes in excess of the Tolerable Upper Intake Level (UL). Data on the prevalence of folate inadequacy and excess FA are usually calculated using nutrient values that reflect the mandated rather than actual levels of fortification. Evidence suggests that manufacturers add extra FA to their fortified products above the established mandated levels, defined as "overage" or the "potential extra amount of FA added to a product during fortification by the food manufacturer to prevent decay/loss during shelf life/storage (Shakur et al. 2009). Results from this study suggested that insufficient dietary intakes of folate in Canadians have been ameliorated due to the fortification policy, although folate inadequacy still exists across all age groups. Fortification and supplement use has resulted in > 10% of supplement users having intakes of FA above the UL, rising to almost 18% when these overage factors were accounted for. Additionally, between 20 and 52% of supplement users had elevated red blood cell folate concentrations, depending on cut-off used. We concluded that supplement users appear to be at an increased risk of FA overconsumption as well as elevated RBC folate. Although previously published work suggests that there is no cause for concern with intakes above the UL (Shakur et al. 2010), we conclude that there is a need for more careful evaluation

of the risks and benefits of FA fortification, particularly over mandated levels. Recent research shows cognitive impairment associated with high RBC folate (Selhub, Rosenberg 2016, Yajnik et al. 2008), and emerging animal studies highlight other adverse outcomes linked with excessive FA (Meadows et al. 2015). With this in mind, we suggest that persons of all ages who may be considering a FA containing supplement should be cautioned about the potential risk involved with FA consumption above the UL. In particular, it may be suggested that vitamins targeted toward children, men, women past child-bearing age and older adults not include FA.

Chapter 6 of this thesis explored new methods in estimating nutrient intakes based on food frequency questionnaire data and parameters generated from the 24 hour dietary recall data, and sought to assess the correlations of estimated nutrient intakes (folate and vitamin B_{12}) based on FFQ data and red blood cell folate and serum B_{12} status. Secondary data analysis was performed using 24 hour dietary recall from the CCHS 2.2's dietary recall component to generate parameters for folate intake from foods as well as B_{12} intake (given the complex relationship of folate and B_{12} status to various health outcomes). The method for estimating relative folate and B_{12} intake took into account the food consumed, age and gender, and varying portion sizes. The parameters were then applied to estimate intakes based on food frequency questionnaire data from 3 combined cycles of the CHMS compared with biomarkers of each nutrient. Results showed that the estimates of folate and B_{12} intakes based on applying the parameters from 24hr recall data to the FFQ data showed lower intakes than estimates previously found using 24 hour dietary recall data, and overall low correlation between dietary intake and blood levels, particularly for vitamin B_{12}. The parameters generated from 24hr recall data could be too simplistic to apply for the estimates of nutrient intakes based on FFQ data. It is possible that

other factors such as bioavailability or genetic interactions play an important role in the relationship between folate and vitamin B_{12} intake and status, and this low correlation may be due to the complexity of these differences.

A limitation of these studies are that the consumption of pulses and soy in the CCHS 2.2 were based on a single reference day. This could mean that individuals who were not identified as a consumer during a single day of intake may still be a pulse or soy consumer. Additionally, lack of information regarding specific types of diets, (i.e. low-carbohydrate, vegetarian or vegan diets) may have been helpful in further examining the demographic of the average pulse or soy consumer or food preparation techniques, which may affect the nutrient content (e.g. added sodium) of the types of foods consumed. It should be noted that the Canadian Nutrient File (CNF) may not be completely accurate in its summary on the nutritional breakdown of various foods or food dishes, and it is possible that nutrient composition may differ between products. Due to the fact that the CNF values for many foods represent a general product (for example canned beans), subtle differences in nutrient composition between variety and brand (e.g. Heinz™ versus Bush Brothers™) are not necessarily reflected in the CNF. In the same way, nutrient composition may also be influenced by growing conditions (such as soil composition) as well as manufacturing and processing differences. It is also impossible to determine whether the 24 hour dietary recall was truly representative of the respondent's normal diet, as respondents may over or under report their food consumption, even with trained experts administering the dietary recall. As the CCHS 2.2 is a self-reported survey and non-sampling errors such as non-response, recall bias and social desirability may affect the validity of results, especially during the 24 hr dietary recall component. Although the five step multiple pass method utilized during

the 24 hr recall has been shown to enhance accuracy and assist the respondent in remembering what and how much food they consumed. Indeed, it has been reported that the average under-reporting of energy intake in the CCHS 2.2 is estimated at 10%, with a greater under-reporting rate among respondents who were overweight or obese, adults compared with teenagers, and women compared with men (Garriguet 2008). Because the CCHS 2.2 was a cross-sectional look at the dietary habits of Canadians, the results should be interpreted with caution. It is entirely possible that the survey would have provided differing results if another time-frame had been chosen, with either higher or lower amounts of pulse or soy consumers. As well, cause and effect cannot be assumed: for example, pulse or soy consumption may be a component of an overall lifestyle. Another limitation is that the CCHS 2.2 was conducted in 2004, and it is possible that dietary patterns have changed over the last decade. Finally, the CCHS 2.2 also does not take into account those residing on Indian reserves, residents occupying any of the three territories, those living in Institutions or members of the Canadian Forces (Health Canada 2012a).

A main limitation of Chapter 6 is that two separate datasets were used to compare folate and vitamin B_{12} intake with blood status. However, our study tried to remedy this by using parameters based on age, gender and cultural background to closely match respondents from both surveys. Similarly, the restricted amount of foods in the CHMS FFQ likely influenced the nutrient intakes calculated in this study, as does the fact that the FFQ did not detail specifics about the food consumed. For example, asking about frequency of consumption of beans does not take into account the variety (eg. black beans vs lentils), which may contain differing amounts of folate, and thus contribute differently to total intake.

A major strength of Chapters 5 and 6 of this thesis was that the prevalence of folate inadequacy as well as toxicity was based on a combination of dietary, supplemental and clinical measures of status. The results of both survey data showed comparable results on folate intakes below Estimated Average Requirement (EAR) and above UL as well as those above the recommended cut-offs. Therefore, the results of this study are generalizable to other similar populations with a similar food supply environment. From a methodological perspective, the work in this dissertation created new approaches to study the relationship between nutrient intake and nutrient status, employing results from different types of dietary recalls to elucidate the topic of nutrient intake and exposure and status.

7.2 Future Directions and Implications

As mentioned previously, many Canadians struggle to meet dietary recommendations, falling below the recommended values of nutrient intakes. Results from this thesis also show that certain Canadians may also be at risk of overconsumption of certain nutrients. This dissertation shows the benefits of consuming certain foods, such as pulses or soy, in regards to enhanced nutrient intake and perhaps a subsequent reduced risk of lifestyle-related diseases among Canadians. However, further research and explanation of the reasons for these effects on potential dietary improvements need to be clarified to fully benefit from the effects of soy and pulses on one's diet.

The folate component of this thesis shows that although FA fortification policies have been successful in combating folate deficiencies (as evidenced by the low prevalence of inadequacy in our study and many others), it may also pose a health risk if it results in usual nutrient intakes in

excess of the Tolerable Upper Intake Level (UL). Our findings suggest a need for more careful assessment of the risks and benefits of food fortification, particularly fortification above mandated levels, and FA supplement use in general population. Results from this research may provide a backbone in terms of future studies regarding updating or refining policies, regulations and recommendations to maximize the benefits of FA while minimizing the risks of overexposure. In the future, it will be necessary to strike a balance between establishing a cut-off that reflects the dietary changes of the population at hand, but also protects this same population from adverse health effects. Further research and studies monitoring excessive folate intake are imperative in order to find a healthy balance to achieve optimal intake and well-designed longitudinal studies are needed to draw definitive conclusions regarding adverse effects of consuming excess FA. Consequently, it is important that universal guidelines be put in place to define high blood folate status, in order to better inform health professionals and the public about FA guidelines and status. Additionally, further work on folate and vitamin B_{12} intake patterns are necessary to clarify the relationship between nutrient intake and nutrient status.

7.3 Chapter 7.0 References

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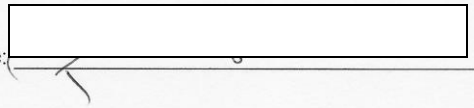
Signature: 

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Adriana Mudryj

Your name: Margaret deGroh

Signature: [redacted]

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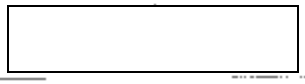
Signature: 

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Sincerely,

Adriana Mudryj

Your name: Harold Aukema

Signature: [redacted]

I am a co-author on manuscript number (please check all that apply):

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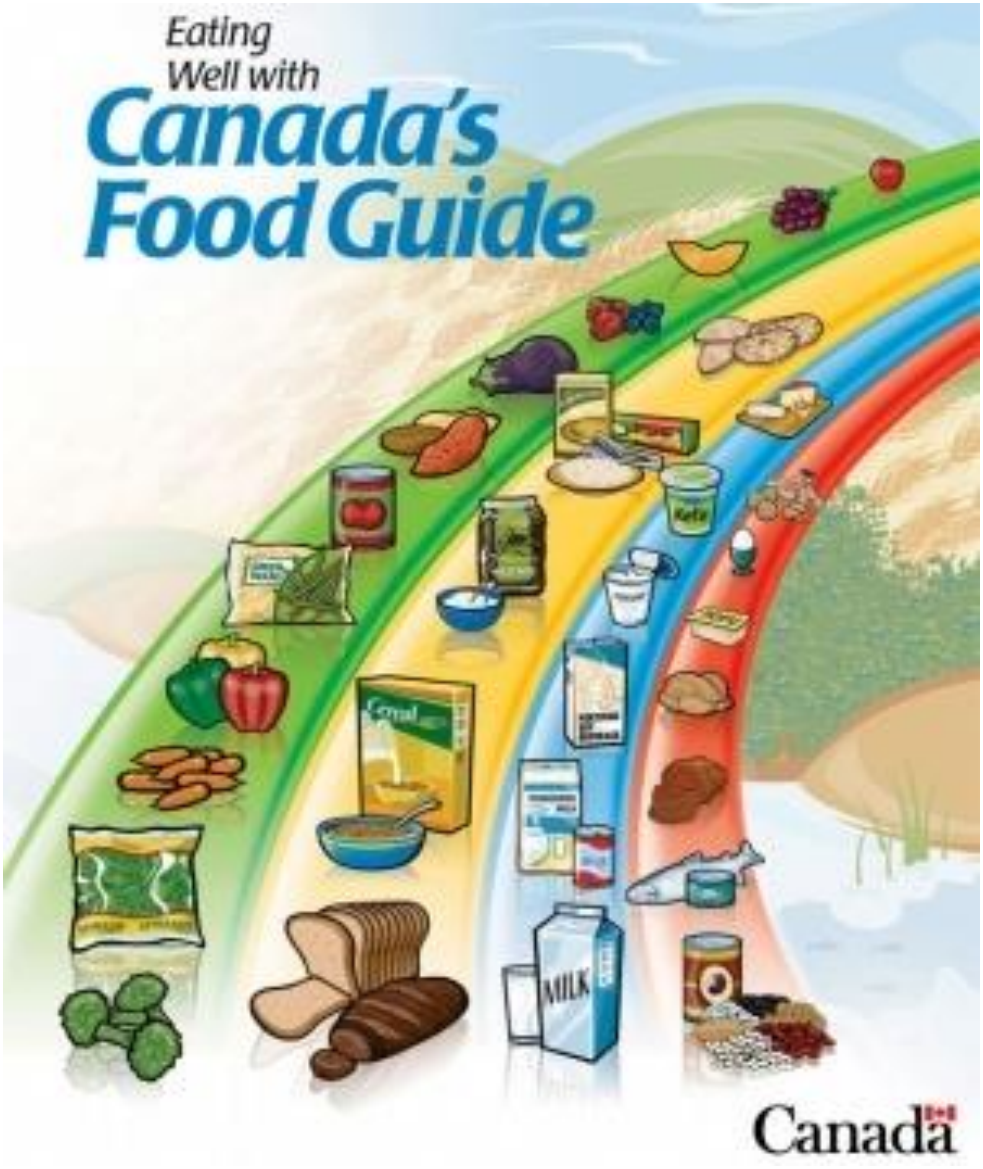
APPENDIX B: Eating Well with Canada's Food Guide: Canada's Food Guide to Healthy Eating
Source: Canada's Food Guide (Health Canada, 2011).



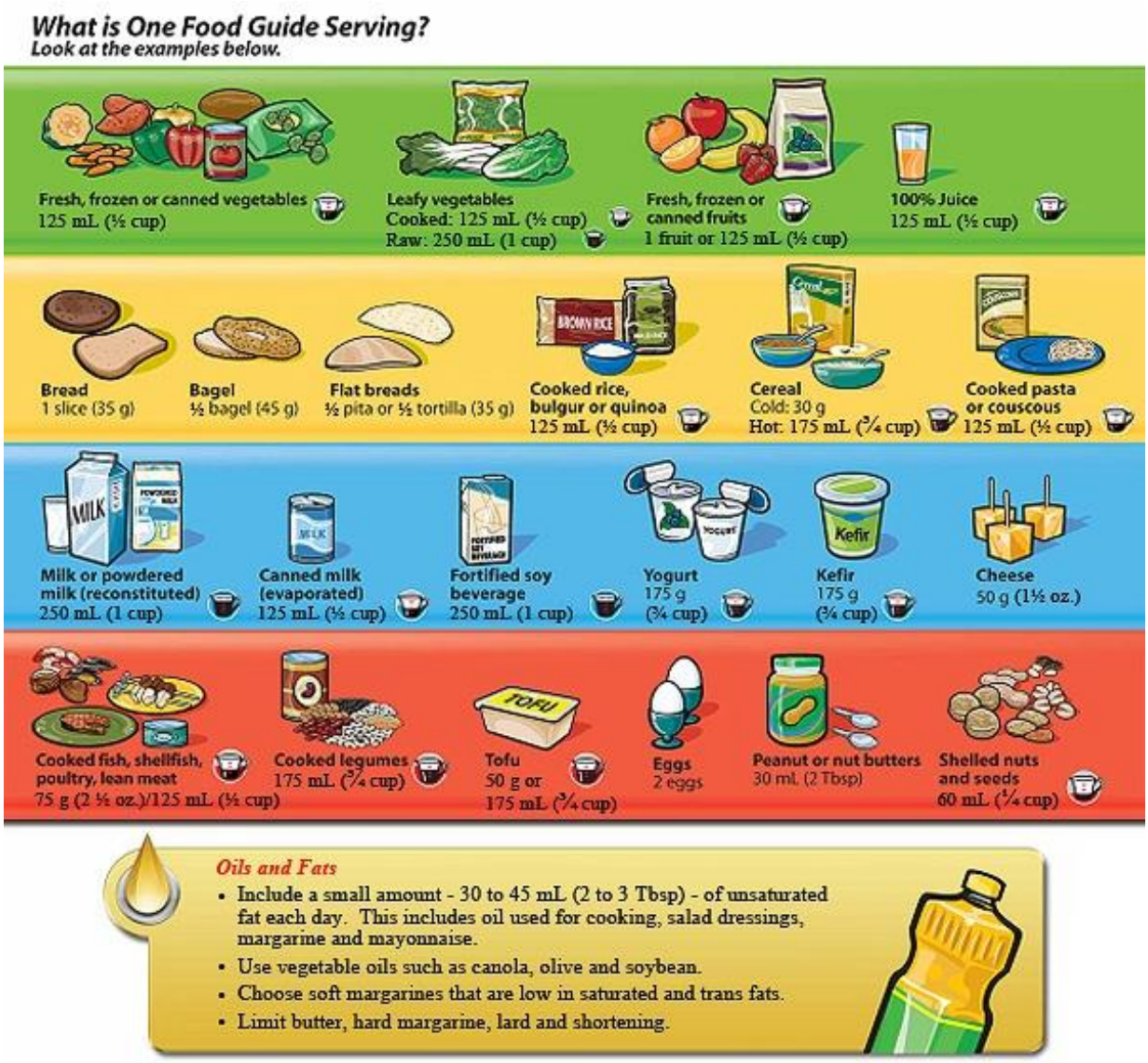
Recommended Number of Food Guide Servings per Day

	Children			Teens		Adults			
Age in Years	2-3	4-8	9-13	14-18		19-50		51+	
Sex	Girls and Boys			Females	Males	Females	Males	Females	Males
Vegetables and Fruit	4	5	6	7	8	7-8	8-10	7	7
Grain Products	3	4	6	6	7	6-7	8	6	7
Milk and Alternatives	2	2	3-4	3-4	3-4	2	2	3	3
Meat and Alternatives	1	1	1-2	2	3	2	3	2	3

The chart above shows how many Food Guide Servings you need from each of the four food groups every day.

