Nutritional Status and Dialysis Modality: Effect on Folate Status 20 Years after the Institution of a National Folic Acid Fortification Program

by

Christine Evelyn Nash

A thesis submitted in conformity with the requirements for the degree of Master of Science

Department of Nutritional Sciences
University of Toronto

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Abstract 

Canadian dialysis patients are supplemented with ≥1mg folic acid/day to prevent folate deficiency, which may not be appropriate post-fortification. Intake and blood concentrations of folate, B₆ and B₁₂ and their association with nutritional status (subjective global assessment [SGA]) and dialysis modality were evaluated in 70 subjects undergoing in-centre hemodialysis (IHD), peritoneal dialysis (PD) or home hemodialysis (HHD). Median total intakes for folate, B₆, and B₁₂ exceeded daily recommendations at 2050µg, 11.7mg, and 9.8µg, respectively. Mean folate (62.5nmol/L) and median B₆ (121mg/L) concentrations were high, while B₁₂ concentrations were within the normal range (532pmol/L). Significant differences were observed between HHD and the PD and IHD groups for median total intakes of folate, B₆ and B₁₂ (p<0.0001), mean serum folate (p=0.003) and SGA (p=0.02). Nutritional status was not correlated to folate status. Current recommendations of high dose supplementation of folic acid and B₆ are likely not indicated in ESKD, irrespective of modality.
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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Abbreviations</td>
<td>ix</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xiv</td>
</tr>
<tr>
<td>List of Appendices</td>
<td>xv</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Chapter 2: Literature Review</td>
<td>4</td>
</tr>
<tr>
<td>2.1 Folate and Folic Acid</td>
<td>4</td>
</tr>
<tr>
<td>2.1.1 Definition, Chemical Structure and Dietary Sources</td>
<td>4</td>
</tr>
<tr>
<td>2.1.2 Absorption and Metabolism</td>
<td>6</td>
</tr>
<tr>
<td>2.1.3 Biochemical Functions</td>
<td>10</td>
</tr>
<tr>
<td>2.1.4 Folate Requirements</td>
<td>15</td>
</tr>
<tr>
<td>2.1.5 Biomarkers of Folate</td>
<td>19</td>
</tr>
<tr>
<td>2.1.6 Folate and Health</td>
<td>24</td>
</tr>
<tr>
<td>2.1.7 Folic Acid Fortification</td>
<td>34</td>
</tr>
<tr>
<td>2.2 Vitamin B₆ and Vitamin B₁₂</td>
<td>36</td>
</tr>
<tr>
<td>2.2.1 Vitamin B₆</td>
<td>36</td>
</tr>
<tr>
<td>2.2.1.1 Definition, Chemical Structure and Dietary Sources</td>
<td>36</td>
</tr>
<tr>
<td>2.2.1.2 Absorption and Metabolism</td>
<td>36</td>
</tr>
<tr>
<td>2.2.1.3 Biochemical Functions</td>
<td>37</td>
</tr>
<tr>
<td>2.2.1.4 Vitamin B₆ Requirements</td>
<td>37</td>
</tr>
<tr>
<td>2.2.1.5 Biomarkers of Vitamin B₆</td>
<td>38</td>
</tr>
<tr>
<td>2.2.1.6 Vitamin B₆ and Health</td>
<td>38</td>
</tr>
</tbody>
</table>
2.2.2 Vitamin B\textsubscript{12} ........................................................................................................ 39
  2.2.2.1 Definition, Chemical Structure and Dietary Sources ............................................. 39
  2.2.2.2 Absorption and Metabolism .................................................................................. 39
  2.2.2.3 Biochemical Functions ......................................................................................... 40
  2.2.2.4 Vitamin B\textsubscript{12} Requirements ........................................................................ 40
  2.2.2.5 Biomarkers of Vitamin B\textsubscript{12} ........................................................................ 40
  2.2.2.6 Vitamin B\textsubscript{12} and Health ........................................................................... 41

2.3 DNA Methylation ............................................................................................................. 41
  2.3.1 Definition, Function and Biological Significance ..................................................... 41

2.4 End-Stage Kidney Disease ............................................................................................ 42
  2.4.1 Treatment of End-Stage Kidney Disease ................................................................. 44
  2.4.2 End-Stage Kidney Disease and Nutritional Status .................................................... 46
  2.4.3 End-Stage Kidney Disease and Health ..................................................................... 49
    2.4.3.1 Diabetes .............................................................................................................. 49
    2.4.3.2 Cardiovascular Disease ..................................................................................... 51
    2.4.3.3 Cancer .............................................................................................................. 51
  2.4.4 DNA Methylation and End-Stage Kidney Disease ................................................... 53
    2.4.4.1 Hyperhomocysteinemia ..................................................................................... 53
    2.4.4.2 Inflammation and Oxidation .............................................................................. 54
    2.4.4.3 Nutritional Considerations ................................................................................ 55
    2.4.4.4 Dialysis Modality .............................................................................................. 56
  2.4.5 End-Stage Kidney Disease and Nutrition ................................................................. 56
    2.4.5.1 Intake Status of Folate, Vitamin B\textsubscript{6} and Vitamin B\textsubscript{12} in Patients with ESKD ........................................................... 58
    2.4.5.2 Biomarker Status of Folate, Vitamin B\textsubscript{6} and Vitamin B\textsubscript{12} in Patients with ESKD........................................................... 59
2.4.5.3 Recommended Intakes of Folate, Vitamin B<sub>6</sub>, and Vitamin B<sub>12</sub> in Patients with ESKD .......................................................... 61

2.4.5.4 Nutritional Status and Dialysis Modality ...................................... 63

Chapter 3: Rationale, Research Questions, Hypotheses and Objectives ............... 65

3.1 Rationale .......................................................................................... 65

3.2 Research Questions .......................................................................... 66

3.3 Hypothesis ....................................................................................... 66

3.4 Objectives ....................................................................................... 66

Chapter 4: Nutritional Status and Dialysis Modality: Their Effect on Folate Status 20
Years after the Institution of a National Folic Acid Fortification Program ............... 67

4.1 Subjects and Methods ...................................................................... 67

4.1.1 Subjects and Study Design ............................................................. 67

4.1.1.1 Recruitment ........................................................................... 68

4.1.1.2 Study Visits .......................................................................... 70

4.1.2 Blood Sample Collection and Analysis .......................................... 71

4.1.2.1 Serum Folate and Plasma Vitamin B<sub>6</sub> .................................. 72

4.1.2.2 Serum Vitamin B<sub>12</sub> and Plasma Total Homocysteine .............. 73

4.1.2.3 CBC, C-Reactive Protein, Serum Albumin, Urea and Creatinine .. 74

4.1.3 Nutrient Intake Data ...................................................................... 74

4.1.4 Assessment of Global Nutritional Status ........................................ 75

4.1.5 Statistical Analysis ....................................................................... 75

4.2 Results ............................................................................................. 77

4.2.1 Subject Characteristics .................................................................. 78

4.2.2 Dialysis Characteristics .................................................................. 79
4.2.3 Dietary and Supplemental Intakes of Folate and Folic Acid, Vitamin B$_6$ and B$_{12}$ ................................................................. 83

4.2.4 Blood Biomarkers of Folate, tHcy, Vitamin B$_6$ and Vitamin B$_{12}$ ............ 87

4.2.5 Ancillary Nutrition and Inflammatory Biomarkers: Creatinine, Urea, Albumin and CRP ................................................................. 89

4.2.6 Global Nutritional Status and Dialysis Modality........................................ 90

4.2.7 Regression Analyses: Relationships between Serum Folate, Plasma Vitamin B$_6$ and Vitamin B$_{12}$ and Nutritional Status and Dialysis Modality in ESKD ...... 91

4.3 Discussion ........................................................................................................ 94

4.3.1 Current Intake and Blood Folate Levels ..................................................... 94

4.3.2 Current Intake and Blood Vitamin B$_6$ and Vitamin B$_{12}$ Levels ............... 102

4.3.3 Strengths and Limitations ........................................................................... 105

Chapter 5: Overall Conclusions and Future Directions ....................................... 108

References ............................................................................................................ 111

Appendix A: Supplemental Data ........................................................................... 147

Appendix B: Consent Form ................................................................................... 162

Appendix C: PD_HHD Food Record Forms ............................................................ 169

Appendix D: IHD Food Record Form .................................................................... 173

Appendix E: Data Collection Forms ..................................................................... 177

Appendix F: Subjective Global Assessment Form ................................................... 189

Appendix G: Medications That Affect Folate Absorption .................................... 190
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-MethylTHF</td>
<td>5-methyltetrahydrofolate</td>
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<tr>
<td>5-MethyleneTHF</td>
<td>5-methylenetetrahydrofolate</td>
</tr>
<tr>
<td>10-formylTHF</td>
<td>10-formyltetrahydrofolate</td>
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<tr>
<td>AI</td>
<td>Adequate intake</td>
</tr>
<tr>
<td>AICAR</td>
<td>Aminoimidazole carboxamide ribonucleotide</td>
</tr>
<tr>
<td>AICARFT</td>
<td>Aminoimidazole carboxamide ribonucleotide formyltransferase</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CHMS</td>
<td>Canadian Health Measures Survey</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DFE</td>
<td>Dietary folate equivalent</td>
</tr>
<tr>
<td>DHF</td>
<td>Dihydrofolate</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate reductase</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary reference intake</td>
</tr>
<tr>
<td>dTMP</td>
<td>Deoxythymidine-5-monophosphate</td>
</tr>
<tr>
<td>dUMP</td>
<td>Deoxyuridine-5-monophosphate</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated average requirement</td>
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<td>EDTA</td>
<td>Ethylenediamine tetraacetic acid</td>
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<tr>
<td>ESKD</td>
<td>End-stage kidney disease</td>
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<tr>
<td>FA</td>
<td>Folic acid</td>
</tr>
<tr>
<td>FPGS</td>
<td>Folylpoly-γ-glutamate synthetase</td>
</tr>
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<td>FR</td>
<td>Folate receptors</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>GAR</td>
<td>Glycinamide ribonucleotide</td>
</tr>
<tr>
<td>GCPII</td>
<td>Glutamate carboxypeptidase II</td>
</tr>
<tr>
<td>GGH</td>
<td>( \gamma )-glutamyl hydrolase</td>
</tr>
<tr>
<td>GARFT</td>
<td>Glycinamide ribonucleotide formyltransferase</td>
</tr>
<tr>
<td>GPI</td>
<td>Glycosyl-phosphatidylinositol</td>
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<tr>
<td>Hcy</td>
<td>Homocysteine</td>
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<tr>
<td>Hhcy</td>
<td>Hyperhomocysteinemia</td>
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<tr>
<td>HHD</td>
<td>Home hemodialysis</td>
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<tr>
<td>IHD</td>
<td>In-centre hemodialysis</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectrometry</td>
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<tr>
<td>LMP</td>
<td>Laboratory Medicine Program</td>
</tr>
<tr>
<td>MRPs</td>
<td>Multidrug resistance-associated proteins</td>
</tr>
<tr>
<td>MS</td>
<td>Methionine synthase</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methylene tetrahydrofolate reductase</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer cell</td>
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<tr>
<td>NTD</td>
<td>Neural tube defect</td>
</tr>
<tr>
<td>PABA</td>
<td>P-aminobenzoic acid</td>
</tr>
<tr>
<td>PCFT</td>
<td>Proton coupled folate transporter</td>
</tr>
<tr>
<td>PD</td>
<td>Peritoneal dialysis</td>
</tr>
<tr>
<td>PEW</td>
<td>Protein-energy wasting</td>
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<tr>
<td><strong>Abbreviation</strong></td>
<td><strong>Full Form</strong></td>
</tr>
<tr>
<td>------------------</td>
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</tr>
<tr>
<td>PLP</td>
<td>Pyridoxal 5'-phosphate</td>
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<tr>
<td>RBC</td>
<td>Red blood cell</td>
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<tr>
<td>RDA</td>
<td>Recommended dietary allowance</td>
</tr>
<tr>
<td>RFC</td>
<td>Reduced-folate carrier</td>
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<tr>
<td>RRF</td>
<td>Residual renal function</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosylmethionine</td>
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<tr>
<td>SAH</td>
<td>S-adenosylhomocysteine</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SGA</td>
<td>Subjective global assessment</td>
</tr>
<tr>
<td>SHMT</td>
<td>Serine hydroxymethyltransferase</td>
</tr>
<tr>
<td>TGH</td>
<td>Toronto General Hospital</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofolate</td>
</tr>
<tr>
<td>tHcy</td>
<td>Plasma total homocysteine</td>
</tr>
<tr>
<td>TS</td>
<td>Thymidylate synthase</td>
</tr>
<tr>
<td>UL</td>
<td>Tolerable upper intake level</td>
</tr>
<tr>
<td>UHN</td>
<td>University Health Network</td>
</tr>
<tr>
<td>UMFA</td>
<td>Unmetabolized folic acid</td>
</tr>
<tr>
<td>Table 2.0</td>
<td>One carbon substitutions and oxidation states of tetrahydrofolates</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Dietary Reference Intakes (DRIs) for folate</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Comparison of folate measurement assays</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Health outcomes associated with inadequate or excess folate intakes</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Prevalence of ESKD in Canada by primary diagnosis, 2017</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Potential mechanisms of malignant transformation in ESKD</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Factors contributing to micronutrient inadequacy in ESKD</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Recommended dietary allowance and calculated vitamin content of diets with different protein intake</td>
</tr>
<tr>
<td>Table 2.8</td>
<td>Current recommendations on daily dose of water-soluble vitamin supplements in ESKD compared to the RDA and UL in the general population</td>
</tr>
<tr>
<td>Table 2.9</td>
<td>Biological half-life and vitamin stores in humans</td>
</tr>
<tr>
<td>Table 4.0</td>
<td>Breakdown of procedures and tests during study visits 1 and 2</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Biomarker measurements</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Subject characteristics</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Dialysis characteristics</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Total dietary intakes: 3-day food record</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Total dietary intakes: 3-day food record in comparison to EAR, renal guidelines</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Total supplemental intakes: Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements</td>
</tr>
</tbody>
</table>
Table 4.7  Total dietary and supplemental intakes: 3-day food records, Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements

Table 4.8  Serum and plasma biomarkers: folate, tHcy, vitamin B₆, and vitamin B₁₂; stratified by modality

Table 4.9  Ancillary nutrition biomarkers: creatinine, urea, albumin, and CRP; stratified by dialysis modality

Table 4.10 Global nutritional status; stratified by dialysis modality
List of Figures

Figure 2.0 Structure of naturally produced folates
Figure 2.1 Structure of folic acid (pteroyl glutamic acid)
Figure 2.2 Scheme of intestinal hydrolysis and absorption of folate
Figure 2.3 Folate absorption and distribution in liver and peripheral tissues
Figure 2.4 Compartmentation of folate-mediated one-carbon metabolism in the cytoplasm, mitochondria and nucleus
Figure 2.5 Overview of folate-mediated one-carbon metabolism; intersections of B\textsubscript{12} and folate metabolism, the methionine cycle, folate cycle, and DNA synthesis showing the methyl folate “trap”
Figure 2.6 Schematic overview of causes and consequences of PEW
Figure 2.7 Global prevalence of protein-energy wasting (PEW) in kidney disease
Figure 2.8 Hazard ratios for death from cancer, noncancer, nonvascular causes among participants with diabetes compared to those without diabetes at baseline
Figure 4.0 Flowchart of study recruitment
Figure 4.1 Pill burden, stratified by dialysis modality
Figure 4.2 Percentage of subjects using Replavite, individual vitamin supplements (folic acid, B\textsubscript{6}, B\textsubscript{12}), oral nutrition supplements
**List of Appendices**

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix A</td>
<td>Supplemental Data</td>
</tr>
<tr>
<td>Appendix B</td>
<td>Consent Form</td>
</tr>
<tr>
<td>Appendix C</td>
<td>PD_HHD Food Record Forms</td>
</tr>
<tr>
<td>Appendix D</td>
<td>IHD Food Record Form</td>
</tr>
<tr>
<td>Appendix E</td>
<td>Data Collection Forms</td>
</tr>
<tr>
<td>Appendix F</td>
<td>Subjective Global Assessment Form</td>
</tr>
<tr>
<td>Appendix G</td>
<td>Medications Affecting Folate Absorption</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

Approximately 4.0 million Canadians (1 in 10 people) are living with chronic kidney disease (1), of which >22,000 individuals have end-stage kidney disease (ESKD) and require dialysis to sustain life. The incidence and prevalence of ESKD has increased by 68% and 128% since 1998 respectively (1), indicating that kidney disease is a significant public health concern in Canada.

The majority of ESKD patients on dialysis in Canada are supplemented with folic acid (FA) at an amount equivalent to that of the tolerable upper intake level or greater (2). Implementation of this protocol years ago was initially aimed to prevent clinical and subclinical folate deficiency, and later to reduce cardiovascular events through the lowering of homocysteine (Hcy) (2). Clinical trials have since negated folic acid’s role in reducing cardiovascular risk (3), and folate deficiency noted in this population was largely based on older studies with small sample sizes prior to folic acid fortification (4–7). While micronutrient losses are greater through dialysis than by urinary and fecal excretion, consumption of a mixed diet containing 60g protein/day has been shown to restore these losses in the conventional (in-centre hemodialysis [IHD]) population (8). Whether this can be extrapolated to those receiving frequent (peritoneal dialysis [PD]) or intensive (home hemodialysis [HHD]) dialysis remains unclear. Furthermore, the degree to which dialysis modality impacts folate status once intake and nutritional status are taken into account is unknown.

Mandatory folic acid fortification in North America was initiated in 1998 and increasing folic acid-containing supplement use has resulted in a low prevalence (<1%) of folate deficiency (red-blood cell [RBC]) folate <305 nmol/L and high folate concentrations (RBC folate >1360 nmol/L) in 40% of the Canadian population (9). Although folic acid is generally regarded as safe and assumed to be primarily beneficial, high intakes and blood levels of folate and folic acid have been linked to several adverse health outcomes (10). Folate plays an essential role in one-carbon transfers involved in nucleotide biosynthesis and biological methylation reactions including DNA methylation (11). DNA methylation plays an important role in gene regulation and genomic stability (12) and aberrant patterns and dysregulation of DNA methylation have
been mechanistically linked to the development of several chronic diseases (11,13). Dialysis patients are characterized by a host of metabolic abnormalities and uremic factors such as hyperhomocysteinemia (Hhcy), inflammation and oxidative stress could result in aberrant DNA methylation (14–17), leading to the development of chronic disease such as cardiovascular disease (CVD) and cancer. Supplementation of folic acid could inadvertently enhance these risks; particularly in those who are well-nourished and consuming adequate micronutrient intakes through dietary sources. Adverse effects of folic acid supplementation have already been observed in the chronic kidney disease population, where subjects with diabetic nephropathy on high doses of B vitamins (follic acid, B_6 and B_12) resulted in a greater decrease of glomerular filtration rate and an increase in cardiovascular events compared with placebo (18). This emphasizes that research to assess folate status in the ESKD population post-fortification is clearly warranted, but to date remains largely unexplored.