Having the amount and type of food recommended and following the tips in *Canada's Food Guide* will help:

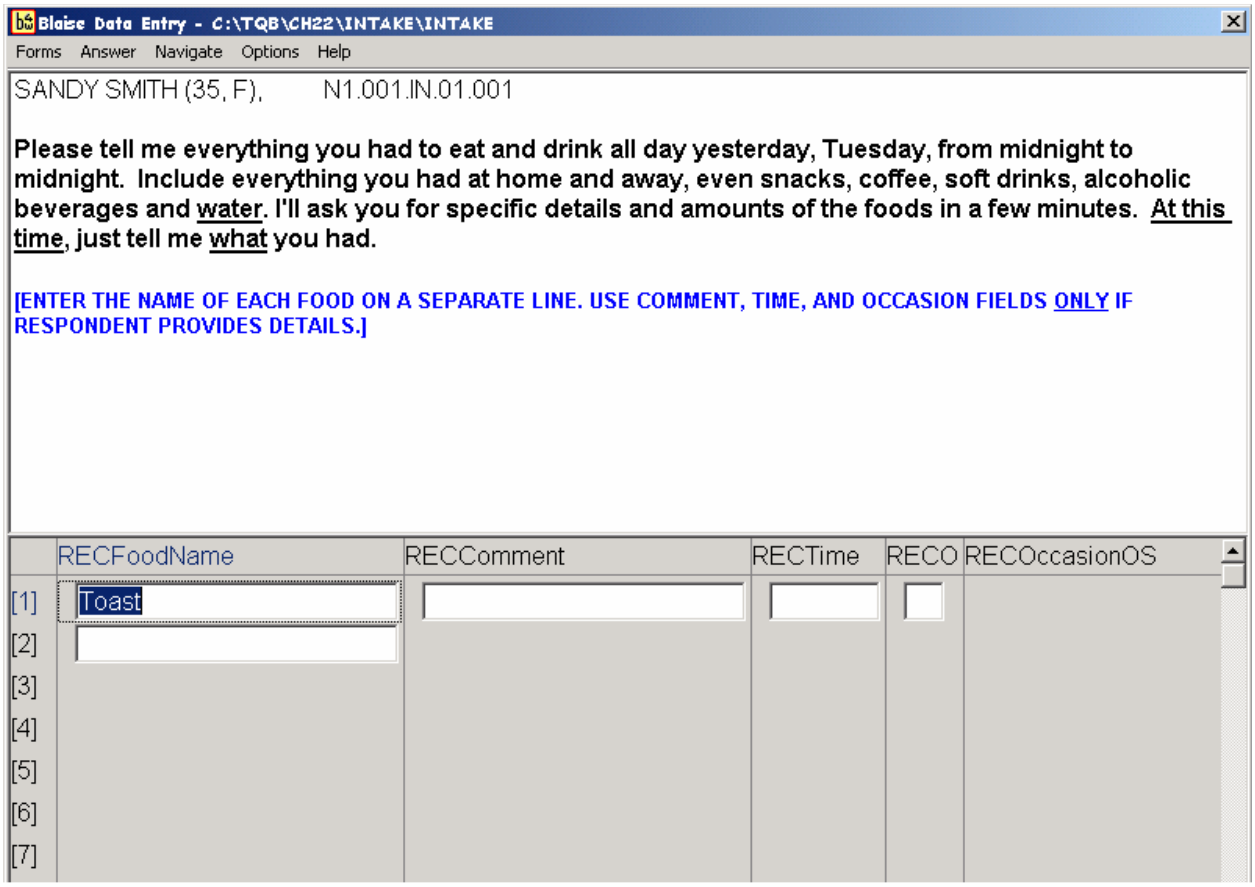
- Meet your needs for vitamins, minerals and other nutrients.
- Reduce your risk of obesity, type 2 diabetes, heart disease, certain types of cancer and osteoporosis.
- Contribute to your overall health and vitality.



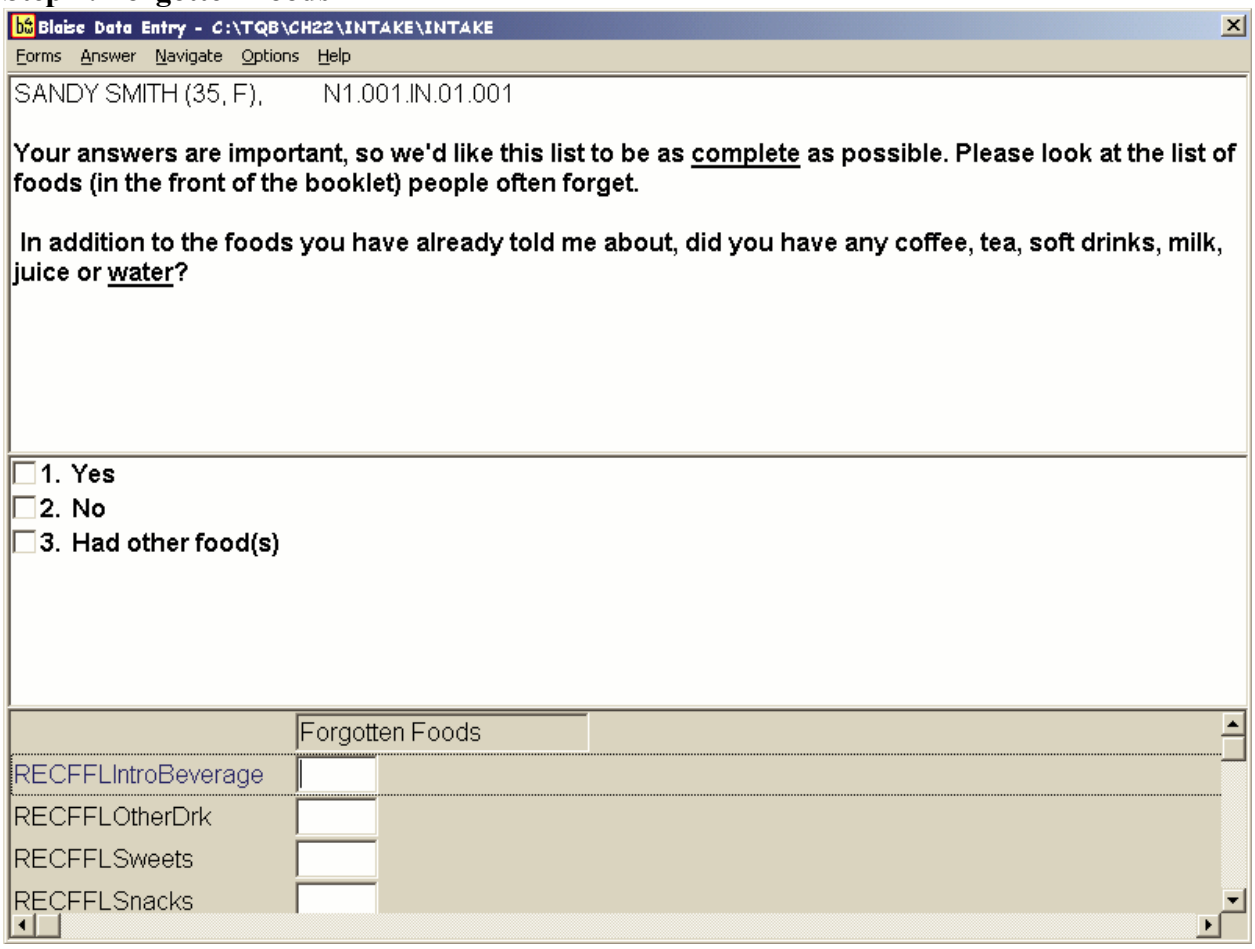
Source: Canada's Food Guide (Health Canada, 2011). Available online at: http://hc-sc.gc.ca/fn-an/food-guide-aliment/index-eng.php

APPENDIX C: Canadian Community Health Survey, Cycle 2.2 (2004) 24-hour Dietary Recall Procedure (Automated Multiple Pass Method)
Source: Statistics Canada, 2007