Supplementation of water-soluble vitamins is known to represent a significant source of one-carbon nutrient intakes in the Canadian dialysis population, yet comprehensive characterization of their dietary and supplementary intakes post-fortification is not well elucidated. As well, Canadian data examining intakes in all three modalities synchronously is lacking. The primary research questions of this study were: 1) What are the current intake and blood levels of folate and folic acid in a Canadian dialysis population 20 years after the advent of a national folic acid fortification program?; 2) Does nutritional status influence blood levels of folate and methyl group donor nutrients (vitamins B_6 and B_12)?; and 3) Does the type of dialysis modality influence blood levels of folate and methyl group donor nutrients (vitamins B_6 and B_12)? The main objectives of this thesis were: 1) to estimate total folate and folic acid intake consumed through food and dietary supplementation in the dialysis population; 2) to describe associations between folate and folic acid intake and blood levels of folate and other methyl group donor nutrients; 3) to examine relationships between nutritional status and intake and blood levels of folate and other methyl donor nutrients; and 4) to examine relationships between dialysis modalities and intake and blood levels of folate and other methyl donor nutrients.

This present study investigated the current intake and blood levels of folate and folic acid in a
Canadian dialysis population in Toronto, Ontario, Canada 20 years after the institution of a national folic acid fortification program, and whether nutritional status and dialysis modality influence blood levels of folate, vitamin B₆ and vitamin B₁₂. Results will provide guidance on dietary recommendations and help update Canadian clinical protocols for folic acid supplementation in the ESKD population. This thesis provides a comprehensive review of the literature (Chapter 2), followed by the study rationale (Chapter 3) and detailed description of the study design, data analysis and discussion of the results (Chapter 4). Overall conclusions of the research findings along with future directions will be presented in Chapter 5.
Chapter 2: Literature Review

2.1 Folate and Folic Acid

2.1.1 Definition, Chemical Structure and Dietary Sources

Folate, a water-soluble B vitamin (B₉), exists in several chemical forms and is the generic term used to describe naturally occurring folates and synthetically manufactured folic acid (19). The primary structure of folate and folic acid is comprised of three components: a 2-amino-4 hydroxy-pteridine moiety (pterin ring), p-aminobenzoic acid (PABA) and glutamate residue(s). The pterin ring is attached to PABA by a methylene bridge, while glutamate residue(s) are linked via γ-peptide bonds (20).

Naturally occurring food folates have reduced pteridine rings and are characterized by their additional glutamate residues, making them polyglutamates (Figure 2.0). In contrast, folic acid exists in the monoglutamate form and is fully oxidized at the N5 and N10 positions (Figure 2.1) (20). These structural differences provide folic acid with a higher stability, and as such, has a greater bioavailability (85-100%) than its naturally occurring counterparts and is the primary form of folate used in both supplement and fortified food products.
The primary structure of folate is a pteridine ring attached to p-aminobenzoic acid (PABA) with one or more glutamate residues attached via γ-peptide bonds. Naturally produced folates are characterized by their reduced pteridine rings and polyglutamated tails. Reprinted with permission. Saber et al, 2017 (20).

The primary structure of folic acid is a pteridine ring attached to p-aminobenzoic acid (PABA) with a single glutamate residue attached via γ-peptide bonds. Folic acid is characterized by its monoglutamylated form and is fully oxidized at the N5 and N10 positions. Reprinted with permission. Saber et al, 2017 (20).

The degree of stability in naturally occurring food folates can vary, depending on the one-carbon substituent (Table 2.0) (21). As food folates are easily altered and prone to oxidation, their bioavailability is influenced by a number of other factors including hydrolysis of polyglutamyl folates, intestinal absorption, and losses during processing and cooking. Mammals are unable to synthesize folates due to their inability to link the pteridine ring with other compounds (22);
therefore, the nutrient must be obtained through dietary sources. Rich sources of natural folate include leafy green vegetables, organ meats, legumes, avocado, asparagus and citrus fruits (19).

Table 2.0 One carbon substitutions and oxidation states of tetrahydrofolates

<table>
<thead>
<tr>
<th>Folate Form</th>
<th>Oxidation State</th>
<th>One-Carbon Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-formyl-THF*</td>
<td>Formate</td>
<td>-CHO</td>
</tr>
<tr>
<td>10-formyl-THF*</td>
<td>Formate</td>
<td>-CHO</td>
</tr>
<tr>
<td>5-formino-THF*</td>
<td>Formate</td>
<td>-CH=NH</td>
</tr>
<tr>
<td>5,10-methenyl-THF*</td>
<td>Formate</td>
<td>=CH-</td>
</tr>
<tr>
<td>5,10-methylene-THF*</td>
<td>Formaldehyde</td>
<td></td>
</tr>
<tr>
<td>5-methyl-THF*</td>
<td>Ethanol</td>
<td>-CH3</td>
</tr>
</tbody>
</table>

*THF = tetrahydrofolate  
Adapted from Shane, 2008 (21) and reprinted with permission.

2.1.2 Absorption and Metabolism

Folate and folic acid are predominantly absorbed in the duodenum and jejunum, as well as across the colon (23). Absorption of folates across the intestinal mucosa can only take place if the nutrient is in the monoglutamate form. Most dietary folates are polyglutamylated derivatives and therefore must be hydrolyzed prior to entering the enterocyte. This action is performed by glutamate carboxypeptidase II (GCPII), an exopeptidase enzyme anchored to the apical brush border membrane in the proximal part of the jejunum, which cleaves C-terminal glutamate residues until a single glutamate residue remains (Figure 2.2) (24,25). As synthetic folic acid exists only in the monoglutamate form, it does not require deconjugation and enters the small intestine unaltered.
Figure 2.2 Scheme of intestinal hydrolysis and absorption of folate. Intestinal absorption of dietary folates; a two-step process involving the hydrolysis of folate polyglutamates to corresponding monoglutamyl derivatives followed by their transport through the intestinal membranes into the enterocyte. GCPII, glutamate carboxypeptidase II; PCFT, proton-coupled folate transporter; RFC1, reduced folate carrier. Adapted from Alemdaroglu, 2008 (26) and reprinted with permission.

The transport of folate into tissues and cells is facilitated by a number of different folate transport systems. These systems are divided into two classes: transmembrane carriers and folate-binding protein (folate receptor)-mediated systems (24). Transmembrane carriers transport folate bidirectionally and include the reduced-folate carrier (RFC), proton coupled folate transporter (PCFT), a family of low-affinity membrane carriers (multidrug resistance-associated proteins [MRPs]), mitochondrial folate transporter and lysosomal folate transporter. Folate-binding protein-mediated systems are responsible for folate transport into the cell by endocytosis and consist of a family of three high-affinity folate receptors (FR) (α, β, and γ) (24).
Monoglutamates are absorbed through transmembrane transport by RFC and PCFT, both of which are highly expressed in the small intestine and considered to be the primary carriers for this form (Figure 2.2) (24,27). RFC is the most widely studied of the transporters; it is a facilitative carrier, and stimulated by high levels of intracellular organic anions to transport folate into cells (24). RFC is ubiquitously expressed in normal adult tissues and has also been found in some tumor cells and fetal tissues (24). A neutral environment (pH 7.4) is required for optimal functioning and it is considered inactive at a pH<6.5 (28). RFC transports folates at the distal intestine due to its more neutral intraluminal pH (27,29). It has a high affinity for reduced folates and antifolates such as methotrexate, but a low affinity for folic acid, thereby rendering it an ineffective transporter for this fully oxidized form.

PCFT functions in an acidic setting. Its transport of folates at the proximal intestine is maximal at pH of ~5.5 (27), and incrementally decreases as pH moves towards a more alkaline environment. PCFT transports with minimal activity at a pH >7.0 (28). Its affinity for reduced folates and folic acid is equivalent, although it does display a higher affinity for 5-methyltetrahyrafolate (5-methylTHF) at pH 6.5 (24,30). The characteristics of PCFT align closely with the properties of intestinal folate transport, suggesting it is likely the primary mechanism by which folates are absorbed. This is further supported by the observation that PCFT is highly expressed in the apical brush border membrane of the proximal jejunum and duodenum, both principal sites for folate absorption. It is also expressed in other tissues including the kidney, liver, spleen, lungs, adrenal glands, placenta and brain (24). MRPs are also expressed on the apical membrane of the small intestine, but serve as folate exporters. They mediate the transport of folate from enterocytes back into the intestinal lumen, opposing the inward transport facilitated by PCFT (29). MRPs have a very low affinity of folates, but high capacity for transport of folates. The mitochondrial folate transporter is responsible for the uptake of reduced folates (5-methylTHF and 5-formylTHF) into the mitochondria, but does not transport folic acid or methotrexate (24). The lysosomal folate transporter is responsible for the uptake of methotrexate polyglutamates by lysosomes, but little is known about the extent to which it contributes to folate transport or turnover (24).
FRs are a family of high-affinity folate-binding proteins encoded by three distinct genes: α, β, and γ. FR-α and β are affixed to cells’ plasma membrane via a glycosyl-phosphatidylinositol (GPI) anchor, while FR-γ lacks a signal for a GPI-anchor attachment and is a secretory protein (25). These proteins facilitate the uptake of folate into cells by receptor-mediated endocytosis; however, their low rate of cycling makes transport by this mechanism a much slower process compared to transmembrane carriers (29). Folate receptors bind with folates on the plasma membrane to form endosomes which then travel to the cytosol and become acidified to dissociate the folate-FR complex (24,28). The FRs have demonstrated a high affinity for a variety of folate forms, with the affinity for folic acid being the highest (24).