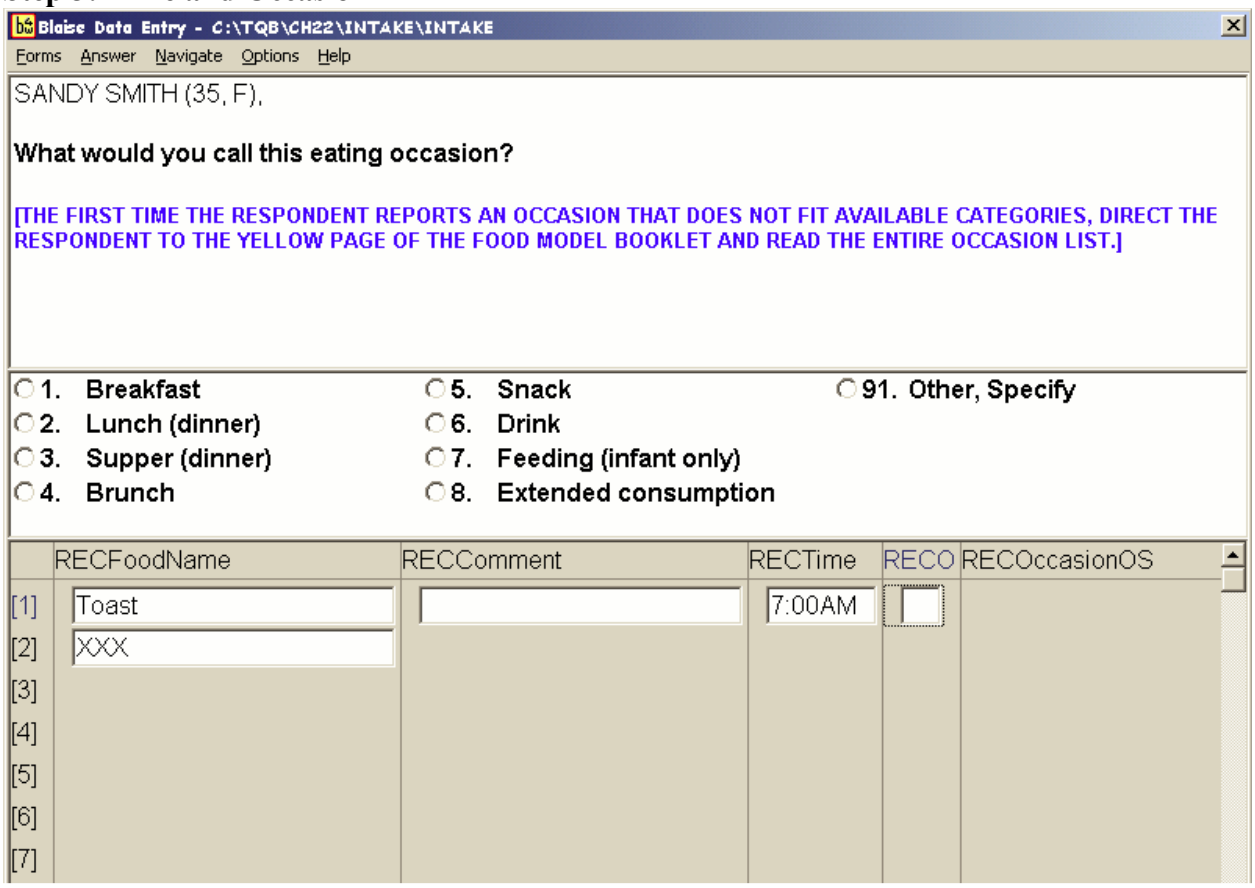
Step 1: Quick List



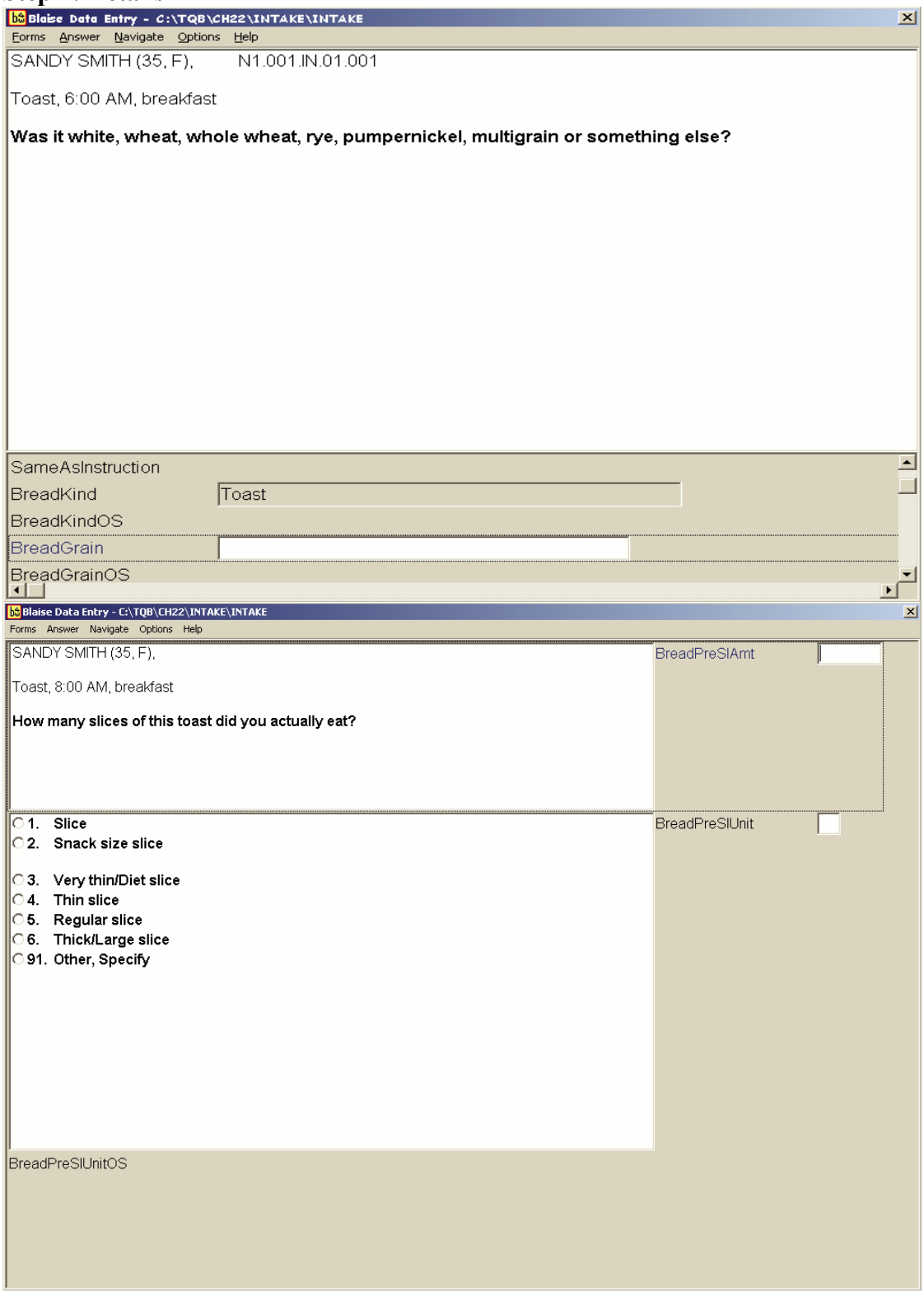
Step 2: Forgotten Foods

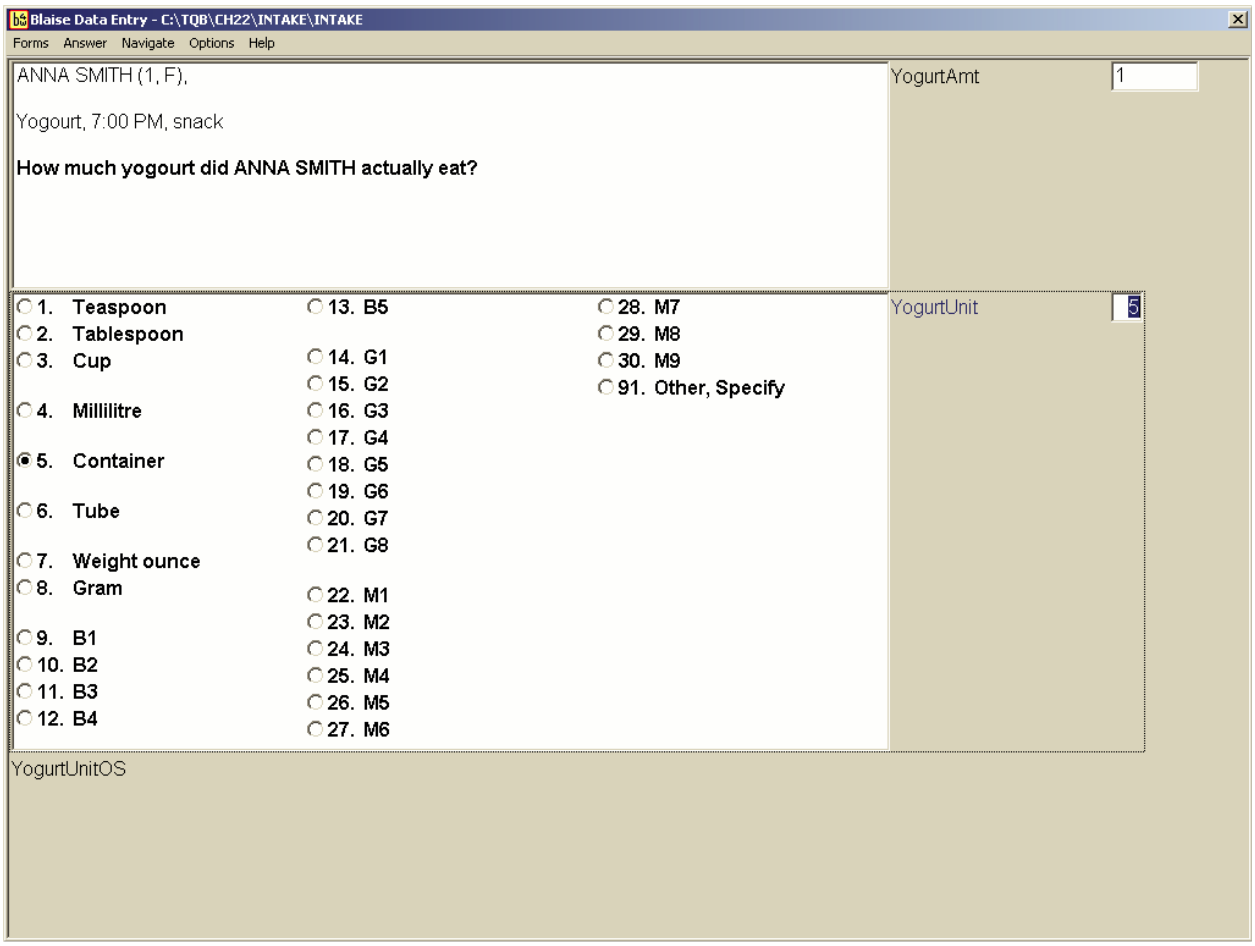


Step 3: Time and Occasion



Step 4: Details

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Forms Answer Navigate Options Help
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Yogourt, 7:00 PM, snack
How much yogourt did ANNA SMITH actually eat?
YogurtAmt
1
1. Teaspoon
2. Tablespoon
3. Cup
4. Millilitre
5. Container
6. Tube
7. Weight ounce
8. Gram
9. B1
10. B2
11. B3
12. B4
13. B5
14. G1
15. G2
16. G3
17. G4
18. G5
19. G6
20. G7
21. G8
22. M1
23. M2
24. M3
25. M4
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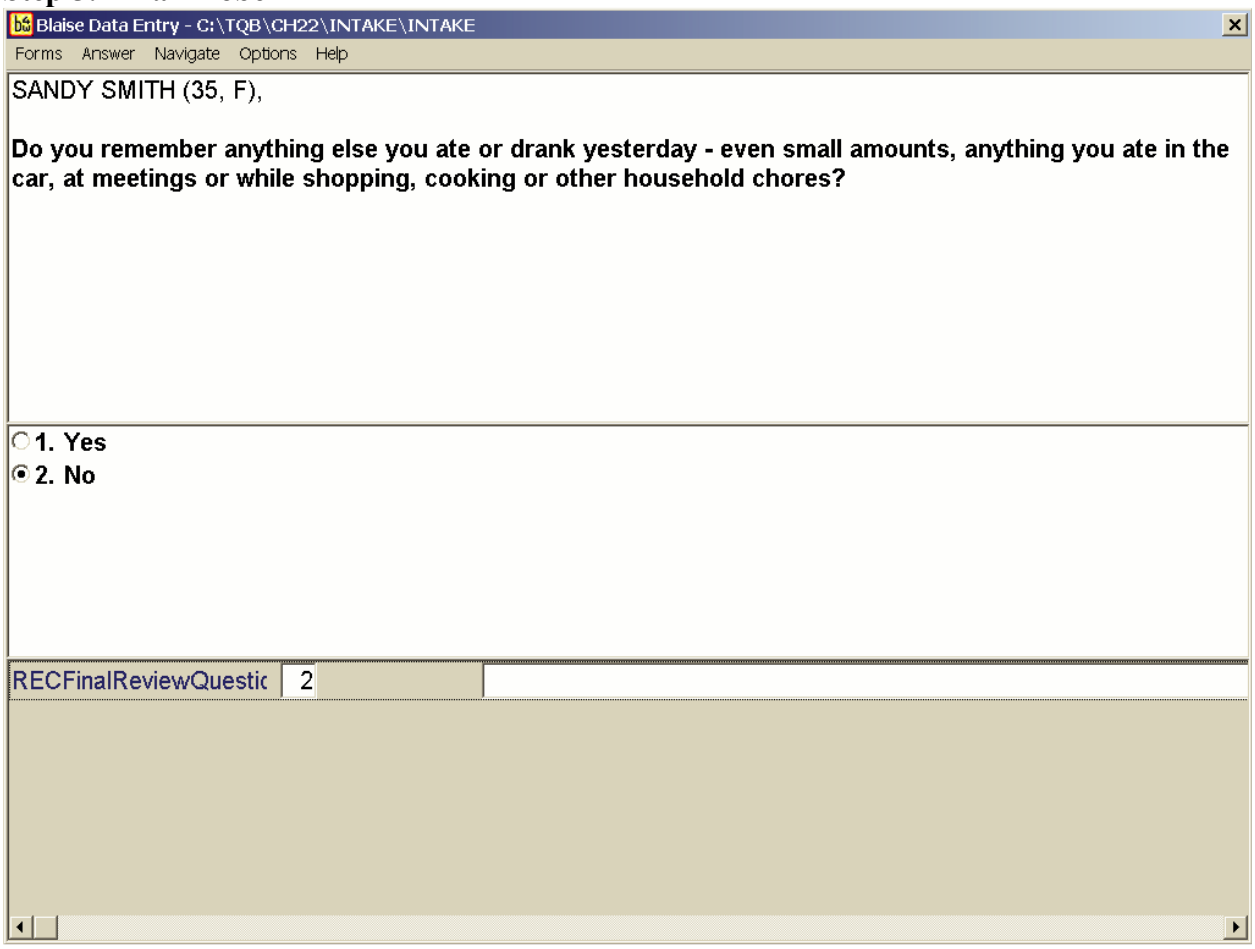
YogurtUnitOS

Step 5: Final Probe



APPENDIX D: Supplementary Data

Table S1. Soy and soy containing food items and the amount of soy protein per 100 g in the Canadian Nutrient File, version 2010.

Food name	**Amount of soy protein (g) per 100 g food product**
Soy milk/beverage (unfortified)	7.95
Soy flour, defatted	24.68
Soy flour, full fat	14.79
Soy flour, low fat	20.02
Miso	1.34
Soy protein concentrate	16.5
Soy protein isolate	22.88
Soybeans, yellow, canned	13
Soybeans, black, canned	11
Soybeans, green, in pod	8
Tofu	12.8
Soy yogurt	8
Soy cheese	7
Soy burger patty	10.99
Soy wieners	11

Table S2a. Male Folate Regression Models.

	DFE[a] from supplement	*Liver**	*Rice*	*Potatoes*	*Fried Potatoes*
Cultural Group					
Caucasian	DFEfromsupp = 1585.8 -863.2 (yes or no) + 3.6 (age)		DFEfromrice = 0.3 + 8.6 (mentions) - 0.01 (age)	DFEfrompotatoes = 0.16 + 19.2 (mentions of consumption) + 0.004 (age)	DFEfromfriedpotatoes = -0.07 + 17.6 (mentions of consumption) + 0.001 (age)
African	DFEfromsupp = 1398.7 - 761 (yes or no) + 4.3 (age)		DFEfromrice = -0.02 + 6.3 (mentions) + 0.02 (age)	DFEfrompotatoes = 0.13 + 11.6 (mentions of consumption) + 0.003 (age)	DFEfromfriedpotatoes = -1.5 + 20.7 (mentions of consumption) + 0.05 (age)
Korean	DFEfromsupp = 1045.2 - 636.8(yes or no) + 7.9 (age)		DFEfromrice = -2.5 + 4.3 (mentions) + 0.2 (age)	DFEfrompotatoes = -1.6 + 9.6 (mentions of consumption) + 0.05 (age)	DFEfromfriedpotatoes = -0.3 + 17.3 (mentions of consumption) + 0.01 (age)
Filipino	DFEfromsupp = 1310.6 - 782.1 (mentions) + 7.8 (age)		DFEfromrice = 4.4 + 7.4 (mentions) = 0.1 (age)	DFEfrompotatoes = 2.2 + 8.9 (mentions) -0.07 (age)	DFEfromfriedpotatoes = 2.0 + 9.7 (mentions of consumption) -0.04 (age)
Japanese	DFEfromsupp = 2448.7 - 1161.1 (yes or no) -3.4 (age)		Dfefromrice = 0.9 + 3.6 (mentions) + 0.02 (age)	DFEfrompotatoes = 0 + 3 (mentions of consumption) + 0 (age)	DFEfromfriedpotatoes = 2.9 + 33.6 (mentions of consumption) - 0.08 (age)
Chinese	DFEfromsupplement = 1615.1 - 880.9 (yes or no) +4.4 (age)		DFEfromrice = -7.7 + 19.3 (mentions) + 0.03 (age)	DFEfrompotatoes = 0.1 + 8.8 (mentions of consumption) +	DFEfromfriedpotatoes = 0.03 + 23.7 (mentions of consumption) - 0

		DFEfromliver = 0.9 + 337 (mentions of consumption) -0.02 (age)		0.004 (age)	(age)
Aboriginal	DFEfromsupplement = 1310.6 - 695.9 (yes or no) + 2.5 (age)		Dfefromrice = -0.04 + 14.5 (mentions) + 0.02 (age)	DFEfrompotatoes = -1.3 + 28.4 (mentions of consumption) + 0.03 (age)	DFEfromfriedpotatoes = 0.5 + 14.7 (mentions of consumption) -0.01 (age)
South Asian	DFEfromsupp = 1430.3 - 750.5 (yes or no) + 2.2 (age)		DFEfromrice = -0.5 + 6.3 (mentions) + 0.04 (age)	DFEfrompotatoes = 0.9 + 6.2 (mentions of consumption) - 0.01 (age)	DFEfromfriedpotatoes = 0.07 + 15.0 (mentions of consumption) + 0 (age)
Southeast Asian	DFEfromsupplement = 1680 - 892.3 (mentions) + 3.6 (age)		DFEfromrice = 1.8 + 11.1 (mentions) + 0.2 (age)	DFEfrompotatoes = 3.4 + 10.5 (mentions of consumption) - 0.1 (age)	DFEfromfriedpotatoes = 0.6 + 18.3 (mentions of consumption) -0.02 (age)
Arab	Dfefromsupplement = 655 - 333.6 (yes or no) + 0.5 (age)		DFEfromrice = 2.5 + 15.7 (mentions) - 0.04 (age)	DFEfrompotatoes = 0.4 + 16.3 (mentions of consumption) - 0.01 (age)	DFEfromfriedpotatoes = -0.6 + 19.2 (mentions of consumption) + 0.02 (age)
West Asian	DFEfromsupp = 1497.9 - 754.4 (yes or no) + 0.4 (age)		DFEfromrice = 1.2 + 6 (mentions) - 0.04 (age)	DFEfrompotatoes = -1.7 +14.3 (mentions of consumption) + 0.05 (age)	DFEfromfriedpotatoes = -0.6 + 19.6 (mentions of consumption) + 0.02 (age)
Latin	DFEfromsupplement = 713.5 - 359.4 (yes or no) + 0.2 (age)		DFEfromrice = -0.02 + 5.1 (mentions) + 0.01 (age)	DFEfrompotatoes = 4.7 + 17.7 (mentions of consumption) - 0.1 (age)	DFEfromfriedpotatoes = 1.1 + 22.2 (mentions of consumption) -0.04 (age)
Other	Dfefromsupplement = 1930.1 - 1054.6		Dfefromrice = 0.8 + 5.6	DFEfrompotatoes = 1.1 + 6.8	DFEfromfriedpotatoes = 0.07 + 10.7

	(yes or no) + 5.8 (age)		(mentions) + 0.02 (age)	(mentions of consumption) - 0.04(age)	(mentions of consumption) -0.002 (age)
Multiple	Dfefromsupplement = 948.4 -543.8 (mentions) + 6 (age)		Dfefromrice = -0.2 + 8.1 (mentions) - 0 (age)	DFEfrompotatoes = 0.8 + 13.9 (mentions of consumption) - 0.02 (age)	DFEfromfriedpotatoes = -0.7 + 21.9 (mentions of consumption) + 0.02 (age)

[a] Dietary Folate Equivalent

* This regression model is for all males, regardless of cultural group, due to lack of valid consumers in each cultural group

Table S2a, continued

	Other Vegetables	*Pasta*	*Fruit*	*Fruit Juice*	*Vegetables*	*Spinach*	*Beans*
Cultural Group							
Caucasian	DFEfromotherveg = 2.3 + 6.4 (mentions) + 0.3 (age)	DFEfrompasta= 4.5 + 216.6 (mentions) - 0.06 (age)	Dfefromfruit = 2.1 + 10.4 (mentions) - 0.1 (age)	DFEfromfjuice = 6.7 + 38.2 (mentions) -0.1 (age)	DFEfromvegjuice = 0.3 + 58.3 (mentions) -0.01 (age)	DFEfromspinach = 0.01 + 77.3 (mentions) + 0 (age)	DFEfrombeans = -0.9 + 97.8 + 0.03 (age)
African	DFEfromotherveg = -9.8 + 9.8 (mentions) + 0.3 (age)	DFEfrompasta = 26.1 + 262 (mentions) - 0.9 (age)	DFEfromfruit = 5.1 + 16.2 (mentions) + 0.01 (age)	DFEfromfruitjuice = -10.8 + 48.9 (mentions) + 0.04 (age)	DFEfromvegjuice = -0.4 + 25.3 (mentions) + 0.01 (age)	DFEfromspinach = 1.6 + 66.4 (mentions) - 0.06 (age)	DFEfrombeans = -2.5 + 71.2 (mentions) + 0.1 (Age)
Korean	DFEfromotherveg = 11.8 + 5.8 (mentions) -0.09 (age)	DFEfrompasta = -3.3 + 93.4 (mentions) + 0.1 (age)	DFEfromfruit = 13.4 + 7.3 (mentions) - 0.3 (age)	DFEfromfruitjuice = 1.3 + 30.2 (mentions) + 0.05 (age)	DFEfromvegjuice = 0.03 +12.7 (mentions) - 0 (age)	Dfefromspinach = -1.1 + 30.1 (mentions) -0.1 (age)	DFEfrombeans = -0.9 + 64.9 (mentions) + 0.03
Filipino	Dfefromotherveg = -16.2 + 11.3 (mentions) + 0.8 (age)	Dfefrompasta = 1.2 + 95.2 (mentions) -0.1 (age)	Dfefromfruit = -41.4 + 50 (mentions) + 0.9 (age)	DFEfromfjuice = 13.5 + 18.8 (mentions) -0.3 (age)	DFEfromvegjuice = 0.03 +12.7 (mentions) - 0 (age)	Dfefromspinach = -0.2 + 30 (mentions) + 0.01 (age)	DFEfrombeans = 6 + 33.3 (mentions) - 0.2 (age)
Japanese	DFEfromotherveg = 1.3 + 3.9 (mentions) + 0.2 (age)	DFEfrompasta = -35.3 + 183.5 (mentions) + 2.5 (age)	Dfefromfruit = -0.2 + 5.6 (mentions) + 0.06 (age)	DFEfromfjuice = -6.1 + 21.5 (mentions) + 0.2 (age)	DFEfromvegjuice = 21.3 + 52.4 - 0.7 (age)	DFEfromspinach = 1.3 + 30.6 (mentions) -0.05 (age)	DFEfrombeans = 0.7 + 17.3 (mentions) - 0.01 (age)
Chinese	DFEfromotherveg = -7.6 + 13.8 (mentions) + 0.8 (age)	DFEfrompasta = -13.9 + 172.9 (mentions)+ 1.1 (age)	DFEfromfruit = 1.7 + 13.6 (mentions) + 0.1 (age)	DFEfromfruitjuice = 0.4 + 33.6 (mentions) + 0 (age)	DFEfromvegjuice = -1.4 + 231.2 (mentions) -0.03 (age)	DFEfromspinach = =7.4 + 96.6 (mentions) + 0.2 (age)	DFEfrombeans = -14.6 + 123.5 (mentions) + 0.4 (age)
Aboriginal	DFEfromotherveg = -1.72 + 5.6 (mentions) + 0.25 (age)	DFEfrompasta= -56.7 + 412.6 (mentions) + 0.27 (age)	Dfefromfruit = 2.56 + 13.1 (mentions) - 0.07 (age)	DFEfromfjuice = -4.1 + 53.8 (mentions) + 0.14 (age)	DFEfromvegjuice = 0.29 + 34.3 (mentions) -0.01 (age)	DFEfromspinach = 0.02 + 21.0 (mentions) - 0.0 (age)	DFEfrombeans = -0.7 + 73.8 (mentions) + 0.02 (age)

South Asian	DFEfromotherveg = 2.3 + 5.9 (mentions) + 0.2 (age)	Dfefrompasta = 4 + 158.6 (mentions) - 0.1 (age)	DFEfromfruit = -1.1 + 16 (mentions) + 0.1 (age)	DFEfromfjuice = -1.1 + 27.3 (mentions) + 0.07 (age)	DFEfromvegjuice = -0.06 + 22.6 (mentions) + 0 (age)	DFEfromspinach = -1.5 + 118.7 (mentions) + 0.07 (age)	Dfefrombeans = 23.6 + 131.4 (mentions) - 0.6 (age)
Southeast Asian	DFEfromotherveg = 21.1 + 6.5 (mentions) + 0.1 (age)	DFEfrompasta = 34.5 + 48 (mentions) - 0.6 (age)	DFEfromfruit = -4 + 20.4 (mentions) - 0.01 (age)	DFEfromfjuice = -3.2 + 38.2 (mentions) + 0.2 (age)	DFEfromvegjuice = -0.06 + 34.5 (mentions) + 0 (age)	Dfefromspinach = 3.8 + 101 (mentions) - 0.1 (age)	Dfefrombeans = 81.9 + 329.6 (mentions) - 2.4 (age)
Arab	DFEfromotherveg = 8 + 2.9 (mentions) + 0.2 (age)	DFEfrompasta = -4.1 + 63.4 (mentions) - 0.08 (age)	Dfefromfruit = -16.2 + 14.5 (mentions) + 0.7 (age)	DFEfromfjuice = -4.1 + 43.6 (mentions) -0.02 (age)	DFEfromvegjuice = -0.01 + 24.4 (mentions) + 0 (age)	Dfefromspinach = 0.05 + 23.1 (mentions) -0 (age)	DFEfrombeans = 5.3 + 69.6 (mentions) - 0.03 (age)
West Asian	DFEfromotherveg = -31.4 + 5.1 (mentions) + 1.5 (age)	DFEfrompasta = -9.9 + 155 (mentions) + 0.3 (age)	DFEfromfruit = 10.9 + 12.6 (mentions) - 0.12 (age)	DFEfromfjuice = 11.8 + 8 (mentions) -0.3 (age)	DFEfromvegjuice= 0 + 26.4 (mentions) - 0 (age)	DFEfromspinach = -0.06 + 7.8 (mentions) + 0 (age)	DFEfrombeans = -66.4 + 173.8 (mentions) +2.2 (age)
Latin	DFEfromotherveg = -5.1 + 7.0 (mentions) + 0.04 (age)	DFEfrompasta = 24.8 +131 (mentions) - 0.6 (age)	DFEfromfruit = -11.3 + 19.4 (mentions) + 0.2 (age)	DFEfjuice = -18.1 + 72.5 (mentions) + 0.5 (age)	DFEfromvegjuice= -0.02 + 65.4 (mentions) + 0 (age)	DFEfromspinach = -1.1 + 147 (mentions) + 0.04 (age)	Dfefrombeans = -10.1 + 155.5 (mentions) + 0.6 (age)
Other	DFEfromotherveg = 2.3 + 6.9 (mentions) - 0.08 (age)	DFEfrompasta = -36.2 + 223.6 (mentions) + 1.2 (age)	Dfefromfruit = -6 + 15.2 (mentions) + 0.2 (age)	DFEfromfjuice = -21.9 + 43.8 (mentions) + 0.5 (age)	Dfefromvegjuice = -0.1 + 42.2 (mentions) + 0 (age)	DFEfromspinach = -23.7 + 333.1 (mentions) + 0.8 (age)	DFEfrombeans = 1.7 + 48.6 (mentions) - 0.05 (age)
Multiple	Dfefromotherveg = 7.6 + 6.2 (mentions) + 0.4 (age)	DFEfrompasta = 1.6 + 187.6 (mentions) -0.3 (age)	DFEfromfruit = -0.07 + 11.2 (mentions) + 0.02 (age)	DFEfromfjuice = 15.9 + 39.1 (mentions) - 0.1 (age)	DFEfromvegjuice = -0.1 + 16.6 (mentions) - 0.1 (age)	DFEspinach = 1 + 60.6 (mentions) -0.05 (age)	Dfefrombeans = -0.7 + 83.1 (mentions) + 0.05 (age)

Table S2a, continued

	Food Group							
	Nuts or Seeds	*Lettuce*	*Tomatoes*	*Eggs*	*Cold Cereal*	*Hot Cereal*	*White Bread*	*Brown Bread*
Cultural Group								
Caucasian	DFEfromnuts = 4.4 + 9.0 (mentions) + 0.05 (age)	DFEfromlettuce = -0.2 + 29.8 (mentions) + 0.08 (age)	DFEfromtomato = -0.2 + 6.2 (mentions) + 0.04 (age)	DFEfromeggs = 1.6 + 15.6 (mentions) + 0.06 (age)	Dfefromcoldcereal = 4.2 + 47.4 (mentions) - 0.06 (age)	DFEfromhotcereal = 0.1 + 4.3 (mentions) - 0 (age)	DFEwhiteb = 13.1 + 88.3 (mentions) - 0.2 (age)	DFEfrombrownb = 0.2 + 37.6 (mentions) + 0.03 (age)
African	DFEfromnuts = 5.4 + 4.1 (mentions) + 0.03 (age)	DFEfromlettuce = 2.4 + 25.1 (mentions) - 0.03 (age)	DFEfromtomato = -1 + 5.6 (mentions) + 0.05 (age)	DFEfromeggs = 3.3 + 15.4 (mentions) + 0.06 (age)	DFEfromcoldcereal = 8.9 + 40.7 (mentions) - 0.16 (age)	DFEfromhotcereal = 0.02 + 0.15 (mentions) - 0. (age)	DFEfromwhiteb = 13.3 + 81.1 (mentions) - 0.05 (age)	DFEfrombrownb = -0.9 + 38.9 (mentions) - 0.01 (age)
Korean	DFEfromnuts = -0.4 + 5.3 (mentions) - 0.02 (age)	DFEfromlettuce = -6.4 + 19.8 *mentions) + 0.2 (age)	DFEfromtomato = 0.2 + 2.8 (mentions) + 0 (age)	Dfeeggs = 13.5 + 19.7 (mentions) - 0.3 (age)	DFEcoldcereal = -6.6 + 24.3 (mentions) + 0.2 (age)	DFEfromhotcereal = 0 + 1.2 (mentions) + 0 (age)	DFEfromwhirebread= -7.6 + 92.8 (mentions) + 0.3 (age)	DFEbrownb= -0.2 + 22.9 (mentions) + 0.01 (age)
Filipino	DFEfromnuts = -1.8 + 47.2 (mentions) + 0.04 (age)	Dfefromlettuce = 0 +12 (mentions) + 0.01 (age)	DFEfromtomato = -0.2 + 5.8 (mentions) + 0.01 (age)	DFEfromeggs = -2 + 14 (mentions) + 0.2 (age)	DFEfromcoldcereals = -2 + 40 (mentions) + 0.0 (age)	DFEfromhotcereals = 0.04 + 8 (mentions) - 0 (age)	DFEwhiteb = 8.8 + 73.3 (mentions) - 0.05 (age)	DFEbrownb = - 0.06 + 23.5 (mentions) + 0 (age)
Japanese	DFEfromnuts = -2.6 + 22.6 (mentions) + 0.05 (age)	DFEfromlettuce = -12.2 + 14.3 (mentions) + 0.5 (age_	DFEfromtomato = -1 + 15.4 (mentions) - 0.1 (age)	Dfefromeggs = -10.7 + 10.9 (mentions) + 0.3 (age)	DFEfromcoldcer = -3.8 + 31.5 (mentions) + 0.08 (age)	DFEfromhotcereal = 0.1 + 1.3 (mentions) - 0 (age)	DFEwhiteb = -2.6 + 31.9 (mentions) + 0.08 (age)	DFEbrownb= 3.1 + 28.4 (mentions) -0.1 (age)
Chinese	DFEfromnuts = -1.5 + 19.8 (mentions) -	DFEfromlettuce = -2.1 + 32.2 (mentions) +	DFEfromtomato = -0.8 + 5.5	DFEfromeggs = 3.4 + 17 (mentions) -	DFEfromcoldcereal = 6.4 + 61.5	DFEfromhotcereal = 0.3 + 6.4	DFEfromwhiteb = 19.2 + 67.1	DFEbrownb = 1.7 + 27.4 (mentions) - 0.05 (age)

	0.1 (age)	0.1 (age)	(mentions) + 0.02 (age)	0.07 (age)	(mentions) -0.2 (age)	(mentions) - 0.01 (age)	(mentions) - 0.2 (age)	
Aboriginal	DFEfromnuts = 10.1 + 17.4 (mentions) -0 (age)	Dfefromlettuce = 0.14 + 18.7 (mentions) - 0.01 (age)	Dfefromtomato = -0.8 + 8.4 (mentions) + 0.03 (age)	DFEfromeggs = 3.6 + 15.7 (mentions) + 0.16 (age)	Dfefromcoldcereal = 3.6 + 45.6 (mentions) - 0.01 (age)	DFEfromhotcereal = 0.2 + 6.7 (mentions) - 0.01 (age)	DFEfromwhiteb = 9.0 + 91.6 (mentions) + 0.06 (age)	DFEbrown = -2.9 + 33.1 (mentions) + 0.07 (age)
South Asian	DFEfromnuts = 2.3 + 4.8 (mentions) + 0 (age)	DFEfromlettuce = -10 + 44.2 (mentions) + 0.6 (age)	DFEfromtomato = 0.02 + 4.5 (mentions) + 0.02 (age)	DFEfromeggs = 5.7 + 13.1 (mentions) - 0.06 (age)	Dfefromcoldcereal = -9.7 + 70.1 (mentions) + 0.2 (age)	DFEfromhotcereal = 0.4 + 2.3 (mentions) - 0.01 (age)	Dfewhiteb = 15.8 + 84.8 (mentions) 0.02 (age)	DFEbrownb = 0.8 + 23.7 (mentions) -0.01 (age)
Southeast Asian	DFEfromnuts = 4.7 + 2.2 (mentions) - 0.05 age)	DFEfromlettuce = -1.5 + 29.2 (mentions) - 0.2 (age)	DFEfromtomato = 2.2 + 8.2 (mentions) =0.05 (age)	DFEfromeggs = 3.4 + 14.2 (mentions) - 0.2 (age)	Dfefromcoldcereal = -6.1 + 43.3 (mentions) + 0.2	Dfefromhotcereal = 0.01 + 0.3 (mentions) - 0(age)	Dfewhiteb = -11.8 + 86.7 (mentions) - 0.3 (age)	DFEbrownb = -0.4 + 27.4 (mentions) + 0.05 (age)
Arab	DFEfromnuts = 1.4 + 11.8 (mentions) - 0.06 (age)	DFEfromlettuce = 2.7 + 11.9 (mentions) + 0 (age)	DFEfromtomato = -3.7 + 8.6 (mentions) + 0.14 (age)	DFEfromeggs = 11.1 + 14.8 (mentions) - 0.2 (age)	DFEfromcoldcereal = 6.3 + 13.2 (mentions) -0.2 (age)	DFEfromhotcereal = 0.01 + 1.1 (mentions) - 0 (age)	Dfefromwhiteb= 5.8 + 77.5 (mentions) + 0.1 (age)	DFEfrombrownb = -1.6 + 16.4 (mentions + 0.1 (age)
West Asian	DFEfromnuts = -0.02 + 6.9 (mentions) + 0 (age)	DFEfromlettuce = -26.4 + 75.9 (mentions) + 0.6 (age)	DFEfromtomato = -4.4 + 8.3 (mentions) + 0.12 (age)	DFEfromeggs = -5.2 + 4.1 (mentions) + 0.6 (age)	DFEfromcoldcer = 0.4 + 48 (mentions) + 0.01 (age)	DFEfromhotcereal = 0 + 7.5 (mentions) + 0 (age)	DFEfromwhiteb = 33.4 + 92.6 (mentions) + 0.02 (age)	DFEfrombrownb= -0.2 + 28.6 (mentions) -0.03 (age)
Latin	DFEfromnuts = 1.4 + 0.9 (mentions) + 0.2 (age)	Dfefromlettuce = -0.5 + 65.6 (mentions) - 0.1 (age)	DFEfromtomato = 0.5 + 6 (mentions) -0.02 (age)	DFEfromeggs = 23.5 + 12.6 (mentions) - 0.4 (age)	DFEcoldcereal = 3.3 + 46.5 (mentions) - 0.04 (age)	DFEfromhotcereal = 0 + 1 (mentions) - 0 (age)	Dfewhiteb = -7.1 + 92.6 (mentions) - 0 (age)	DFEbrownb = 2.2 + 27.8 (mentions) -0.1 (age)
Other	DFEfromnuts	Dfefromlettuce	Dfefromtom	Dfefromeggs	DFEfromcoldc	DFEfromho	DFEwhiteb =	Dfebrownb = 1.8 -32.6

	= 1.2 + 12.6 (mentions) - 0.02 (age)	= -14.6 + 46.6 (mentions) + 0.5 (age)	ato = 0.4 + 5.5 (mentions) - 0 (age)	= -3.5 + 13.5 (mentions) +0.1 (age)	ereal = 0.3 + 59.4 (mentions) - 0.01 (age)	tcereal = 0.9 + 6.1 (mentions) - 0.05 (age)	0.09 + 82.3 (mentions) + 0(age)	(mentions) -0.1 (age)
Multiple	Dfefromnuts = 2.8 + 4.1 (mentions) - 0.06 (age)	DFEfromlettuce = 1.4 + 23.4 (mentions) - 0.01 (age)	DFEfromtomato = 1.1 + 5.2 (mentions) - 0 (age)	Dfefromeggs = -1 + 15.9 (mentions) + 0.2 (age)	Dfefromcoldcereal = -1.9 + 48 (mentions) + 0.2 (age)	DFEfrom hotcereal = 0.3 + 17.4 (mentions) - 0.01 (age)	DFEfromwhiteb= -1.2 + 94.6 (mentions) - 0.1 (age)	DFEfrombrownb = 0.7 + 30.5 (mentions) + 0 (age)

Table S2b. Male Vitamin B_{12} Regression Models.