Monoglutamylated 5-methylTHF is the primary folate form in portal circulation following intestinal absorption (Figure 2.3). The majority of circulating folate is taken up by the liver, a principal storage site containing roughly half of the body’s folate pool (31). Here, it is metabolized back to its polyglutamated form via the folylpoly-γ-glutamate synthetase (FPGS) enzyme in order to retain or “trap” folate within the cell. Polyglutamylated derivatives are considered to be the more optimal substrates than its monoglutamylated counterparts for one-carbon metabolism (29). To meet systemic folate needs, folate must be converted back to its monoglutamated form before its release into circulation. This action is performed by γ-glutamyl hydrolase (GGH), a lysosomal peptidase that cleaves terminal glutamate residues of the polyfolylglutamates to form monoglutamyl folate (32). FPGS is a key component of maintaining intracellular folate retention, whereas both FPGS and GGH are integral to the homeostasis and regulation of cellular folate pools (24,25).
2.1.3 Biochemical Functions

Folate plays an essential role in one-carbon transfers that are key to nucleotide biosynthesis and biological methylation reactions. Its involvement in nucleotide (de novo purine and thymidylate) biosynthesis makes folate a critical co-factor in the synthesis, stability and integrity, and repair of DNA (10). Folate also facilitates the transfer of one carbon units involved in the remethylation of Hcy to methionine, as well as amino acid interconversions and biological methylation
reactions (19,21). Folate-mediated one-carbon metabolism occurs within three inter-reliant cellular compartments (cytoplasm, mitochondria and nucleus), with each one carrying out specific metabolic roles (Figure 2.4). (24,33). Cytoplasmic one-carbon metabolism is involved in the remethylation of Hcy to methionine and the synthesis of purine and thymidylate. Mitochondrial and nucleic folate metabolism synthesize formate and thymidylate, respectively (21). Metabolic intermediates such as serine, glycine and formate are readily transported between compartments to meet the needs of the folate-dependent biosynthetic pathways, while folate coenzymes remain in situ (24,34). (Figure 2.4)
Figure 2.4 Compartmentation of folate-mediated one-carbon metabolism in the cytoplasm, mitochondria and nucleus. One-carbon metabolism in the cytoplasm is required for de novo synthesis of purines and thymidylate, and for remethylation of homocysteine to methionine. One carbon metabolism in mitochondria generates one carbon units for cytoplasmic one carbon metabolism by generating formate from serine and glycine. One carbon metabolism in the nucleus synthesizes dTMP from dUMP and serine. Adapted from Anderson, 2009 (35) and reprinted with permission.

Dietary-derived folates exist primarily in 5-methylTHF and 10-formylTHF forms and can therefore participate in one-carbon metabolism upon entry into the cell. In contrast, folic acid requires a 2-step reaction which is catalyzed by the enzyme dihydrofolate reductase (DHFR) (24). Folic acid is first reduced to dihydrofolate (DHF); a process noted to be slow and inefficient (36). DHFR further reduces DHF to tetrahydrofolate (THF), where it is then methylated to 5-methylTHF, the predominant circulating form of folate (Figure 2.5) (10). This process occurs primarily in the liver and to some degree in the small intestine (34). The low and
highly variable activity of DHFR in the liver causes the enzyme to become easily saturated by folic acid, resulting in the presence of unmetabolized folic acid (UMFA) in circulation (37,38).

THF is the most effective substrate for the enzyme FPGS and subsequent polyglutamylation of folate for retention in the cell. As such, both dietary and synthesized folate in their 5-methylTHF forms are converted to THF by vitamin B₁₂-dependent methionine synthase (MS) (Figure 2.5) (21,29). THF is further converted to 5,10-methylenetetrahydrafolate (5,10-methyleneTHF) via serine hydroxymethyltransferase (SHMT), which utilizes vitamin B₆ as a cofactor to accept a methyl group from serine to produce 5,10-methyleneTHF and glycine (21). 5,10-methyleneTHF is a key substrate in folate metabolism; it can be used directly for the synthesis of thymidylate, oxidized to 10-formyltetrahydrafolate (10-formylTHF) for purine synthesis, or irreversibly reduced to 5-methylTHF by the rate-limiting enzyme methylenetetrahydrofolate reductase (MTHFR) for methionine regeneration (Figure 2.5) (21).

Thymidylate is the precursor to thymine; a pyrimidine nucleobase, essential for DNA synthesis and repair (21). The formation of thymidylate occurs when methylation of deoxyuridine-5-monophosphate (dUMP) is carried out by 5,10-methyleneTHF to form deoxythymidine-5-monophosphate (dTMP; thymidylate) (39). This reaction is catalyzed by thymidylate synthase (TS), a rate-limiting enzyme which also creates DHF in the process. DHF can then be reduced back to THF via DHFR.

Folate co-enzymes are required in two of the ten-step purine biosynthesis process (39). 5,10-methyleneTHF is first oxidized to 10-formylTHF in order to donate a one-carbon unit each to aminooimidazole carboxamide ribonucleotide (AICAR) and glycaminamide ribonucleotide (GAR). This process is catalyzed by the enzymes aminooimidazole carboxamide ribonucleotide formyltransferase (AICARFT) and glycaminamide ribonucleotide formyltransferase (GARFT), resulting in the production of THF as well as purine intermediaries (Figure 2.4) (39).
In the methionine cycle, Hcy is remethylated through donation of a methyl group from 5-methylTHF (34). This transfer is catalyzed by vitamin B₁₂-dependent MS to synthesize methionine, which is then converted to S-adenosylmethionine (SAM) through an ATP-dependent transfer of adenosine to methionine via adenosyltransferases (MAT1A and MAT2A) (40). The precursory role of methionine is a critical element in the methylation pathway, as SAM is a universal methyl donor in >100 methylation reactions including DNA, RNA, proteins, neurotransmitters and phospholipids (25,39). These methylation reactions result in the production of S-adenosylhomocysteine (SAH), a known potent product inhibitor of SAM-dependent methyltransferases. SAH is converted by S-adenosylhomocysteine hydrolase to Hcy and adenosine forms, where they are metabolized or transported out of the cell to prevent SAH accumulation and maintain normal DNA methylation (21). Hcy can be remethylated back to methionine utilizing the methyl group from 5-methylTHF or irreversibly degraded to cysteine via the transsulfuration pathway (29). Alternatively, Hcy can be remethylated through a folate-independent pathway. Here, choline is oxidized to produce betaine, which then donates a methyl group to Hcy through the assistance of betaine-homocysteine methyltransferase; a process specific to the liver and kidney (Figure 2.5) (41).
Figure 2.5 Overview of folate-mediated one carbon metabolism. Intersections of B12 and folate metabolism, the methionine cycle, folate cycle, and DNA synthesis showing the methyl folate “trap”. The key intersection of B12 and folate occurs at the MS reaction in which the one-carbon methyl group MethylTHF is transferred to Hcy to form methionine. In B12 deficiency, folate becomes trapped as methylTHF. Administration of FA can temporarily overcome this block through DHFR reduction to THF. Adapted from Green, 2017 (42) and reprinted with permission.

2.1.4 Folate Requirements

Folates derived from natural and synthetic folate forms vary considerably in their degree of absorption (43,44). As such, a new unit termed dietary folate equivalents (DFE) was developed by the Food and Nutrition Board of the Institute of Medicine; and incorporated into the newly introduced 1997 Dietary Reference Intakes (DRIs) for folate. Use of DFE accounts for the higher bioavailability of synthetic folic acid found in supplements (85-100%) and
fortified foods (85%) compared to natural food folates (50%) (45,46). DFE equivalents convert all folate forms to an amount equal to natural food folates using a calculation that reflects the greater bioavailability of folic acid either as a supplement or a fortificant (19):

- $1 \, \mu g \text{ DFE} = 1 \, \mu g \text{ food folate} + (1 \, \mu g \times 1.7)$

Other calculations can be utilized, depending on the type of conversion required (46,47):

- $1 \, \mu g \text{ DFE} = 1 \, \mu g \text{ food folate}$
- $1 \, \mu g \text{ DFE} = 0.6 \, \mu g \text{ folic acid from fortified foods OR supplements consumed with food}$
- $1 \, \mu g \text{ DFE} = 0.5 \, \mu g \text{ folic acid from supplements taken on an empty stomach}$

Dietary reference intakes (DRIs) is an umbrella term coined for a set of nutrient reference values used in the dietary assessment and planning of healthy individuals and groups in North America (48). These recommendations, stratified by age and sex, are determined by the Food and Nutrition Board of the Institute of Medicine and include the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI) and Tolerable Upper Intake Level (UL) (19). The EAR is an amount of nutrient required to meet 50% of the population’s needs, and can be used in the assessment of intakes for groups or individuals. In contrast, RDA estimations are intended for the planning of individual diets and based on the average daily intake sufficient to meet nutrient requirements for the majority of the population (97-98%) (19,48). The RDA is determined from the EAR and takes expected nutrient variation into account. If distribution of requirements for the nutrient is normal, the following calculation is used (19):

- $\text{RDA} = \text{EAR} + 2 \, \text{standard deviations (SD)}_{\text{EAR}}$

If distribution of requirements for the nutrient demonstrates a higher variability, thereby preventing calculation of an SD, the RDA is set using an assumed 10% coefficient of variation (CV) method (19):
- RDA = EAR + 2 (0.1 x EAR)  \[\text{OR} \]  RDA = 1.2 x EAR

A larger CV is utilized if there is a higher degree of variation. If distribution of requirements is skewed, other methods are employed to determine the RDA (19). In the event an EAR, and subsequent RDA, is unable to be established due to lack of data, an AI is used. AI estimates the average daily nutrient intake required for prevention of inadequacy, and is set using approximations from both observational and experimental study data (19). With regards to excessive nutrient intake, the UL identifies a maximum amount that can be ingested by the majority of the healthy population without experiencing adverse effects. If intakes exceed the UL, the risk of adverse effects also increases (19,45,48).