	Food Group							
	B_{12} from supplement	*Liver**	*Offal**	*Bacon*	*Hot Dogs*	*Red Meat[a]*	*Saltwater Fish[b]*	*Freshwater Fish[c]*
Cultural Group								
Caucasian	B12fromsupp = 74.8 - 43.1 (yes or no) + 0.3 (age)			B12frombacon = -0.02 + 0.5 (mentions) + 0 (age)	B12fromhotdog = 0 + 1 (mentions) + 0 (age)	B12frommeat = 0.2 + 1.3 (mentions) + 0.01 (age)	B12fromsaltwfish = 0.4 + 2.2 (mentions) + 0.01 (age)	12
African	B12fromsupp = 27.9 - 15.6 (yes or no) + 0.1 (age)			B12frombacon = 0 + 0.5 (mentions) + 0 (age)	B12fromhotdog = 0.03 + 0.6 (mentions) - 0 (age)	B12frommeat = -0.4 + 1.2 (mentions) + 0.04 (age)	B12fromsaltwfish = -0.3 + 3.4 (mentions) + 0.01 (age)	B12fromfreshwfish = -0.02 + 4.4 (mentions) + 0 (age)
Korean	B12fromsupp = 17.7 - 11.5 (yes or no) + 0.2 (age)			B12frombacon = 0 + 0.6 (mentions) - 0 (age)	B12fromhotdog = 0 + 0.7 (mentions) + 0 (age)	B12frommeat = -2.8 + 1.4 (mentions) + 0.2 (age)	B12fromsaltwfish = 0.01 + 0.4 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 0.01 (mentions) + 0 (age)
Filipino	B12fromsupp = 24.1 --15.1 (yes or no) + 0.2 (age)			B12frombacon = -0.02 + 0.8 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.7 (mentions) + 0 (age)	B12frommeat = 0.04 + 0.7 (mentions) + 0.01 (age)	B12fromsaltwfish = 0.3 + 3 (mentions) - 0.01 (age)	B12fromfreshwfish = -0.02 + 3 (mentions) + 0 (age)
Japanese	B12fromsupp = 79.5 - 38.4 (mentions) - 0.07 (age)			B12frombacon = -0.01 + 0.2 (mentions) + 0 (age)	B12fromhotdog = 0.1 + 0.8 (mentions) - 0 (age)	B12frommeat = -1 + 0.6 (mentions) + 0.03 (age)	B12fromsaltwfish = 0 + 2.1 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 0.01 (mentions) + 0 (age)
Chinese	B12fromsupp = 40.1 - 21.9 (yes or no) + 0.1 (age)			B12frombacon = 0.09 + 0.5 (mentions) -0 (age)	B12fromhotdog = 0.01 + 0.3 (mentions) - 0 (age)	B12frommeat = 1.9 + 0.8 (mentions) - 0.03 (age)	B12fromsaltwfish = 0.03 = 1.9 (mentions) - 0 (age)	B12fromfreshwfish = -0.1 + 2.9 (mentions) + 0 (age)
Aboriginal	B12fromsupp = 42.2 - 25.3			B12frombacon = -0.06 + 0.6	B12fromhotdog = -0.02 + 0.99	B12frommeat = 0.13 + 1.3	B12fromsaltwfish = -0.05 + 1.9	B12fromfreshwfish = 0.04 + 1.4

	(yes or no to supp use) + 0.26 (age)	B12fromliver = 0.12 + 61.4 (mentions) - 0 (age)	B12fromoffal = 0.02 + 6.4 (mentions) -0 (age)	(mentions) + 0 (age)	(mentions) + 0(age)	(mentions) + 0.02 (age)	(mentions) + 0 (age)	(mentions) -0 (age)
South Asian	B12fromsupp = 150.9 -74.5 (yes or no) - 0.06 (age)			B12frombacon = 0.01 + 0.3 (mentions) -0 (age)	B12fromhotdog = 0.01 + 0.5 (mentions) - 0 (age)	B12frommeat = -0.6 + 2.4 (mentions) + 0.01 (age)	B12fromsaltwfish = -0.1 + 3.1 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 0.8 (mentions) - 0 (age)
Southeast Asian	B12fromsupp = 41.1-21.3 (yes or no) + 0.05 (age)			B12frombacon = -0.02 + 1.4 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.7 (mentions) + 0 (age)	B12frommeat = -0.2 + 0.8 (mentions) + 0.01 (age)	B12fromsaltwfish = 0.1 + 4.3 (mentions) - 0 (age)	B12fromfreshwfish = 0.2 + 2.1 (mentions) -0.01 (age)
Arab	B12fromsupp = 23.3 - 13.1 (yes or no) + 0.2 (age)			B12frombacon = 0 + 0.02 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.5 (mentions) - 0 (age)	B12frommeat = 0.8 + 0.3 (mentions) + 0 (age)	B12fromsaltwfish = -0.07 + 1.9 (mentions) + 0(age)	B12fromfreshwfish = 0 + 3.3 (mentions) - 0 (age)
West Asian	B12fromsupp = 92.7 - 46.8 (yes or no) + 0.03 (age)				B12fromhotdogs = 0 + 0.5 (mentions) + 0 (age)	B12frommeat = 0.7 + 0.7 (mentions) + 0.1 (age)	B12fromsaltwfish = 0 + 1.2 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 2.4 (mentions) + 0 (age)
Latin	B12fromsupp = 18.2 - 10 (yes or no)			B12frombacon = 0.03 + 0.4 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.4 (mentions) + 0 (age)	B12frommeat = -0.5 + 1.3 (mentions) + 0.02 (age)	B12fromsaltwfish = -0.01 + 0.7 (mentions) + 0 (age)	B12fromfreshwfish = - + 2.9 (mentions) + 0 (age)
Other	B12fromsupp = 63 - 34.2 (yes or no) + 0.2 (age)			B12frombacon = -0.05 + 0.7 (mentions) + 0 (age)	B12fromhotdog = 0 + 1.3 (mentions) + 0 (age)	B12frommeat = 0.6 + 1 (mentions) - 0.01 (age)	B12fromsaltwfish = 0.02 + 0.8 (mentions) - 0 (age)	B12fromfreshwfish = 0 + 3.9 (mentions) + 0 (age)
Multiple	B12fromsupp = 41.7 - 27.3 (mentions) + 0.6 (age)			B12frombacon = 0.03 + 0.5 (mentions) - 0 (age)	B12fromhotdog = 0 + 0.7 (mentions) + 0 (age)	B12frommeat = 0.1 + 1.1 (mentions) + 0.03 (age)	B12fromsaltwfish = -0.04 + 2.9 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 0.5 (mentions) + 0 (age)

* This regression model is for all males, regardless of cultural group, due to lack of valid consumers in each cultural group

[a] Red meat encompasses beef, hamburger, pork or lamb

[b] Saltwater fish includes (but is not limited to) salmon, tuna, fish sticks and other saltwater varieties
[c]Freshwater fish includes (but is not limited to) trout, walleye, pickerel and other freshwater varieties

Table Table S2b, continued

	Food Group					
	Shellfish[a]	*Eggs*	*Frozen Yogurt/Ice Cream*	*Milk*	*Cottage Cheese*	*Yogurt*
Cultural Group						
Caucasian	B12fromshellfish = -0.4 + 6.1 (mentions) - 0.03 (age)	B12fromeggs = 0.3 + 0.08 (mentions) + 0 (age)	B12fromfrozenyogurt = -0.1 + 0.6 (mentions) - 0 (age)	B12frommilk = 0.9 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = -0.3 + 0.7 (mentions) + 0 (age0	B12fromyogurt = 0.4 + 0.4 (mentions) + 0 (age)
African	B12fromshellfish = -0.6 + 16.3 (mentions) + 0.2 (age)	B12fromeggs = 0.04 + 0.2 (mentions) + 0 (age)	B12fromfrozenyogurt = -0.03 + 0.5 (mentions) + 0 (age)	B12frommilk = 0.5 + 0.5 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = -0.02 + 0.5 (mentions) + 0 (age)
Korean	B12fromshellfish = -0.03 - 1.3 (mentions) = 0 (age)	B12fromeggs = 0.2 + 0.2 (mentions) -0 (age)	B12fromyogurt = 0 + 0.3 (mentions) + 0 (age)	b12frommilk = 0.7 + 0.5 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = 0 + 0.9 (mentions) -0 (age)
Filipino	B12fromshellfish = 0.4 + 1.7 (mentions) -0.01 (age)	B12fromeggs = 0.01 + 0.1 (mentions) + 0 (age)	B12fromfrozenyogurt = 0 + 0.3 (mentions) - 0 (age)	B12frommilk = 0.3 + 0.5 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.1 (mentions) + 0 (age)	B12fromyogurt = 0 + 0.3 (mentions) + 0 (age)
Japanese	B12fromshellfish = -14.3 + 21.2 (mentions) + 0.3 (age)	B12fromeggs = -0.13 + 0.14 (mentions) + 0 (age)	B12fromfrozenyogurt = -0.06 + 0.2 (mentions) + 0 (age)	B12frommilk = 0.6 = 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = 0 + 1.2 (mentions) + 0 (age)
Chinese	B12fromshellfish = 2.3 + 4.2 (mentions) -0.06 (age)	B12fromeggs = 0.1 + 0.2 (mentions) -0 (age)	B12fromfrozenyogurt = 0 + 0.5 (mentions) + 0(age)	B12frommilk = 0.6 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = 0 + 0.8 (mentions) -0 (age)
Aboriginal	B12fromshellfish = 2.1 +19.8	B12fromeggs = 0.06 = 0.2	B12fromfrozenyogurt = -0.02 + 0.4	B12frommilk = 0.5 + 0.5	B12fromcottagecheese = 0 + 0.08 (mentions)	B12fromyogurt = 0 + 0.5

	(mentions) - 0.06 (age)	(mentions) + 0 (age)	(mentions) + 0 (age)	(mentions) - 0.02 (age)	- 0 (age)	(mentions) -0 (age)
South Asian	B12fromshellfish = -0.05 + 2.7 (mentions) + 0 (age)	B12fromeggs = 0.1 + 0.2 (mentions) -0 (age)	B12fromfrozenyogurt = 0 + 0.4 (mentions) + 0 (age)	B12frommilk = 0.5 + 0.6 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.2 (mentions) + 0 (age)	B12fromyogurt = -0.06 + 0.8 (mentions) + 0 (age)
Southeast Asian	B12fromshellfish = 1.9 + 2.4 (mentions) -0.03 (age)	B12fromeggs = 0.02 + 0.2 (mentions) - 0 (age)	B12fromfrozenyogurt = 0.04 + 0.2 (mentions) -0 (age)	B12frommilk = 0.01 + 0.5 (mentions) - 0 (age)	B12fromcottagecheese = 0 + 0.2 (mentions) + 0 (age)	B12fromyogurt = 0 + 0.6 (mentions) + 0 (age)
Arab	B12fromshellfish = -0.01 + 0.6 (mentions) + 0 (age)	B12fromeggs = 0.2 + 0.2 (mentions) -0 (age)	B12fromfrozenyogurt = -0.01 = 0.5 (mentions) + 0 (age)	B12frommilk = 0.09 + 0.5 (mentions) - 0 (age)	B12fromcottagecheese = 0 + 0.07 (mentions) + 0 (age)	b12fromyogurt = -0.03 + 0.4 (mentions0 + 0 (age)
West Asian	B12fromshellfish = 0 + 0.8 (mentions) - 0 (age)	B12fromeggs = -0.05 + 0.03 (mentions) + 0.01 (age)	B12fromfrozenyogurt = 0.01 + 0.4 (mentions) - 0(age)	B12frommilk = 0.5 + 1.6 (mentions) + 0.01 (age)	B12fromcottagecheese = 0 + 0.2 (mentions) + 0 (age)	B12fromyogurt = -0.13 + 2 (mentions) + 0 (age)
Latin	b12fromshellfish = -0.09 + 5.1 (mentions) + 0 (age)	B12fromeggs = 0.2 + 0.2 (mentions) -0 (age)	B12fromfrozenyogurt = 0 + 0.5 (mentions) + 0(age)	B12frommilk = 0.8 + 0.5 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = 0.06 + 0.3 (mentions) - 0 (age)
Other	B12fromshellfish = -0.2 + 1.7 (mentions) + 0 (age)	B12fromeggs = -0.07 + 0.2 (mentions) + 0 (age)	B12fromfrozenyogurt = 0 + 0.4 (mentions) + 0 (age)	B12frommilk = 0.6 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.2 (mentions) + 0 (age)	B12fromyogurt = 0.01 + 0.5 (mentions) - 0 (age)
Multiple	B12fromshellfish = 0 + 1.8 (mentions) + 0 (age)	B12fromeggs = 0.05 + 0.2 (mentions) + 0 (age)	B12fromfrozenyogurt = -0.01 + 0.4 (mentions) + 0 (age)	B12frommilk = 0.6 + 0.4 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.07 (mentions) - 0 (age)	B12fromyogurt = -0.01 + 0.6 (mentions) + 0 (age)

[a]Shellfish includes (but is not limited to) shrimp, mussels, scallops, lobster, clams, oyster or crab

Table S3a. Female Folate Regression Models.

	DFE[a] from supplement	*Liver**	*Rice*	*Potatoes*	*Fried Potatoes*
Cultural Group					
Caucasian	DFEfromsupplement = 1806.2 - 974.5 (mentions) + 3.6 (age)	DFEfromliver = -0.4 + 225.7 (mentions of consumption) + 0.01 (age)	DFEfromrice = 0.3 + 9.2 (mentions) - 0 (Age)	DFEfrompotatoes = 0.3 + 14.1(mentions of consumption) - 0.001 (age)	DFEfromfriedpotatoes = -0.06 + 14.3 (mentions of consumption) + 0 (age)
African	DFEfromsupp = 2119.6 - 1121.5 (yes or no) + 4.6 (age)		DFEfromrice = 0.3 + 5.4 (mentions) - 0.01 (age)	DFEfrompotatoes = -0.2 + 19.7 (mentions of consumption) - 0.008 (age)	DFEfromfriedpotatoes = 0.8 + 14.5 (mentions of consumption) -0.02 (age)
Korean	Dfefromsupp = 907.1 -505.4 (yes or no) + 2.9 (age)		DFEfromrice = -10.8 + 6.5 (mentions) + 0.5 (age)	DFEfrompotatoes = 0.4 + 6.7(mentions of consumption) - 0.04(age)	DFEfromfriedpotatoes = 0 + 9.1 (mentions of consumption) + 0 (age)
Filipino	DFEfromsupp = 1504.8 - 842 (yes or no) + 4.7 (age)		DFEfromrice = -4.4 + 7.6 (mentions) + 0.08 (age)	DFEfrompotatoes = -1 + 6.8 (mentions of consumption) + 0.04(age)	DFEfromfriedpotatoes = 0.02 + 12.2 (mentions of consumption) -0 (age)
Japanese	DFEfromsupp = 2257.3 - 1143.5 (yes or no) + 1.0 (age)		DFEfromrice = 28.4 +103.1 (mentions) - 2.3 (age)	DFEfrompotatoes = -0.2 + 5.3 (mentions of consumption) + 0.004 (age)	DFEfromfriedpotatoes = 0 +10.5 (mentions of consumption) -0 (age)
Chinese	DFEfromsupp = 1547.5 - 790.1 (yes or no) + 0.9 (age)		DFEfromrice = -3.4 + 6.3 (mentions) +	DFEfrompotatoes = 0.4 + 6.9 (mentions of	DFEfromfriedpotatoes = -0.5 + 22.1 (mentions of

			0.05 (age)	consumption) - 0.01 (age)	consumption) + 0.01 (age)
Aboriginal	DFE_supp = 1687.5 -951.9 (yes or no to FA supplement) + 6.7 (age)		DFEfromRice = 1.63 + 19.9 (mentions) - 0.05 (age)	DFEfrompotatoes = -0.3 + 11.4 (mentions of consumption) + 0.03 (age)	DFEfromfriedpotatoes = -0.2 + 15 (mentions of consumption) + 0.006 (age)
South Asian	DFEfromsupp = 942.6 - 532.5 (mentions) + 3.7 (age)		DFEfromrice = -1.2 + 5.7 (mentions) + 0.04 (age)	DFEfrompotatoes = -0.04 + 7.4 (mentions of consumption) + 0.004 (age)	DFEfromfriedpotatoes = -0.1 + 9.7 (mentions of consumption) + 0.03 (age)
Southeast Asian	DFEfromsupp = 1437.5 - 731.9 (yes or no) + 0.8 (age)		DFEfromrice = -0.4 + 5 (mentions) + 0.1 (age)	DFEfrompotatoes = 0.8 + 6.6 (mentions of consumption) - 0.04 (age)	DFEfromfriedpotatoes = 0.4 + 11.1 (mentions of consumption) -0.01 (age)
Arab	DFEfromsupp = 1539.3 - 921.6 (yes or no) + 10.5 (age)		DFEfromrice = -0.7 + 4.6 (mentions) + 0.04 (age)	DFEfrompotatoes = -0.8 + 19.8 (mentions of consumption) + 0.03 (age)	DFEfromfriedpotatoes = -0.3 + 12.7 (mentions of consumption) + 0.009 (age)
West Asian	DFEfromsupplement = 2800.6 - 1360.2 (mentions) -2.4 (age)		DFEfromrice = -24.2 + 9 (mentions) + 0.7 (age)	DFEfrompotatoes = -6 + 23.2 (mentions of consumption) + 0.2 (age)	DFEfromfriedpotatoes = -0.02 + 3.3 (mentions of consumption) + 0 (age)
Latin	DFEfromsupp = 1119 - 618.8 +4.5 (age)		DFEfromrice = -0.7 + 3.4 (mentions) + 0.06 (age)	DFEfrompotatoes = 0.4 + 9.7 (mentions of consumption) - 0.008 (age)	DFEfromfriedpotatoes = -0.5 + 14.6 (mentions of consumption) + 0.02 (age)
Other	DFEfromsupp =		DFEfromrice	DFEfrompotatoes	DFEfromfriedpotatoes

	1892.2 - 987.6 (yes or no) + 2.3 (age)		= -0.9 + 6.9 (mentions) + 0.03 (age)	= -1 + 8.4 (mentions of consumption) + 0.03 (age)	= -0.3 + 13.4 (mentions of consumption) + 0.006 (age)
Multiple	DFEfromsupp = 1048 - 617.6 (yes or no) +6.6 (age)		Dfefromrice = 3.4 + 8.4 (mentions) - 0.02 (age)	DFEfrompotatoes = 1.3 + 11 (mentions of consumption) - 0.06 (age)	DFEfromfriedpotatoes = 0.05 + 9.6 (mentions of consumption) -0.003 (age)

[a] Dietary Folate Equivalent

* This regression model is for all females, regardless of cultural group, due to lack of valid consumers in each cultural group

Table S3a, continued.