The RDA for folate is 400 µg/day DFE for adults ≥19 years to ensure maintenance of RBC folate, as well as serum/plasma folate and plasma total homocysteine (tHcy) concentrations (Table 2.2) (19). RBC folate is considered to be the primary indicator of folate adequacy, as it reflects storage within the red blood cells, whose lifespan is ~120 days. Therefore, RBC folate is a proxy of long-term folate status and proxy of tissue folate status (48,24). As such, metabolic studies looking at folate status in response to fixed diets were the cornerstone from which inferences were drawn. A key study supporting the development of EAR recommendations for this age group found an intake of 320 µg dietary folate was incapable of maintaining sufficient RBC levels in 50% of the participants after seventy days. Secondary indicators’ serum folate and tHcy were also found to be suboptimal in 50% of the group (49). These findings were confirmed in other experimental studies; therefore, an EAR of 320 µg (and subsequent RDA) was derived (50,51).

For women of childbearing age (15-45 years), the Food and Nutrition Board of the Institute of Medicine recommends supplementing with 400 µg folic acid from either fortified food products or supplements at least 2-3 months prior to conception (19). In Canada, current recommendations for women of childbearing age (12-45 years) include the use of a multivitamin
containing 400 μg/day of folic acid three months prior to pregnancy (52,53). This is owing to a strong body of evidence identifying an inverse relationship between increased folic acid intake during the periconceptional period and decreased prevalence of neural tube defects (NTDs) (54,55). NTDs are a consequence of malformation or failure of the neural tube to close; a process that occurs within the third or fourth week of gestation (56). Both countries recommend a larger dose of folic acid for women previously affected by a NTD-related pregnancy (52,53,57). During pregnancy, folate requirements remain high to support the many physiological changes taking place, including fetal development and metabolic processes that accompany its growth. As such, it is recommended that women continue to supplement with 400 μg folic acid in addition to consuming a folate-rich diet (19,52,53). The RDA for pregnant women is also increased from 400 μg to 600 μg DFE (Table 2.1); an amount derived from a single controlled metabolic study and supporting population-based studies, where RBC and serum folate levels were maintained through daily ingestion of 600 μg folic acid (58). Findings also revealed that tHcy levels were not reflective of folate status throughout the pregnancy, and is therefore not recommended as an indicator during the gestational period (58).

The UL for folic acid is set at 1000 μg/day for adults (19+ years) and women who are pregnant or lactating (Table 2.1), but pertains solely to folic acid found in fortified foods and supplements (19). This recommendation is based on a series of case reports and experimental animal studies, which identified a progressive, neurological decline in vitamin B_{12}-deficient individuals consuming folic acid supplements in excess of 5 mg/day, despite correction of megaloblastic anemia (24,48). It is recognized that high intakes of folic acid may in fact conceal or “mask” the hematological effects of vitamin B_{12} deficiency (24,59). This occurs when folic acid is able to bypass the “methyl-folate trap” and correct the anemia through its conversion to THF and 5,10-methyleneTHF (Figure 2.5) (24,48). However, when MTHFR irreversibly reduces 5,10-methyleneTHF to 5-methylTHF, it is unable to be further converted to THF due to reduced ability of the vitamin B_{12}-dependent MS. 5-methylTHF is thereby “trapped” in its current form and to a large extent not usable by the cell for one-carbon metabolism (24,48). In addition to accumulation of 5-methylTHF, a limited pool of MS is unable to regenerate methionine from Hcy, thereby causing a buildup of the intermediary amino acid and subsequent disruption to the
methylation cycle (24). Additional concerns for excess folic acid intake include resistance to antifolate drugs, epigenetic dysregulation and reduced natural killer (NK) cell cytotoxicity (60,61).

Achieving adequate nutrient balance in the different groups within the general population can be challenging. Recommendations such as the DRIs can help guide health professionals in their client assessments and plans for maintenance of good health and prevention of chronic disease.

Table 2.1 Dietary Reference Intakes (DRIs) for folate (µg)

<table>
<thead>
<tr>
<th>Life-Stage Group</th>
<th>EAR (µg/day DFE)</th>
<th>RDA (µg/day DFE)</th>
<th>AI (µg/day DFE)</th>
<th>UL (µg/day FA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 months</td>
<td>N/A*</td>
<td>N/A*</td>
<td>65</td>
<td>ND**</td>
</tr>
<tr>
<td>7-12 months</td>
<td>N/A*</td>
<td>N/A*</td>
<td>80</td>
<td>ND**</td>
</tr>
<tr>
<td>1-3 years</td>
<td>120</td>
<td>150</td>
<td>N/A</td>
<td>300</td>
</tr>
<tr>
<td>4-8 years</td>
<td>160</td>
<td>200</td>
<td>N/A</td>
<td>400</td>
</tr>
<tr>
<td>9-13 years</td>
<td>250</td>
<td>300</td>
<td>N/A</td>
<td>600</td>
</tr>
<tr>
<td>14-18 years</td>
<td>330</td>
<td>400</td>
<td>N/A</td>
<td>800</td>
</tr>
<tr>
<td>19+ years</td>
<td>320</td>
<td>400</td>
<td>N/A</td>
<td>1000</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>520</td>
<td>600</td>
<td>N/A</td>
<td>1000</td>
</tr>
<tr>
<td>Lactation</td>
<td>450</td>
<td>500</td>
<td>N/A</td>
<td>1000</td>
</tr>
</tbody>
</table>

*N/A = not available  **N/D = not determinable  Adapted from Suitor, 2000; IOM, 1998 (19,45).

2.1.5 Biomarkers of Folate

Serum, plasma and RBC are the most common biomarkers used to measure folate status in the clinical, research and population health settings (62). Serum and folate assays are more responsive to interventions with folic acid, either in fortified foods or in supplements (63). Both
exhibit prandial variation, and are therefore highly influenced by recent dietary folate/folic acid and supplemental folic acid intake (64). Repeated measures are recommended to reflect a more accurate picture of trends in individual intake, particularly for interventional studies (62). In contrast, RBC folate remains stable. Folate enters the red cells during bone marrow development, where the majority is retained for the lifespan of the cells, approximately 120 days (62). RBC folate represents average folate repletion over this time period, and is therefore considered a more reliable index and long-term indicator (~3 months) of folate status in tissue stores (24). As such, this measure is considered to be the gold standard for determination of folate status (65,64).

Fasting tHcy can also be used as an inverse functional indicator of folate status (24). However, it is considered to be a non-specific marker, as Hcy is influenced by several factors, including vitamins B6 and B12, genetic variations (i.e. MTHFR polymorphism) and impaired renal function (24,66). Lifestyle factors such as smoking, high intakes of coffee and/or alcohol and lack of exercise can also impact blood levels (68,24).

Identification of circulating UMFA in serum and plasma has gained considerable interest in the post-fortification era. Although not a clinical biomarker, UMFA reflects the amount of excess folic acid in the bloodstream that has not been converted to other folate forms or removed by the body through urinary excretion (67). An increased prevalence of UMFA has been noted in blood samples of several populations; but interestingly, is not limited to countries with mandatory fortification policies (68). For example, in Germany, which does not have mandatory fortification, Obeid et al (2010) measured UMFA concentrations in a cohort of pregnant women (n=87) and their cord blood (n=29). Detectable levels of UMFA were observed in 44% and 55% of women’s circulation and cord blood, respectively, and was unexpectedly not explained by supplement use (69). In the United States, where mandatory fortification has been in place since 1998, data was extracted from the 2001-2002 NHANES population survey and revealed ~40% of older adults (≥60 years) had detectable UMFA blood levels and was significantly higher in supplement users versus non-users (67). Detectable levels have been identified across the lifespan including children, adolescents, adults (70,71), pregnant women and umbilical cord
blood (69), and 4-day old infants (72). To date, the effect of UMFA on health remains to be elucidated.