	Food Group						
	Other vegetables	*Pasta*	*Fruit*	*Fruit Juice*	*Vegetable Juice*	*Spinach*	*Beans*
Cultural Group							
Caucasian	DFEfromotherveg = 6.0 + 6.1 (mentions) + 0.2 (age)	DFEfrompasta = 3.9 + 162.0 (mentions) - 0.1 (age)	DFEfromfruit = 3.7 _ 9.8 (mentions) + 0.01 (age)	DFEfromfjuice = 4.9 + 29.8 (mentions) - 0.07 (age)	DFEfromvegjuice = 0.03 + 42.1 (mentions) - 0 (age)	DFEfromspinach = 1.2 + 94.5 (mentions) -0.02 (age)	DFEfrombeans = 0.8 + 91.6 (mentions) - 0.02 (age)
African	DFEfromotherveg = -2.5 + 5 (mentions) + 0.4 (age)	DFEfrompasta = -3.2 + 130.9 (mentions) + 0.08 (age)	DFEfromfruit = 1.5 + 9.4 (mentions) + 0.05 (age)	DFEfromfjuice = -57.3 + 73.8 (mentions) + 1.05 (age)	DFEfromvegjuice = 0.2 + 14.1 (mentions) -0.01 (age)	DFEfromspin = -3.2 + 55.2 (mentions) + 0.1 (age)	DFEfrombeans = 5.4 + 175.5 (mentions) - 0.3 (age)
Korean	DFEotherveg = 52.5 + 1.8 (mentions) + 0.7 (age)	DFEfrompasta = 6.2 + 115.4 - 0.2 (age)	DFEfromfruit = 26.6 + 20.3 (mentions) -0.6 (age)	DFEfromfjuice = -16.2 + 41 (mentions) + 0.4 (age)	DFEfromvegjuice = 0.03 +12.7 (mentions) - 0 (age)	DFEfromspin = 2 + 41.2 (mentions) -0.07 (age)	DFEfrombeans = -0.3 + 55.2 (mentions) + 0 (age)
Filipino	DFEfromotherveg = 19.5 + 3.6 (mentions) + 0.12 (age)	DFEfrompasta = -8.4 + 161.1 (mentions) + 0.1 (age)	DFEfromfruit = -8.5 + 17.7 (mentions) + 0.2 (age)	DFEfromfruitjuice = 19 + 31.5 (mentions) -0.4 (age)	DFEfromvegjuice = 0.03 +12.7 (mentions) - 0 (age)	Dfefromspinach = -7.7 + 56.1 (mentions) + 0.2 (age)	DFEfrombeans = -3.2 + 85.9 (mentions) + 0.1 (age)
Japanese	DFEfromotherveg = -12.1 + 7.4	DFEfrompasta =	Dfefromfruit = 5.5 + 7.1	DFEfromfjuice = -6.8 + 14.8	DFEfromvegjuice = 21.3 + 52.4 - 0.7 (age)	DFEfromspinach = -12.6 + 49.7	DFEfrombeans = 0.7 + 17.3 (mentions) -

	(mentions) + 0.3 (age)	-26.3 + 11.5 (mentions) + 0.5 (age)	(mentions) -0.07 (age)	(mentions) + 0.2 (age)		(mentions) + 0.2 (age)	0.01 (age)
Chinese	Dfefromotherveg = 49.5 + 3.2 (mentions) + 0.1 (age)	DFEfrompasta = 2.9 + 118.5 (mentions) +0.2 (age)	DFEfromfruit = 1.1 + 13.2 (mentions) + 0.2 (age)	Dfefromfjuice = -4.4 + 27.1 (mentions) - 0.02 (age)	DFEfromvegjuice = 0.01 + 39.9 (mentions) - 0(age)	DFEfromspinach = 0.5 + 126 (mentions) - 0.07 (age)	Dfefrombeans = 5.9 + 46.8 (mentions) -0.09 (age)
Aboriginal	DFEfromotherveg = -4.1 + 6.0 (mentions)+ 0.33 (age)	DFEfrompasta = 3.2 + 152.4 (mentions) - 0.05 (age)	DFEfromfruit = 1.0 + 13.0 (mentions) + 0 (age)	DFEfromfjuice = -6.9 + 30.1 (mentions) + 0.15 (age)	DFEfromvegjuice = 0.31 + 37.7 (mentions) - 0.01 (age)	DFEfromspinach = 0.93 + 92.7 (mentions) - 0 (age)	Dfefrombeans = 0.69 + 56.3 (mentions) - 0.02 (age)
South Asian	DFEfromotherveg = -2 + 6 (mentions) + 0.3 (age)	DFEfrompasta = -4.2 + 136.9 (mentions) + 0.1 (age)	Dfefromfruit = 2.5 + 9.3 (mentions) + 0.1 (age)	DFEfromfruitjuice = 7.5 + 24 (mentions) -0.3 (age)	DFEfromvegjuice = 0.3 + 17.1 (mentions) - 0.01 (age)	DFEfromspinach = -3.9 + 114.1 (mentions) + 0.2 (age)	DFEfrombeans = 14.3 + 151.5 (mentions) - 0.4 (age)
Southeast Asian	DFEfromotherveg = 8.4 + 5.8 (mentions) + 0.5 (age)	DFEfrompasta= 31.8 + 169.2 (mentions) -1.2 (age)	DFEfromfruit = 9.4 + 12.5 (mentions) -0.2 (age)	DFEfromfruitjuice = -5.3 + 19.3 (mentions) + 0.1 (age)	DFEfromvegjuice = -0.06 + 34.5 (mentions) + 0 (age)	DFEfromspinach = 0.8 + 152.8 (mentions) -0.01 (age)	DFEfrombeans = -6 + 80.4 (mentions) + 0.2 (age)

Arab	DFEfromotherveg = 4.8 + 5.5 (mentions) + 0.3 (age)	DFEfrompasta = 5.2 + 96 (mentions) -0.2 (age)	DFEfromfruit = 6.6 + 13.4 (mentions) - 0.4 (age)	DFEfromfjuice = -38.8 + 55.3 (mentions) + 1.7 (age)	DFEfromvegjuice = -0.01 + 24.4 (mentions) + 0 (age)	DFEfromspinach = 1.6 + 28.4 (mentions) -0.06 (age)	DFEfrombeans = 3.2 + 85.4 (mentions) -0.16 (age)
West Asian	DFEfromotherveg = 8.6 + 7.2 (mentions) + 0.01 (age)	DFEfrompasta = 45.2 + 175.7 (mentions) -1.3 (age)	DFEfromfruit = -17.1 + 17.6 (mentions) + 0.07 (age)	DFEfromfjuice = 13.3 + 5 (mentions) -0.1 (age)	DFEfromvegjuice= 0 + 26.4 (mentions) - 0 (age)	DFEfromspinach = 21.1 + 155.4 (mentions) - 0.6 (age)	DFEfrombeans = 6.9 + 47.6 (mentions) -0.2 (age)
Latin	DFEfromotherveg = -19.3 + 8.2 (mentions) + 0.4 (age)	DFEfrompasta = 3.2 +128.8 (mentions) -0.3 (age)	DFEfromfruit = -7 + 20.3 (mentions) + 0.08 (age)	DFEfromfjuice = 14.4 + 38.2 (mentions) - 0.6 (age)	DFEfromvegjuice= -0.02 + 65.4 (mentions) + 0 (age)	DFEfromspinach = -1.6 + 362.5 (mentions) + 0.05 *age)	DFEfrombeans = -2.9 + 174.3 + 0.2 (age)
Other	Dfeotherveg = 28.4 + 3.8 (mentions) -0.05 (age)	DFEfrompasta = -10.9 + 147.3 (mentions) + 0.3 (age)	DFEfromfruit = 2.8 + 13.8 (mentions) -0.1 (age)	DFEfromfjuice = 7.4 + 27.1 (mentions) -0.2 (age)	DFEfromvegjuice = 1.3 + 46.1 (mentions) - 0.04 (age)	DFEfromspinach = -0.4 +131.3 (mentions) + 0.02 (age)	DFEbeans = 4.4 + 80.2 (mentions) - 0.1 (age)
Multiple	DFEfromotherveg = 13.6 + 3.2 (mentions) -0.07 (age)	DFEfrompasta = 22.7 + 146.9 (mentions) -0.7	DFEfromfruit = 3.7 + 10.4 (mentions) -0.04 (age)	DFEfromfjuice = 4.9 + 21.5 (mentions) - 0.1 (age)	Dfefromvegjuice = -0.01 + 69.3 (mentions) + 0 (age)	DFEfromspinach = -1.9 + 79.5 (mentions) + 0.08 (age)	DFEfrombeans = 12.2 + 230 (mentions) -0.5 (age)

		(age)					

Table S3a, continued.

Food Group								
	Nuts or Seeds	*Lettuce*	*Tomatoes*	*Eggs*	*Cold Cereal*	*Hot Cereal*	*White Bread*	*Brown Bread*
Cultural Group								
Caucasian	DFEfromnuts = 3.4 + 7.4 (mentions) + 0.02 (age)	DFEfromlettuce = 5.3 + 31 (mentions) + 0.01 (age)	DFEfromtomato = -0.04 + 5.5 (mentions) + 0.03 (age)	DFEfromeggs = 0.4 + 12.6 (mentions) + 0.05 (age)	DFEfromcoldcereal = 2.3 + 36.3 (mentions) - 0.03 (age)	Dfefromhotcereal = 0.01 + 4.1 (mentions) + 0 (age)	DFEfromwhiteb = 17 + 70.4 (mentions) - 0.3 (age)	DFEfrombrownb = 1.4 + 32.8 (mentions) - 0.01 (age)
African	DFEfromnuts = -2.6 + 3.5 (mentions) + 0.2 (age)	DFEfromlettuce = -6.4 + 28.2 (mentions) + 0.2 (age)	DFEfromtomato = -0.6 + 5.8 (mentions) + 0.02 (age)	DFEfromeggs = 0.4 + 12 (mentions) + 0.01 (age)	DFEfromcoldcer = -4.2 + 39.5 (mentions) + 0.1 (age)	DFEfromhotcereal = 0.05 + 2.8 (mentions) -0 (age)	DFEwhiteb = -0.4 +64.3 (mentions) + 0.03 (age)	DFEfrombrownb= 0.8 + 34.2 (mentions) -0.02 (age)
Korean	DFEfromnuts = -1.8 + 5.1 (mentions) _ 0.07 (age)	DFEfromlettuce = 5.3 + 21.7 (mentions) - 0.04 (age)	DFEfromtomato = -1.3 + 7.4 (mentions) + 0.07 (age)	DFEfromeggs = -11 + 10.4 (mentions) + 0.4 (age)	DFEfromcoldcereal = 0.5 + 37.3 (mentions) - 0.01 (age)	DFEfromhotcereal = 0 + 1.2 (mentions) + 0 (age)	DFEfromwhiteb = -22.7 + 67.1 (mentions) + 0.4 (age)	DFEfrombrownb= 0.4 + 29.7 (mentions) -0.01 (age)
Filipino	DFEfromnuts = -5.6 + 18.2 (mentions) + 0.1 (age)	DFEfromlettuce = -2.5 + 15.8 (mentions) + 0.1 (age)	Dfefromtomato = 3.4 + 4.1 (mentions) + 0.04 (age)	DFEfromeggs = 0.4 + 5.5 (mentions) + 0.01 (age)	DFEfromcoldcereal = -0.06 + 27.9 (mentions) + 0.03 (age)	DFEfromhotcereal = -0.4 + 16 (mentions) + 0.02 (age)	Dfefromwhiteb = 16.9 + 67.9 (mentions) - 0.1 (age)	Dfefrombrownb = 3 + 32.2 (mentions) -0.1 (age)
Japanese	DFEfromnuts = -2.6 + 22.6 (mentions) + 0.05 (age)	Dfefromlettuce = -1.8 + 32 (mentions) +	DFEfromtomato = -1.8 + 6.7 (mentions)	DFEfromeggs = 6.6 + 0.5 (mentions)	DFEfromcoldcereal = -5.4 + 31.3 (mentions) +	DFEfromhotcereal = 0.1 + 1.3 (mentions) -	DFEwhiteb= -56.2 + 76.1 (mentions) + 1 (age)	Dfebrownb = 0.06 + 23.1 (mentions) + 0 (age)

		0.02 (age)	+ 0.02 (age)	+ 0.9 (age)	0.1 (age)	0 (age)		
Chinese	DFEfromnuts = 0.9 + 8.4 (mentions) + 0.03 (age)	Dfefromlettuce = 4.3 + 43.1 (mentions) - 0.2 (age)	DFEfromtomato = 1.9 + 3.9 (mentions) - 0.02 (age)	DFEfromeggs = -2.3 + 13.6 (mentions) + 0.1 (age)	DFEfromcoldcereal = 0.5 + 37.3 (mentions) - 0.01 (age)	DFEfromhotcereal = 0.03 + 3.4 (mentions) - 0 (age)	DFEwhiteb = -16.4 + 72.1 (mentions) + 0.3 (age)	DFEbrownb = 1.2 + 21.5 (mentions) -0.02 (age)
Aboriginal	DFEfromnuts = 5.57 + 33.9 (mentions) + 0 (age)	DFEfromlettuce = 4.5 + 18.6 (mentions) - 0.03 (age)	DFEfromtomato = -0.6 + 4.61 (mentions) + 0.05 (age)	DFEfromeggs = 4.2 + 14.8 (mentions) + 0.02 (age)	DFEfromcoldcereal= 6.39 + 38.5 (mentions) - 0.12 (age)	DFEfromhotcereal = -0.16 + 1.2 (mentions) + 0.01 (age)	DFEwhiteb = 24.35 + 63.8 (mentions) - 0.6 (age)	DFEbrownb = 3.1 + 30.6 (mentions) -0.05 (age)
South Asian	DFEfromnuts = 2.8 + 7.4 (mentions) - 0.03 (age)	Dfefromlettuce = -9 + 28.7 (mentions) + 0.5 (age)	Dfefromtomato = -0.05 + 4.5 (mentions) + 0.02 (age)	DFEfromeggs = -0.8 + 10.6 (mentions) + 0.05 (age)	DFEfromcoldcereal = -1.8 + 45.8 (mentions) + 0.01 (age)	DFEfromhotcereal = -0.5 + 3.1 (mentions) + 0.02 (age)	DFEwhiteb = 16.5 + 72.1 (mentions) - 0.3 (age)	DFEfrombrownb= 0.1 + 20.9 (mentions) + 0 (age)
Southeast Asian	DFEfromnuts = 5 + 7.2 (mentions) - 0.05 (age)	Dfefromlettuce = 7.4 + 23.6 (mentions) - 0.2 (age)	DFEfromtomato = 2.3 + 3.3 (mentions) - 0.02 (age)	DFEfromeggs = 10.6 + 5.4 (mentions) -0.03 (age)	DFEfromcoldcereal = 1.1 + 40.7 (mentions) + 0 (age)	DFEfromhotcereal = -0.01 + 1.1 (mentions) + 0 (age)	DFEwhiteb = -7.5 + 108.2 (mentions) - 0.1 (age)	DFEbrownb = 0.2 + 28.4 (mentions) -0.01 (age)
Arab	DFEfromnuts = -9.2 + 0.09 (mentions) + 0.64 (age)	DFEfromlettuce = -9.5 + 76.9 (mentions) + 0.08 (age)	DFEfromtomato = 3.2 + 4.4 (mentions) + 0.03 (age)	Dfefromeggs = 8.4 + 14.2 (mentions) -0.2 (age)	DFEfromcoldcereal = -11.4 + 40.4 (mentions) + 0.4 (age)	DFEfromhotcereal = -0.02 + 0.4 (mentions) + 0 (age)	DFEwhiteb = -26.7 + 117.5 (mentions) + 0.8 (age)	DFEfrombrownb= 2.7 + 65 (mentions) - 0.1 (age)
West Asian	DFEfromnuts = 4.3 + 10.9 (mentions) - 0.05 (age)	DFEfromlettuce = 18.2 + 54.8 (mentions) -1 (age)	DFEfromtomato = 3.5 + 5.6 (mentions) - 0.03 (age)	DFEfromeggs = 8.5 + 4.3 (mentions) + 0 (age)	DFEfromcoldcereal = 4.8 + 69.3 (mentions) - 0.1 (age)	DFEfromhotcereal = 0 + 7.5 (mentions) + 0 (age)	DFEwhiteb = 21.3 + 74.6 (mentions) -1 (age)	DFEfrombrownb = 8.7 + 26.4 (mentions) - 0.2 (age)