There are three methods for the analysis of folates in serum, plasma, RBC and other biological fluids: the microbiological, protein-binding and chromatographic assays (Table 2.2) (24). The microbiological method was introduced over 50 years ago to measure folate and uses lactic acid bacteria (e.g. lactobacillus rhamnosus) to quantify the amount in the sample (24,64). It is a simple, yet precise measure, as bacteria are unable to grow in the medium without the presence of folate and responds proportionally to the amount in the sample. The response of l.rhamnosus is different, depending on length of the polyglutamyl chain. A full response is seen with mono-, di-, and triglutamates, and then starts to diminish in proportion to increasing glutamate residues (64). However, it lacks ability to differentiate between different folate forms and only measures total folate (24,64). Protein binding assays have been in use since the 1970’s and use folate-binding proteins primarily from milk or milk fractions to bind and separate folate from samples (24,64). Contrary to the microbiological assay, which measure the metabolic use and growth of folate, protein-binding assays measure the competition between labeled (standard) and unlabeled (biological sample) folates (24). Folate affinity is dependent upon the folate form and as such, responds according to the one-carbon substituent and number of glutamate residues present in the sample (24). Overall, the protein-binding assay provides quick results, but is highly influenced by elements such as dilution techniques, pH and protein content (24,64). Its narrow detection range also limits the protein-binding assays’ utility in populations with fortification policies (64). Mass spectrometry was introduced more recently and has the capacity to measure individual folate forms, including folic acid (24,64,65). High-performance liquid chromatography (HPLC) rapidly separates individual folate types from the biological sample and is known for its reproducibility. The isotope-dilution/liquid chromatography/tandem mass spectrometry (LC/MS/MS) produces the highest level of accuracy amongst assays when employed and correlates well to the microbiological assay method (65). However, instrumentation is expensive and requires experienced staff to operate it. It also necessitates a high degree of sample cleaning (64,65).
Table 2.2 Comparison of folate measurement assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Assay Principle</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiological</td>
<td>Growth of bacteria (<em>L. rhamnosus</em>) is proportional to amount of folate in sample</td>
<td>Gold standard for overall folate status</td>
<td>Unable to distinguish between individual folate forms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Highly sensitive</td>
<td>Low precision</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inexpensive</td>
<td>Can be affected by antibiotics / antifolates</td>
</tr>
<tr>
<td>Protein-binding</td>
<td>Folate binding proteins “extract” folate from samples, can be radio- or non-radiolabeled</td>
<td>Quick results</td>
<td>Narrow detection range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-throughput analysis</td>
<td>Binding proteins respond differently, depending on folate forms in the sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good precision</td>
<td></td>
</tr>
<tr>
<td>Chromatographic</td>
<td>Individual forms of folate are separated and quantified based on their interaction with the adsorbent material. May be quantified by measuring the mass to charge ratio.</td>
<td>Highly sensitive</td>
<td>Extensive sample cleaning</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Is able to separate and measure individual folate forms</td>
<td>Interconversions of folate forms needed for correct interpretation of data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Highly selective</td>
<td>Expensive specialized equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Requires trained personnel</td>
</tr>
</tbody>
</table>

Adapted from Bailey, 2010 (24) and Plumptre, 2016 thesis (73).

Widespread variability among folate assay methods and the aforementioned factors has caused difficulty in achieving standardization, particularly with RBC folate (74). For example, a study by Owen et al (2003) compared the Bio-Rad Quantaphase II radio assay with five automated serum and RBC folate assays and found differences of up to 40% and 250%, respectively (75). Another study examined the methodologies used from twenty laboratories to determine folate results from serum and whole blood samples. They found a 35% inter-laboratory variability for red cell folate, compared to 27.6% for serum folate (76). To date, the microbiological assay remains the gold standard for assessment of serum and RBC folate, and is the foundation behind folate adequacy cut-offs (62). This method is also recommended by WHO for assessment of folate status at the population level (77). However, the close correlation between LC-MS/MS
(described above) and microbiological serum folate measures allows this newer method to be used and produce results similar to the microbiological assay (62). In contrast, RBC folate measures can yield differing concentrations and are often not analogous to each other (78). Adjustment equations have been developed to allow for comparative analyses, but interpretive caution should be taken when applied to datasets outside of its origin (33,78). Future harmonization of laboratory processes (e.g. standardized reagent use in microbiologic assays) has been proposed to aid in the generation of comparable folate concentrations across groups (77).

RBC folate has been identified as the primary marker for determination of folate adequacy (19) and NTD risk in women of reproductive age (77). In the presence of deficiency, both megaloblastic changes and a rise in tHcy concentrations have been noted; as such, several studies have been conducted to ascertain an appropriate cut-off. For RBC folate, investigators performed dose-response studies using low folate diets in varying amounts and determined 305 nmol/L (140 ng/ml) was sufficient to prevent the emergence of disease indicators (79–83). A cut-off of >906 nmol/L has been recognized for maximal protection against NTDs (84); although two large studies (n=249,045) found >1000 nmol/L provided a substantial risk reduction (85). Many studies have validated an elevated tHcy in the presence of confirmed folate deficiency, supporting the rationale to incorporate this intermediate metabolite as a secondary indicator (49,50,86). A serum folate value <7 nmol/L (3 ng/ml) suggests there is a deficit at the time of blood draw, yet intake variability can make it difficult to quantify true folate status (19). Performing repeated measures over time or employing other folate indices in conjunction with serum folate allows for a higher accuracy of interpretation (19). All three cut-offs (RBC [<305 nmol/L], RBC-NTD [<905 nmol/L], serum folate [<7 nmol/L]) have been derived using the microbiological assay (62,84). In 2015, the World Health Organization updated their cut-off values for RBC folate to reflect updated guidelines on concentrations considered optimal for the prevention of NTDs (<905 nmol/L). Previous 2005 guidelines for serum/plasma and RBC folate in all ages excluding women of reproductive age were maintained (hematological indicator, macrocytic anemia: RBC [<226.5 nmol/L], serum/plasma folate [<6.8 nmol/L]); hematological indicator, Hcy: RBC (<340 nmol/L), serum/plasma folate (<10 nmol/L) (77).
The notable rise in both serum/plasma and RBC folate concentrations post-fortification and recent public health concerns regarding potential adverse effects of folic acid supplementation have brought into question whether cutoffs should be developed for high folate concentration. This has not been formally established; however, several studies have examined various cut-off points in relation to supplementary folic acid intake. Using the BioRad immunoassay, the 1999-2004 National Health and Nutrition Examination Survey (NHANES) identified a serum folate concentration cutoff >45 nmol/L and RBC folate concentration cutoff ≥1360 nmol/L (97th percentile) as being indicators of high folate status (87). A more recent NHANES (2005-2010) proposed RBC cutoffs of 1820 nmol/L (90th percentile), 2150 nmol/L (95th percentile) and 2490 nmol/L (97.5th percentile), respectively (88). These values were adjusted to the microbiologic assay (89). In the 2007-2009 Canadian Health Measures Survey (CHMS), the Canadian population surveyed was noted to have RBC folate concentrations above 1450 nmol/L (16%), 1800 nmol/L (6%), and 2150 nmol/L (2%). These cutoffs were estimated from the 2005-2010 NHANES and adjusted from Immulite 2000 immunoassay to microbiologic assay (88).

2.1.6 Folate and Health

Folate is an indispensable metabolic component in all living organisms and cell types due to its critical role in one-carbon metabolism. Numerous studies have observed the importance of a balanced equilibrium between inputs and outputs of the folate metabolic pathway, and that folate inadequacy or excess may result in deleterious health effects (11,90). Genetic, pharmacological and environmental factors have a similar capacity to alter or disrupt the folate metabolic pathway (90–92). Given that the critical role of folate is nucleotide biosynthesis and biological methylation reactions, an aberrant or disrupted folate metabolic pathway may contribute to the development and/or progression of several human diseases including cardiovascular disease, cancer, neurodegenerative diseases, birth defects, and metabolic diseases such as obesity and diabetes (93–97). It has also been implicated in an accelerated decline of kidney function in patients with diabetic nephropathy (18). Health outcomes associated with inadequate and excess intakes of folate are summarized in Table 2.3.
Table 2.3 Health outcomes associated with inadequate or excess folate intakes

<table>
<thead>
<tr>
<th></th>
<th>Inadequate Intake</th>
<th>Excess Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Convincing</strong></td>
<td>Megaloblastic anemia</td>
<td>Masking of $B_{12}$ Deficiency</td>
</tr>
<tr>
<td></td>
<td>Neural tube defects (NTDs)</td>
<td></td>
</tr>
<tr>
<td><strong>Probable</strong></td>
<td>Cancer initiation</td>
<td>Cancer progression</td>
</tr>
<tr>
<td></td>
<td>Cardiovascular:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Coronary heart disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Stroke</td>
<td></td>
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<tr>
<td></td>
<td>Cognitive impairment:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Dementia</td>
<td></td>
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<tr>
<td></td>
<td>• Alzheimer’s disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Parkinson’s disease</td>
<td></td>
</tr>
<tr>
<td><strong>Insufficient Evidence</strong></td>
<td>Neuropsychiatric disorders</td>
<td>Resistance to antifolate drugs</td>
</tr>
<tr>
<td></td>
<td>Other congenital disorders</td>
<td>Reduced natural killer cell cytotoxicity</td>
</tr>
</tbody>
</table>

Table modified from Fard 2018 thesis (98).

**Folate Inadequacy**

**Convincing**

An overwhelming body of evidence supports the link between folate deficiency and the development of megaloblastic anemia and NTDs (19).

**Megaloblastic anemia**

Chronic folate inadequacy through suboptimal folate intake, poor intestinal absorption, alcohol
abuse, amongst others, is associated with megaloblastic anemia (24). Megaloblastic states are a result of decreased DNA synthesis. Over time this leads to impaired maturation of nuclei, which is characterized by large, structurally abnormal, immature red blood cells (megaloblasts) in the bone marrow. These abnormalities cause the bone marrow to produce fewer red blood cells; many of which die prior to their maturation (24). An early symptom is pallor of the skin, and can progress to weakness, fatigue, shortness of breath and difficulty in concentrating (19,99). There is a wealth of evidence supporting the relationship between the development of megaloblastic anemia and folate deficiency; as such, it has been used in the establishment of folate-deficiency cutoffs in Canada and the United States (88). Megaloblastic anemia due to dietary deficiencies is rare in folate-fortified countries (24).