Latin	DFEfromnuts = 4.8 + 7.3 (mentions) - 0.12 (age)	DFEfromlettuce = -36 + 70.7 (mentions) + 1 (age)	DFEfromtomato = -2.7 + 6.2 (mentions) + 0.1 (age)	DFEfromeggs = -10.5 + 16.5 (mentions) + 0.3 (age)	DFEfromcoldcereal = 0.2 + 52.7 (mentions) - 0.03 (age)	DFEfromhotcereal = -0.04 + 4.4 (mentions) + 0 (age)	DFEwhiteb = -13.1 + 69.4 (mentions) + 0.9 (age)	DFEbrownb = 3.4 + 18.2 (mentions) -0.1 (age)
Other	DFEnuts = -0.02 + 9.7 (mentions) + 0.02 (age)	DFEfromlettuce = -2.7 + 30.2 (mentions) + 0.2 (age)	DFEfromtomato = 2 + 4.6 (mentions) - 0 (age)	DFEfromeggs = 0.01 + 13 (mentions) + 0.5 (age)	DFEfromcoldcereal = -0.9 + 38.7 (mentions) + 0.5 (age)	DFEfromhotcereal = 0.4 + 1.8 (mentions) - 0.01 (age)	DFEwhiteb = -14.6 + 86.9 (mentions) + 0.2 (age)	DFEfrombrownb= 0.5 + 30.5 (mentions) + 0.03 (age)
Multiple	DFEfromnuts = 1 + 10.2 (mentions) + 0.1 (age)	DFElettuce = -4.5 + 76 (mentions) - 0.4 (age)	DFEtomato = -0.7 + 4.5 (mentions) + 0.07 (age)	DFEfromeggs = 3.3 + 13 (mentions) -0.04 (age)	DFEfromcoldcereal = 2.7 + 23.9 (mentions) +0.01 (age)	DFEfromhotcereal = 0 + 7.8 (mentions) + 0 (age)	DFEwhiteb = 9.5 + 66.2 (mentions) - 0.1 (age)	DFEbrownb = -0.4 + 38.8 (mentions) -0.03 (age)

Table S4a. Female Vitamin B_{12} Regression Models:

	Food Group							
	B_{12} from supplement	*Liver**	*Offal**	*Bacon*	*Hot Dogs*	*Red Meat[a]*	*Saltwater Fish[b]*	*Freshwater Fish[c]*
Cultural Group								
Caucasian	B12fromsupp = 101.1 - 56.1 (yes or no) + 0.3 (age)	B12fromliver = -0 + 86.2 (mentions)	B12fromoffal = 0 + 2.6	B12frombacon = -0.01 + 0.4 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.8 (mentions) + 0 (age)	B12frommeat = 0.03 + 0.9 (mentions) + 0 (age)	B12fromsaltwfish = 0 + 2.4 (mentions) + 0 (age)	B12fromfreshwfish = -0.01 + 4.7 (mentions) + 0 (age)
African	B12fromsupplement = 161.1 - 75.5 (yes or no) -0.4 (age)			B12frombacon = 0.01 + 0.4 (mentions) - 0 (age)	B12fromhotdog = -0.02 + 1.2 (mentions) + 0 (age)	B12frommeat = 0 + 1.2 (mentions) + 0 (age)	B12fromsaltwfish = -0.02 + 2.3 (mentions) + 0 (age)	B12fromfreshwfish = -0.02 + 7.6 (mentions) + 0 (age)
Korean	B12fromsupp = 25.6 - 14 (yes or no) + 0.06 (age)			B12frombacon = 0 + 0.3 (mentions) -0 (age)	B12fromhotdog = 0 + 0.7 (mentions) + 0 (age)	B12frommeat = -0.05 + 1.6 (mentions) + 0 (age)	B12fromsaltwfish = -0.7 + 1.7 (mentions) + 0.1 (age)	B12fromfreshwfish = 0 + 0.01 (mentions) + 0 (age)
Filipino	B12fromsupp = 22.4 -13.4 (yes or no) + 0.1 (age)			B12frombacon = -0.03 + 0.5 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.6 (mentions) - 0 (age)	B12frommeat = -0.06 + 0.8 (mentions) + 0.01 (age)	B12fromsaltwfish = 0.05 + 2.6 (mentions) -0 (age)	B12fromfreshwfish = 0.2 + 1.8 (mentions) - 0.01 (age)
Japanese	B12fromsupp = 43.2 -22.3 (yes or no) + 0.05 (age)			B12frombacon = -0.01 + 0.2 (mentions) + 0 (age)	B12fromhotdog = 0.1 + 0.8 (mentions) - 0 (age)	B12frommeat = 0.2 + 0.5 (mentions) + 0 (age)	B12fromsaltwfish = 0 + 1.2 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 0.01 (mentions) + 0 (age)
Chinese	B12fromsupp = 36.8 - 18.7 (yes or no) + 0.02 (age)			B12frombacon = -0.02 + 0.4 (mentions) +	B12fromhotdog = 0 + 0.7 (mentions) - 0 (age)	B12frommeat = 0.4 + 0.5 (age) + 0 (age)	B12fromsaltwfish = -0.1 + 1.5 (mentions) + 0 (age)	B12fromfreshwfish = -0.03 + 1.0 (mentions) + 0 (age)

		+ 0 (Age)	(mentions) + 0 (age)	0 (age)				
Aboriginal	B12_supp = 172.2 - 122.5 (yes or no to B12 supplement) +2.3 (age)			B12frombacon = -0.02 + 0.32 (mentions) + 0 (age)	B12_from_HotDogs = 0.04 + 0.83 (mentions) -0 (age)	B12frommeat = -0.04 + 0.73 (mentions) + 0.01 (age)	B12fromsaltwfish = 0.01 + 3 (mentions) - 0 (age)	B12fromfreshwfish = -0.01 + 7 (mentions) + 0 (age)
South Asian	B12fromsupplement = 225.7 - 121.3 (mentions) + 0.5 (age)			B12frombacon = -0.2 + 0.4 (mentions) = 0 (age)	B12fromhotdog = 0 + 0.3 (mentions) + 0 (age)	B12frommeat = -0.1 + 1.3 (mentions) + 0 (age)	B12fromsaltwfish = -0.05 + 2.5 (mentions) + 0(age)	B12fromfreshwfish = 0.01 + 1.1 (mentions) -0 (age)
Southeast Asian	B12fromsupplement = 25.2 -12.9 (yes or no) + 0.02 (age)			B12frombacon = 0.04 + 0.9 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.7 (mentions) + 0 (age)	B12frommeat = 0.6 + 0.5 (mentions) -0 (age)	B12fromsaltwfish = 0.2 + 2.7 (mentions) -0.01 (age)	B12fromfreshwfish = 0.2 + 2.1 (mentions) -0.01 (age)
Arab	B12fromsupp = 26.4 - 16.1 (yes or no) + 0.2 (age)			B12frombacon = 0 + 0.02 (mentions) + 0 (age)	B12fromhotdog = 0 + 0.5 (mentions) - 0 (age)	B12frommeat = 0.01 + 1.63 (mentions) + 0 (age)	B12fromsaltwfish = -0.06 + 4.5 (mentions) + 0 (age)	B12fromfreshwaterfish = 0 + 1.6 (mentions) + 0 (age)
West Asian	B12fromsupplement = 70.2 - 32.9 (yes or no) - 0.1 (age)				B12hotdog = 0 + 0.5 (mentions) - 0 (age)	B12frommeat = 0.4 + 1.6 (mentions) -0.01 (age)	B12fromsaltwfish = -0.1 + 4.3 (mentions) + 0 (age)	B12fromfreshwfish = 0 + 2.4 (mentions) + 0 (age)
Latin	B12fromsupp = 81 - 42.3 (yes or no) + 0.14 (age)			B12frombacon = 0 + 0.1(mentions) + 0 (age)	B12fromhotdog = 0.04 + 0.9 (mentions) - 0 (age)	B12frommeat = 0.05 + 0.9 (mentions) + 0 (age)	B12fromsaltwfish = 0.01 + 1.1 (mentions) - 0 (age)	B12fromfreshwfish = - + 2.9 (mentions) + 0 (age)
Other	B12fromsupp = 257.9 - 127.3 (yes or no) -0.09 (age)			B12frombacon = 0 + 0.4 (mentions) + 0 (age)	B12hotdog = 0.02 + 0.9 (mentions) + 0 (age)	B12frommeat = 0.2 + 0.6 (mentions) + 0 (age)	B12fromsaltwfish = 0.01 + 1.3 (mentions) - 0(age)	B12fromfreshwfish = 0 + 3.9 (mentions) + 0 (age)
Multiple	B12fromsupp = 29 - 16.3 (yes or no) +			B12frombacon = -0.04 +	B12fromhotdog = -0 + 0.5	B12frommeat = -0.02 + 0.7	B12fromsaltwfish = 0 + 2.7	B12fromfreshwfish = 0 + 0.5

	0.1 (age)			0.4 (mentions) + 0 (Age)	(mentions) + 0 (age)	(mentions) + 0 (age)	(mentions) - 0 (age)	(mentions) + 0 (age)

* This regression model is for all females, regardless of cultural group, due to lack of valid consumers in each cultural group

[a] Red meat encompasses beef, hamburger, pork or lamb

[b] Saltwater fish includes (but is not limited to) salmon, tuna, fish sticks and other saltwater varieties

[c]Freshwaster fish includes (but is not limited to) trout, walleye, pickerel and other freshwater varieties

Table S4a, continued

	Food Group					
	Shellfish[a]	*Eggs*	*Frozen Yogurt/Ice Cream*	*Milk*	*Cottage Cheese*	*Yogurt*
Cultural Group						
Caucasian	B12fromshellfish = 0.02 + 4.6 (mentions) + 0 (age)	B12fromeggs = 0.02 + 0.13 (mentions) + 0 (age)	B12fromfrozenyogurt = -0.01 + 0.4 (mentions) + 0 (age)	B12frommilk = 0.5 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = -0.01 + 0.6 (mentions) + 0 (age)
African	B12fromshellfish = 0 + 0.3 (mentions) + 0 (age)	B12fromeggs = 0.01 + 0.14 (mentions) + 0 (age)	B12fromfrozenyogurt = -0.01 + 0.6 (mentions) + 0 (age)	B12frommilk = 0.4 + 0.3 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.05 (mentions) + 0(age)	B12fromyogurt = 0 + 0.4 (mentions) - 0 (age)
Korean	B12fromshellfish = 0.6 + 1.2 (mentions) - 0.03 (age)	B12fromeggs = -0.06 + 0.2 (mentions) - 0 (age)	B12fromfrozenyogurt = 0.02 + 0.4 (mentions) - 0 (age)	B12frommilk = 0.6 + 0.2 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = 0 + 1.3 (mentions) - 0 (age)
Filipino	B12fromshellfish = 1.9 + 1.7 (mentions) -0.04 (age)	B12fromeggs = 0.01 + 0.08 (mentions) + 0 (age)	B12fromfrozenyogurt = 0 + 0.3 (mentions) - 0 (age)	B12frommilk = 0.6 + 0.4 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.01 (mentions) + 0 (age)	B12fromyogurt = 0 + 0.7 (mentions) - 0 (age)
Japanese	B12fromshellfish = 0 + 0.6 (mentions) + 0 (age)	B12fromeggs = 0.2 + 0.04 (mentions) + 0.01 (age)	B12fromfrozenyogurt = 0 + 0.2 (mentions) + 0 (age)	B12frommilk = 0.08 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = -0.02 + 0.9 (mentions) + 0 (age)
Chinese	B12fromshellfish = 0.5 + 3 (mentions) + 0.04 (age)	B12fromeggs = -0.2 + 0.4 (mentions) + 0 (age)	B12fromfrozenyogurt = 0 + 0.3 (mentions) - 0 (age)	B12frommilk = 0.3 + 0.5 (mentions) - 0 (age)	B12fromcottagecheese = 0 + 0.3 (mentions) = 0 (age)	B12fromyogurt = -0.02 + 0.7 (mentions) + 0 (age)
Aboriginal	B12fromshellfish = -0.1 + 9.9	B12fromeggs = 0.1 + 0.16	B12fromfrozenyogurt= -0.1 + 0.6 (mentions) +	B12frommilk = 0.4 + 0.4	B12fromcottagecheese = 0 + 0.1 (mentions) +	B12fromyogurt = -0 + 0.6

	(mentions) + 0 (age)	(mentions) -0 (age)	0 (age)	(mentions) - 0.01 (age)	0(age)	(mentions) + 0 (age)
South Asian	B12fromshellfish = 0.01 + 0.4 (mentions) - 0 (age)	B12fromeggs = 0.01 + 0.12 (mentions) + 0 (age)	b12fromfrozenyogurt = 0 + 0.4 (mentions) + 0 (age)	B12frommilk = 0.4 + 0.5 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.08 (mentions) - 0 (age)	B12fromyogurt = -0.01 + 0.6 (mentions) + 0 (age)
Southeast Asian	B12fromshellfish = 0 + 1 (mentions) + 0 (age)	B12fromeggs = 0.5 = 0.07 (mentions) - 0 (age)	B12fromfrozen = -0.02 + 0.5 (mentions) - 0 (age)	B12frommilk = 0.5 + 0.1 (mentions) - 0 (age)	B12fromcottagecheese = 0 + 0.03 (mentions) + 0 (age)	B12fromyogurt = 0 + 0.5 (mentions) + 0 (age)
Arab	B12fromshellfish = -0.01 + 0.6 (mentions) + 0 (age)	B12fromeggs = 0.3 + 0.09 (mentions) - 0(age)	B12fromfrozenyogurt = -0.01 + 0.4 (mentions) + 0 (age)	B12frommilk = 0.8 + 0.13 (mentions) - 0.02 (age)	B12fromcottagecheese = 0 + 0.06 (mentions) + 0 (age)	B12fromyogurt = 0.03 + 0.2 (mentions) - 0 (age)
West Asian	B12fromshellfish = 0 + 0.8 (mentions) - 0 (age)	B12fromeggs = 0.2 + 0.05 (mentions) - 0 (age)	B12fromfrozenyogurt = -0.08 + 0.2 (mentions) + 0 (age)	B12frommilk = 0.7 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.2 (mentions) + 0 (age)	B12fromyogurt = -0.06 + 0.6 (mentions) + 0(age)
Latin	B12fromshellfish = 0.02 + 1 (mentions) - 0 (age)	B12fromeggs = -0.2 + 0.2 (mentions) + 0.1 (age)	B12fromfrozenyogurt = -0.01 + 0.3 (mentions) + 0 (age)	B12frommilk = 0.2 + 0.4 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.5 (mentions) + 0 (age)	B12fromyogurt = 0.03 + 0.7 (mentions) - 0 (age)
Other	B12fromshellfish = -0.06 + 1.6 (mentions) - 0 (age)	B12fromeggs = 0 + 0.2 (mentions) + 0 (age)	B12fromfrozenyogurt = 0.02 + 0.3 (mentions) + 0 (age)	B12frommilk = 0.6 + 0.3 (mentions) - 0.01 (age)	B12fromcottagecheese = 0 + 0.7 (mentions) + 0 (age)	B12fromyogurt = -0.03 + 0.5 (mentions) - 0 (age)
Multiple	B12fromshellfish = 0.01 + 0.6 (mentions) - 0(age)	B12fromeggs = 0.06 + 0.15 (mentions) - 0 (age)	B12fromfrozenyogurt = 0.01 + 0.3 (mentions) - 0 (age)	B12frommilk = 0.3 + 0.3 (mentions) - 0.01 (age)	B12fromcottagecheese = 0.01 + 0.5 (mentions) + 0 (age)	B12fromyogurt = -0.02 + 0.5 (mentions) + 0 (age)

[a]Shellfish include (but are not limited to): shrimp, mussels, scallops, lobster, clams, oyster or crab

Table S5. Overall daily portion sizes using results from the 24 hr dietary recall component of the Canadian Community Health Survey, Cycle 2.2 (CCHS 2.2)