**Neural Tube Defects (NTDs)**

An inverse relationship has been demonstrated between risk of NTDs and both maternal folate/folic acid intake and blood folate concentrations (54,55). Current evidence is sufficient to support the recommendations for periconceptual folic acid supplementation and folate intake in order to reduce NTD risk (19). A more detailed review of the evidence will discussed later on in this thesis.

**Probable**

**Cardiovascular**

Epidemiologic studies have identified Hhcy as an independent risk factor for coronary heart disease and stroke (100). This is supported by in vivo studies, which suggest a mechanistic link may exist between Hcy and the promotion of thrombus formation and endothelial damage (94). It has been well established that low blood concentrations and dietary intake of folate and other methyl group donor nutrients (e.g. vitamins B2, B6 and B12, choline, and betaine) involved in the Hcy remethylation pathway significantly contribute to elevated plasma thcy concentrations. This association has prompted many clinical and epidemiological trials to investigate whether
folic acid supplementation alone or in combination with other methyl group donor supplementation is effective in the prevention of cardiovascular events and/or in the reduction of cardiovascular-related mortality (101–105). The Homocysteine Studies Collaboration pooled thirty prospective and retrospective trials and observed for every 25% reduction in plasma tHcy, there was an 11% and 19% decreased risk in coronary heart disease and stroke, respectively (106). However, a more recent systematic review of fifteen randomized controlled trials (71,422 participants) reported no differences between treatment and placebo groups on homocysteine-lowering interventions from supplemental folic acid alone or in combination with other B vitamin forms. A small difference was noted for stroke in the homocysteine-lowering intervention group using folic acid alone or in combination with other B vitamin forms compared to placebo (107). Larger trials comparing high-dose vs. low-dose homocysteine-lowering interventions are required to fully elucidate the extent of these relationships (107).

Cognition

Epidemiologic studies suggest an inverse association between folate status and various cognitive states, including depression, impairment and dementia (108). However, the number and quality of studies is limited.

i. Alzheimer’s Disease

Epigenetic alterations, particularly to changes in DNA methylation, are thought to play an important role in Alzheimer’s disease development and progression. This is not surprising, as inverse associations have been previously noted between age and global loss of DNA methylation (109,110). Dietary folate and its role in providing methyl groups for regulation of DNA methylation has generated much interest, as have other one-carbon constituents. B-vitamin inadequacies and Hhcy have been linked to impaired spatial memory (111), cognitive decline and loss of brain volume (96,112). Those living with Alzheimer’s disease are noted to have low blood folate levels and increased plasma tHcy when compared to age-matched healthy controls (113,114), but epidemiological studies have been conflicting in their findings. A recent meta-
analysis found both folate and vitamin B_{12} deficiencies increased with age and correlated to Alzheimer’s disease risk (115), while another identified significantly lower folate and vitamin B_{12} levels in Alzheimer’s disease subjects. Interestingly, these deficits were not related to malnutrition and was in advance of the protein-energy malnutrition associated with Alzheimer’s disease (116). A large, prospective cohort study followed subjects for approximately four years and found no associations between Alzheimer’s disease and intakes of folate and vitamins B_{6} and B_{12} (117). In the Baltimore Longitudinal Study of Aging, participants were followed for fourteen years and found folate intakes above the RDA provided a protective effect for Alzheimer’s disease. A meta-analysis (1,574,299 subjects) analyzing adherence to the Mediterranean diet and incidence of chronic diseases found the diet, which is B-vitamin rich, had a protective effect against Alzheimer’s disease and Parkinson’s disease (118). On the contrary, the Cache study (2009) determined intakes from food and supplemental forms of B vitamins were unrelated to dementia or incidence of Alzheimer’s disease (119). The contradictions in findings highlight the complexity involved in successfully singling out specific nutrients of interest in the highly interdependent one-carbon metabolism pathway. Due to methodological limitations with the published studies, no definitive conclusions can be drawn at this time. Further investigations are warranted to determine what role folate has, if any, on the onset and progression of this disease.

**ii. Parkinson’s Disease**

It has been purported that DNA methylation may be altered via gene-environment interactions; as such, methyl group donor nutrients such as folate and other one-carbon nutrients have garnered much interest. Higher Hcy levels (10-30%) have been consistently reported in patients with Parkinson’s disease when compared to controls (120). This, in part, could be explained by Levodopa treatment which patients receive to treat Parkinson’s symptoms at all stages of the condition (121). Levodopa methylation occurs via the catechol-O-methyltransferase enzyme and requires a high utilization of SAM as a methyl donor, thereby depleting the methyl groups’ reserve and increasing SAH formation (122). Significant correlations have been found between neurodegenerative markers (Amyloid Precursor Protein and SNCA) and SAH concentration (123). The increased hydrolysis of SAH to Hcy results in a greater concentration of tHcy, which
is thought to have a toxic effect on dopaminergic neurons (124,125). Animal models have demonstrated strong correlations between folate deprivation, elevated tHcy concentrations and neurodegeneration (126,127), whereas human studies have been limited with mixed results (128–130). It is purported that B vitamins, particularly folate, may have an impact on Parkinson’s disease through its regulation of Hcy, and that therapeutic dosing may be required in the presence of neurodegeneration (131). More research in the form of interventional trials is necessary to better elucidate this relationship.

Insufficient Evidence

Neuropsychiatric Disorders

Folates may influence the nervous system in several ways, including its role in the synthesis of SAM (which acts as a methyl donor in the central nervous system), purine and thymidylate synthesis, as well as its effect on monoamine metabolism (132). As such, researchers have explored the relationship between folate status and a number of neuropsychiatric conditions. Prevalence of folate deficiency has been identified in 15-51% of psychiatric patients, including those with depression, psychosis, schizophrenia and mania, amongst others (133). It is unclear whether the deficiency contributes to neuropsychiatric illnesses or is in fact, a result of factors encompassing the conditions, such as folate-depleting medications, decreased intake, or lifestyle factors (134). Studies have examined the efficacy of folic acid supplementation on various outcomes and observed improvements in a variety of disorders: shortened hospital stays, improved psychometric tests, as well as clinical and social improvements when using standardized measures (133). For example, two separate double-blind, placebo-controlled studies found participants with depression and schizophrenia, respectively, improved with folic acid treatment compared to placebo (135,136). While these studies and others have been encouraging, a definitive connection has not yet been proven. More research is needed to establish causality between the two.
Other Congenital Disorders

Unlike the well-established inverse relationship between maternal folate status and NTD risk, the correlation between maternal folate status on other congenital disorders such as orofacial clefts and congenital heart defects is less clear (137). There have been a number of studies investigating periconceptional use of folic acid-containing multivitamins, and whether they can exert a protective effect similar to NTDs. Evidence to date is much more compelling for congenital heart defects. An RCT performed in Hungary evaluated the efficacy of a periconceptional multivitamin containing 800 mcg folic acid on NTDs and after further analysis, found it provided a 50% risk-reduction for heart defects (138). This finding has been supported in several other studies (139–142), yet remains unclear if the protective effect is driven independently by folic acid or in combination with other nutrients. Studies examining the association between maternal multivitamins and the occurrence of orofacial clefts have shown mixed results (143–149). More folic acid-specific interventional studies are required to determine its role in the occurrence and reoccurrence of orofacial clefts using varying doses (137).

Folate Excess

Convincing

Masking of B₁₂ Deficiency

It has been well documented that excess folic acid intake has the potential to mask B₁₂ deficiency (24). In the presence of B₁₂ deficiency, methylene-THF regeneration is disrupted and folate is “trapped” in its methyl folate form, as B₁₂ is required for its conversion to THF in the MS reaction (Figure 2.6) (42). This “folate trapping” causes a deficiency of folate. High intakes of folic acid can correct the inadequacy and enable the continuation of DNA synthesis, thereby reversing the anemia. However, it allows the B₁₂ deficiency to progress undetected until neurological symptoms begin to manifest (24). Approximately 20-30% of reported B₁₂
deficiencies have reported neurological damage in the absence of anemia (150). This is of particular concern in high risk populations such as the elderly, where a number of studies have found low vitamin B\textsubscript{12} to be associated with an increased risk of cognitive impairment (151–154).

**Insufficient Evidence**

**Antifolate Resistance**

Antifolates are used primarily in the treatment of inflammatory conditions (e.g. rheumatoid arthritis) and cancer (92,152). Their mechanism of action involves the interference with enzymes involved in folate metabolism, which can lead to disruption to the metabolic pathway. Excess folate and folic acid may compete with antifolates, potentially causing a decrease in the drug’s strength and efficacy (92,156). A study by van Ede et al (2001) conducted a randomized, double-blind, placebo-controlled study which studied the effects of folic or folinic acid supplementation on toxicity and efficacy of methotrexate in 424 subjects with rheumatoid arthritis. They observed higher doses of methotrexate were required in the supplemented groups to achieve a similar clinical response (folic acid; p<0.001; folinic acid; p=0.017) (157). This finding has not been uniformly consistent (156,158,159); a recent systematic review was undertaken to assess if folate supplementation reduces methotrexate benefits; however, results remained inconclusive due to the vast differences in markers of disease activity (160).