	Portion Sizes (g ± SE)		
Food Group	**Overall**	**Supplement Users**	**Non-Supplement Users**
Folate			
Liver	131.3 ± 17.2	120.2 ± 13.5	132.9 ± 22.8
Eggs	26.8 ± 0.5	28.2 ± 1.1	26.4 ± 0.6
Beans	57.2 ± 4.7	77.4 ± 11.9	49.1 ± 4.5
Tomatoes	55.9 ± 0.8	56.0 ± 1.6	55.9 ± 0.9
Lettuce	48.2 ± 1.9	51.6 ± 2.6	47.0 ± 2.4
Spinach	48.2 ± 1.9	51.6 ± 2.6	47.0 ± 2.4
Pasta	167.2 ± 3.6	157.9 ± 6.6	170.0 ± 4.4
Rice	170.5 ± 3.6	158.8 ± 5.4	174.1 ± 4.5
Nuts and Seeds	26.6 ± 1.1	25.8 ± 1.8	27.0 ± 1.4
Fruits	106.1 ± 1.7	101.7 ± 2.3	107.6 ± 2.1
Fruit Juices	205.2 ± 3.2	203.4 ± 6.1	205.9 ± 3.9
Potatoes	128.8 ± 2.3	121.5 ± 4.0	131.1 ± 2.7
Fried Potatoes	97.1 ± 2.1	99.4 ± 5.9	96.4 ± 2.1
Vegetables	36.7 ± 0.6	37.4 ± 1.0	36.5 ± 0.7
Vegetable Juices	242.9 ± 9.6	247.8 ± 18.3	241.2 ± 11.3
White Bread	50.1 ± 0.7	48.0 ± 1.4	50.6 ± 0.7
Brown Bread	48.0 ± 0.6	47.5 ± 1.0	48.2 ± 0.6
Cereal (hot or cold)	69.7 ± 2.2	70.8 ± 2.9	69.3 ± 2.8
B_{12}			
Hot Dogs	51.4 ± 1.6	51.2 ± 3.5	51.5 ± 1.8
Bacon	25.5 ± 0.8	22.9 ± 1.2	26.3 ± 1.0
Red Meat	86.8 ± 1.2	86.3 ± 2.4	87.0 ± 1.4
Liver	131.3 ± 17.2	132.4 ± 18.2	130.9 ± 23.3
Offal	54.8 ± 8,1	41.8 ± 17.2	59.6 ± 9.8
Eggs	26.8 ± 0.5	28.1 ± 1.0	26.4 ± 0.6
Freshwater Fish	112.4 ± 12.1	116.0 ± 21.1	111.7 ± 13.8
Saltwater Fish	92.3 ± 3.8	84.2 ± 4.9	95.7 ± 5.0
Shellfish	73.2 ± 5.1	65.1 ± 6.9	75.3 ± 6.1
Cottage Cheese	72.6 ± 5.3	84.8 ± 18.2	67.2 ± 6.7
Yogurt	143.8 ± 6.0	93.4 ± 3.1	139.6 ± 3.7
Ice cream or frozen yogurt	107.8 ± 2.2	93.4 ± 3.1	113.0 ± 2.9
Milk	129.9 ± 1.5	131.5 ± 3.0	129.4 ± 1.8

Males			
Portion Sizes (g ± SE)			
Food Group	**Overall**	**Supplement Users**	**Non-Supplement Users**
Folate			
Liver	141.8 ± 29.2	121.1 ± 58.5	144.9 ± 33.2
Eggs	30.1 ± 0.8	30.3 ± 1.7	30.1 ± 1.0
Beans	67.0 ± 8.1	91.0 ± 17.9	57.9 ± 8.1
Tomatoes	57.7 ± 1.1	58.9 ± 2.6	57.4 ± 1.3
Lettuce	46.6 ± 3.1	48.0 ± 3.0	46.2 ± 3.9
Spinach	46.6 ± 3.1	48.0 ± 3.0	46.2 ± 3.9
Pasta	194.6 ± 6.3	186.9 ± 12.4	196.7 ± 7.4
Rice	198.2 ± 6.0	188.1 ± 9.2	201.0 ± 7.5
Nuts and Seeds	32.4 ± 1.9	31.8 ± 3.4	32.6 ± 2.3
Fruits	111.8 ± 3.2	108.6 ± 4.6	112.8 ± 3.9
Fruit Juices	228.4 ± 5.2	228.3 ± 10.9	228.5 ± 6.1
Potatoes	148.1 ± 3.6	142.9 ± 7.6	149.4 ± 4.0
Fried Potatoes	106.8 ± 3.4	114.3 ± 11.1	104.9 ± 3.3
Vegetables	38.6 ± 0.8	38.2 ± 1.4	38.7 ± 0.9
Vegetable Juices	267.8 ± 17.0	271.9 ± 36.4	266.7 ± 19.4
White Bread	54.2 ± 0.9	51.7 ± 2.0	54.8 ± 1.0
Brown Bread	52.8 ± 0.8	51.3 ± 1.2	53.3 ± 1.0
Cereal (hot or cold)	80.0 ± 4.0	79.1 ± 5.4	80.4 ± 5.0
B_{12}			
Hot Dogs	57.1 ± 2.5	60.6 ± 6.0	56.1 ± 2.7
Bacon	29.1 ± 1.2	23.9 ± 2.0	30.5 ± 1.5
Red Meat	100.6 ± 1.9	103.1 ± 3.8	100.0 ± 2.2
Liver	141.8 ± 29.2	121.1 ± 58.5	144.9 ± 33.2
Offal	56.7 ± 16.4	NA	
Eggs	30.1 ± 0.8	30.4 ± 1.7	30.1 ± 1.0
Freshwater Fish	112.2 ± 19.8	148.7 ± 33.9	106.4 ± 21.6
Saltwater Fish	98.4 ± 4.7	97.3 ± 7.33	98.8 ± 5.8
Shellfish	80.7 ± 7.9	61.6 ± 8.5	85.0 ± 9.5
Cottage Cheese	68.1 ± 7.6	75.4 ± 11.7	64.8 ± 9.7
Yogurt	162.9 ± 14.3	193.7 ± 49.8	151.7 ± 7.2
Ice cream or frozen yogurt	120.7 ± 3.9	93.8 ± 5.8	128.2 ± 4.5
Milk	141.2 ± 2.3	144.1 ± 5.1	140.4 ± 2.7

Females			
Portion Sizes (g ± SE)			
Food Group	**Overall**	**Supplement Users**	**Non-Supplement Users**
Folate			
Liver	121 ± 17.1	119.8 ±14.0	121.6 ± 25.6
Eggs	23.4 ± 0.6	26.6 ± 1.4	22.1 ± 0.7
Beans	50.1 ± 5.6	68.1 ± 15.4	42.7 ± 4.6
Tomatoes	54.2 ± 1.1	53.8 ± 2.2	54.3 ± 1.4
Lettuce	49.6 ± 2.3	54.4 ± 4.0	47.7 ± 2.8
Spinach	49.6 ± 2.3	54.4 ± 4.0	47.7 ± 2.8
Pasta	139.9 ± 3.4	133.2 ± 5.3	142.2 ± 4.3
Rice	144.4 ± 4.1	134.8 ± 5.8	147.6 ± 5.2
Nuts and Seeds	21.7 ± 1.2	21.6 ± 2.0	21.7 ± 1.6
Fruits	100.9 ± 1.5	96.7 ± 2.2	102.6 ± 1.9
Fruit Juices	183.0 ± 3.5	183.9 ± 6.0	182.6 ± 4.3
Potatoes	109.1 ± 2.8	105.2 ± 4.3	110.5 ± 3.5
Fried Potatoes	85.3 ± 2.1	85.2 ± 4.0	85.3 ± 2.4
Vegetables	34.9 ± 0.8	36.7 ± 1.4	34.2 ± 1.0
Vegetable Juices	217.7 ± 10.6	228.9 ± 17.3	213.3 ± 13.3
White Bread	45.3 ± 1.0	44.4 ± 1.8	45.5 ± 1.1
Brown Bread	43.5 ± 0.7	44.9 ± 1.5	42.8 ± 0.7
Cereal (hot or cold)	59.8 ± 1.8	64.1 ± 3.1	57.8 ± 2.0
B_{12}			
Hot Dogs	43.8 ± 1.8	40.4 ± 3.0	45.0 ± 2.2
Bacon	21.1 ± 0.8	22.1 ± 1.5	20.7 ± 0.9
Red Meat	70.5 ± 1.3	71.4 ± 2.9	70.2 ± 1.5
Liver	121.0 ± 17.1	136.0 ± 22.3	110.8 ± 24.8
Offal	53.5 ± 9.4	NA	
Eggs	23.4 ± 0.6	26.2 ± 1.3	22.2 ± 0.7
Freshwater Fish	112.7 ± 11.6	84.5 ± 21.4	119.2 ± 13.7
Saltwater Fish	85.6 ± 5.8	73.3 ± 6.6	92.6 ± 8.1
Shellfish	65.7 ± 6.4	67.8 ± 10.1	65.0 ± 7.7
Cottage Cheese	75.6 ± 7.0	91.2 ± 9.7	68.8 ± 8.9
Yogurt	131.3 ± 2.7	131.8 ± 4.2	131.1 ± 3.4
Ice cream or frozen yogurt	95.3 ± 2.1	93.2 ± 3.5	96.3 ± 2.7
Milk	118.9 ± 2.0	121.9 ± 4.0	117.7 ± 2.2

NA: Not Applicable: Insufficient numbers prohibit release of these values from the Research Data Centre

Table S6. B_{12} intake ± SE (µg) of Canadians by supplement use based on results from the CCHS 2.2

Life Stage Group	Overall (N=32,776)	Supplement Users (N= 8683)	Non-Supplement Users (N=24,093)
< 8	NA	NA	NA
Male 9 to 13 (n= 2080)	4.6 ± 1.7	10.7 ± 8.2	4.6 ± 1.8
Female 9 to 13 (n= 1980)	3.4 ± 1.3	13.1 ± 39.9	3.3 ± 1.1
Male 14 to 18 (n= 2288)	5.7 ± 2.6	20.9 ± 14.3	5.7 ± 2.5
Female 14 to 18 (n= 2256)	3.3 ± 1.5	32.8 ± 80.6	3.4 ± 1.5
Male 19 to 30 (n=1804)	5.4 ± 2.8	49.9 ± 189.4	5.2 ± 2.5
Female 19 to 30 (n= 1854)	3.5 ± 1.5	65.1 ± 171.8	3.4 ± 1.6
Male 31 to 50 (n= 2596)	5.4 ± 2.9	55.7 ± 139.6	5.3 ± 2.9
Female 31 to 50 (n= 2686)	3.9 ± 2.5	66.1 ± 142.9	4.0 ± 2.7
Male 51 to 70 (n= 2550)	4.9 ± 2.5	64.2 ± 149.2	4.9 ± 2.5
Female 51 to 70 (n= 3200)	3.9 ± 2.2	82.4 ± 204.8	4.0 ± 2.1
Male 71 and over (n= 1520)	4.6 ± 3.3	97.7 ± 218.6	4.4 ± 3.6
Female 71 and over (n=2610)	3.6 ± 2.3	71.8 ± 173.6	3.4 ± 1.7
Total (N= 32,776)	4.5 ± 2.7	55.5 ± 157.2	4.5 ± 2.8

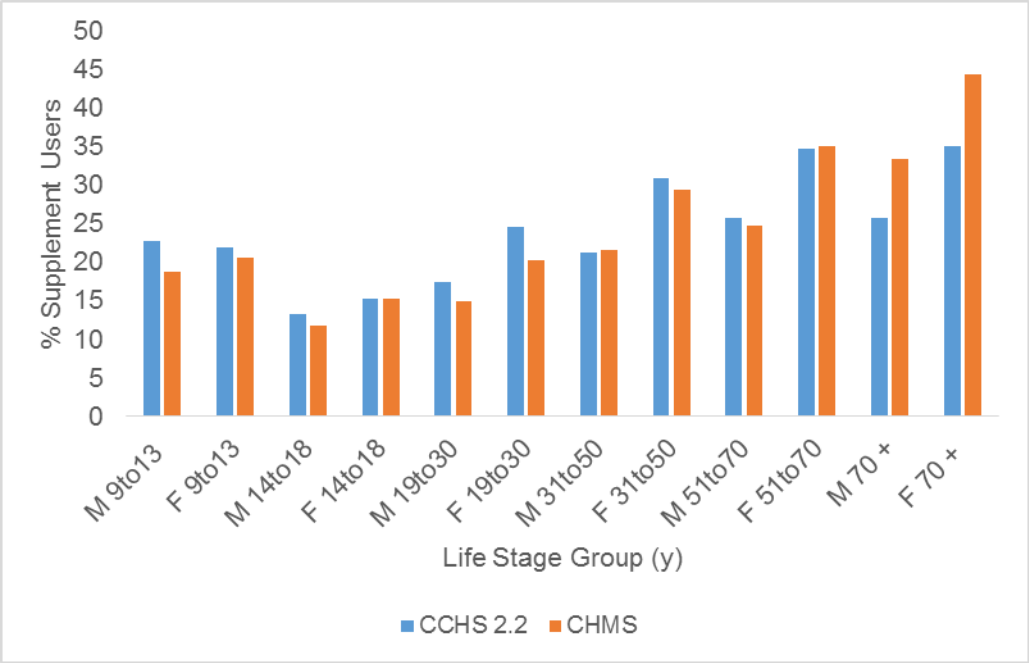
Table S7. Plasma B_{12} levels ± SE (nmol/L) of Canadians by supplement use[a] based on results from the CHMS

	Overall	Supplement Users	Non-Supplement Users
Males (n = 7528)	331.9 ± 3.2	398.5 ± 6.9***	314.1 ± 3.7
Females (n=7795)	355.9 ± 5.1	434.1 ± 9.9***	325.5 ± 7.0
Total (N = 15,323)	343.7 ± 3.6	418.7 ± 6.7***	319.5 ± 4.4

[a] Using supplement data from 3 cycles of the Canadian Health Measures Survey

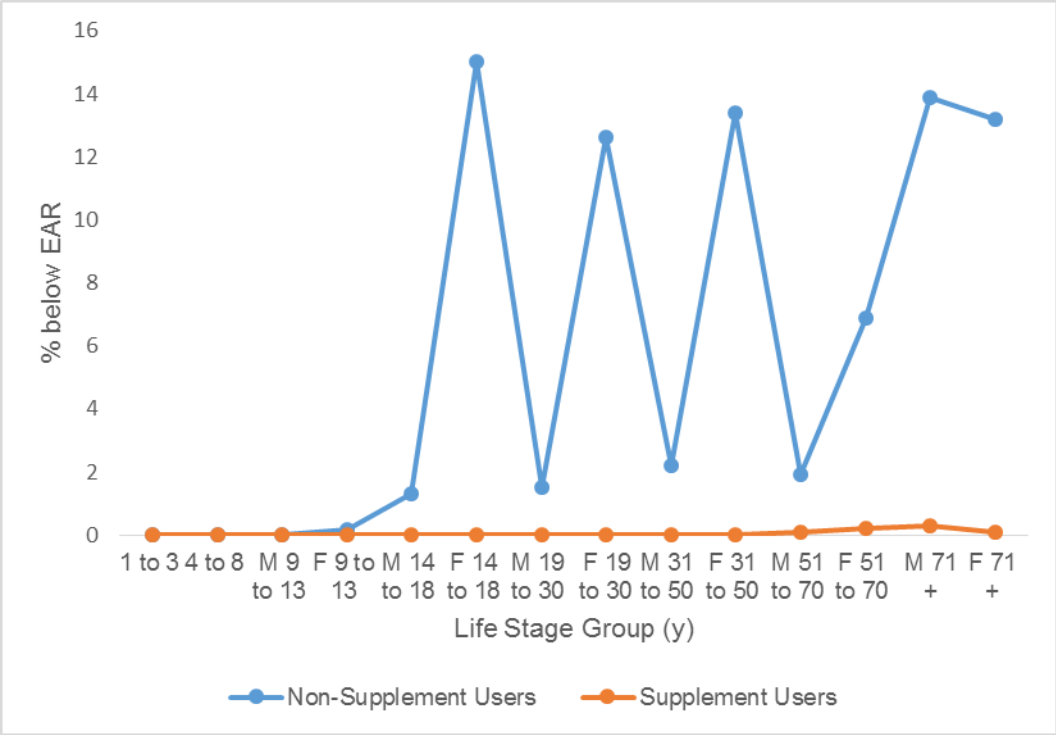
*** Significantly higher when compared to non-supplement users $p < 0.001$

Fig. S1. B_{12} supplement use by life stage group[a]



[a] Using supplement data from the Canadian Community Health Survey, Cycle 2.2 and 3 cycles of the Canadian Health Measures Survey

Fig. S2. Prevalence of B_{12} inadequacy by life stage group[a]



[a] Based on proportion of respondents below the Estimate Average Requirement using 24 hour dietary data from the Canadian Community Health Survey, Cycle 2.2

Table S8. Prevalence (%) of elevated red blood cell (RBC) folate at proposed cut-offs and with conversion factor[a]

Characteristic	< 305 nmol/L	> 1450 nmol/L		> 1800 nmol/L		> 2150 nmol/L	
		As Is	Converted*	As Is	Converted*	As Is	Converted*
Male (n= 7713)	NA	23.8	8.8	9.2	2.3	3.1	<1.0
Female (n=8041)		32.3	14.3	14.8	5.0	6.3	1.8
Overall (n=15,754)		28.0	11.5	12.0	3.6	4.7	1.3
Supplement Users							
Male (n= 1556)	NA	40.0	20.5	21.8	7.4	NA	NA
Female (n=1978)		52.4	28.8	29.8	12.3		
Overall (n=3534)		47.0	25.2	26.3	10.2		
Non Supplement Users							
Male (n= 6157)	NA	20.0	6.1	6.3	1.1	NA	NA
Female (n=6063)		25.6	9.5	9.8	2.5		
Overall (n=12,220)		22.7	7.7	8.0	1.8		

[a]*Converted microbiological assay concentration = -22.95 x (0.81) x Immulite 2000 assay concentrations (Colapinto et al, 2015)

NA: Data suppressed, numbers too small for release