**Reduced Natural Killer Cell Cytotoxicity**

NK cells are part of the innate immune system located primarily in blood, lymphoid organs, liver and lungs (161). They play an integral role in the destruction of virus-infected cells and limiting cancer cell progression. In the post-fortification era, reduced NK cell cytotoxicity has been attributed to increased circulating concentrations of UMFA (61). In a cohort of healthy postmenopausal women, plasma UMFA was detected in 78% of the blood samples and NK cytotoxicity was 23% lower in study subjects with detectable levels of UMFA. As well, an
inverse U-shaped curve was seen between dietary folate/folic acid supplement intake and NK cell cytotoxicity in those with detectable levels of UMFA (61). Similarly, a prospective study on healthy Brazilian adults (n=30) evaluated the effect of high dose (5mg) folic acid supplementation on folate concentrations and immune response. Results showed significant increases in both blood folates and UMFA concentrations, as well as a reduction in both the number and cytotoxicity of NK cells (162). These findings are supported by animal models which have also observed reduced NK cell cytotoxicity and altered immune response when fed a high folic acid diet (163,164).

**Dual-Modulatory Role: Inadequacy and Excess**

**Probable**

**Cancer**

Folate has been thought to play an important role in cancer development and progression. A large body of evidence collectively suggests an inverse association between suboptimal folate status and risk of certain cancers including colorectum, oropharynx, esophagus, stomach, pancreas, breast, cervix, ovary and lungs, as well as neuroblastoma and leukemia (60,165–167). However, the nature and magnitude of this purported inverse association have not been equivocally established (60).

Animal and clinical studies suggest that folate may in fact have a dual, modulatory effect on the development and progression of cancer, depending on the stage of cell transformation and the dose and timing of folate intervention (167). In normal tissues, folate deficiency appears to increase, while modest levels of folic acid may decrease, the risk of neoplastic transformation. In contrast, in transformed cells, folate deficiency suppresses or inhibits, while folic acid supplementation may promote the progression of (pre)neoplastic foci (10). Biological mechanisms underpinning the dual role effects on folate on the development and progression of
cancer are likely related to the role of nucleotide biosynthesis and biological methylation reactions (168–171).

Clinical trials examining the effect of folic acid supplementation on cancer risk have to date provided conflicting results. Double-blinded placebo-controlled randomized clinical trials investigating folic acid supplementation and the risk of recurrent colon adenomas, a well-established precursor of colon cancer, as the primary endpoint, observed an increase (172), decrease (173) or null effect (174,175). An increased risk was demonstrated in the Aspirin/Polyp Prevention Study, where subjects with a history of colorectal adenomas and on folic acid supplementation had a 67% increased risk of advanced lesions (172). Additional analyses also revealed folic acid supplementation to be associated with an increased risk of prostate cancer in this study (176). The WENBIT study (Norway) investigated the effect of folic acid (800 µg/d) and other B vitamins (B₆ 40 mg/day and/or B₁₂ 400 µg/d) on cardiovascular outcomes through homocysteine-lowering for a period of 2-5 years. Interestingly, researchers noted a total increase in cancer incidence and mortality by 21% and 38%, respectively, over a 36 month period (177). In another meta-analysis, which reviewed eight randomized controlled trials (37,485 subjects) using folic acid supplementation on the primary outcome of cardiovascular disease, no significant effects on cancer incidence or mortality over a median timeframe of five years was found (178). A separate meta-analysis of thirteen trials also found a null effect of folic acid supplementation on both cardiovascular (primary endpoint) and colorectal cancer risk in patients with prior adenomas. Yet, a non-significant trend towards colorectal cancer risk was observed in three of the trials involving patients with previous adenomas, as well as in all thirteen trials (179). A surprising temporal trend of increased colorectal cancer incidence in Canada, United States and Chile during the post-fortification era was reported in two ecologic studies; suggesting folic acid fortification may be, in part, responsible for this rise (180,181). However, two large prospective studies conducted in the United States post-fortification did not find a tumor-promoting effect to be associated with folic acid supplementation, but did identify that a moderate folate consumption provided a reduction of risk for colorectal cancer (182,183). The role of folate in cancer development and progression remain unresolved.
2.1.7 Folic Acid Fortification

Adequate intake of folate in either its natural or synthetic form is sufficient to circumvent the majority of NTDs (184,185). An early, non-randomized, interventional study in the United Kingdom supplied 3.6 mg folic acid periconceptually (≤1 month) to women with a previous NTD-affected pregnancy and observed a significant 86% reduction in NTD risk (186). These findings led to observational studies (187,188) and subsequent randomized controlled trials (138,189), which identified a strong relationship between periconceptional folic acid supplementation and reduced NTD rates in the periconceptional period. One such trial was a large (n=1195), multicenter, double-blind, randomized controlled study led by the United Kingdom Medical Research Council, which investigated the recurrence risk of NTDs in the United Kingdom (17 centers) and six other countries (16 centers). A 72% protective effect in women at risk of having a NTD-recurrence was observed with 4 mg folic acid (189); a finding supported in another large (n=4753), randomized controlled trial conducted in Hungary, which found a smaller dose of 0.8 mg to be 100% NTD-protective (138). Based on this evidence, a recommendation of 0.4 mg folic acid/day (in addition to food folate) for all women capable of becoming pregnant, was established in 1992 and 1993 by the United States government (190) and Health Canada (191) respectively. These recommendations were also included in the IOM’s 1998 Dietary Reference Intakes for Folate (19).

Despite folic acid recommendations periconceptually, it has been noted that 40% of pregnancies are unplanned (19); a concern, as development of the embryotic nervous system occurs between the third and fifth weeks, and often before the woman is aware of the pregnancy (19). Additionally, strategic approaches through dietary and supplementary means are limited due to a multitude of barriers including behavior change, accessibility, affordability and sustainability (192). As such, mandatory fortification of folic acid was introduced in November 1998 (United States and Canada) as an additional population-based strategy to increase total daily intake and blood levels of folate. The addition of 150 μg folic acid/100g in white wheat flour and cornmeal and 200 μg folic acid/100g in enriched pasta was mandated in Canada (193), whereas the U.S. incorporated 140 μg folic acid/100g into all three grain products (white-wheat flour, cornmeal
and enriched pasta) (194). This has led to an approximate 46% (Canada; 1993-2002) and 36% (U.S.; 1996-2006) reduction in NTDs compared to pre-fortification rates (195,196). The success of this public health strategy has been paralleled by other countries with mandatory fortification programs (197).

Dietary intake and blood levels of folate and folic acid in North America have significantly increased post-fortification. United States population-based surveys have reported a 136% and 57% increase in serum and RBC folate concentrations, respectively, since mandatory fortification compared with pre-fortification levels (89) and have observed <1% of the population to be folate deficient (198). As well, the prevalence of low serum and RBC folate concentrations significantly decreased in women of childbearing age pre to post fortification (serum folate: 21% to <1%; RBC folate: 38% to 5%) (89). In the 2007-2009 CHMS, it was reported >40% of the Canadian population have RBC folate concentrations exceeding 1360 nmol/L, a value considered to be high (97.5\(^{th}\) percentile; NHANES 1999-2004) (9). Folic acid as a fortificant was intended to add a further 100-200 µg folic acid to total daily folate intakes (199). In Canada, folic acid amounts have been observed as being ~50% higher in fortified products than what had been mandated (200), suggesting total daily folic acid intake may be much greater than originally predicted.

Despite the low prevalence of serum and RBC folate post-fortification, a certain component of the North American population (including women of childbearing age) remains below a RBC folate concentration of 906 nmol/L (201); the level considered to provide a maximal amount of protection against NTD-affected pregnancies. In a large case-control study in Ireland (n=56 049), researchers noted a dose-response relationship between RBC folate concentrations and risk of NTD (84), and determined there was more than an eight-fold difference in NTD-risk between women with RBC folate <340 nmol/L versus those with RBC folate levels ≥906 nmol/L (84). Insufficient case numbers prevented analysis of potential additional benefits beyond the aforementioned maximal level of protection.
Prior to fortification of folic acid, United States population data found more than 90% of women aged 20-59 years of age were below the optimal NTD-protection level (202). Post-fortification, an increase was observed in median RBC folate concentrations (587 nmol/L), yet the percentage below the NTD-protection level remained unchanged (202). In Canada, 22% of women aged 15-45 years old had levels below 906 nmol/L (203) in the post-fortification era, with supplemental folic acid use identified as being the biggest predictor of women attaining optimal RBC folate concentrations above the NTD-protection level (203). These findings reinforce the importance of folic acid fortification, as well as periconceptional folic acid supplementation, to help mitigate risk of NTDs.

2.2. Vitamin B₆ and Vitamin B₁₂

2.2.1 Vitamin B₆

2.2.1.1 Definition, Chemical Structure and Dietary Sources

Vitamin B₆ is a water-soluble vitamin that exists in several forms, including pyridoxine, pyridoxal, and pyridoxamine, as well as their 5-phosphate forms pyridoxal, pyridoxine and pyridoxamine phosphate, respectively (19). Pyridoxal phosphate (PLP) is the major form of vitamin B₆ and is a coenzyme for >100 enzymes. Vitamin B₆ can be found in beef, organ meats, fish, chickpeas and non-citrus fruits, and its bioavailability is approximately 75% when consumed from a mixed diet (19).

2.2.1.2 Absorption and Metabolism

Vitamin B₆ is absorbed across the jejunum via phosphatase-mediated hydrolysis. The resulting non-phosphorylated forms are then transported into the mucosa by nonsaturable passive diffusion (19). The liver is the main storage site for this vitamin, where it is then converted back to its phosphorylated forms by pyridoxal kinase. PLP is highly protein-bound, with albumin being the
primary PLP-binding protein in plasma. If capacity has been exceeded, PLP is quickly hydrolyzed into its nonphosphorylated forms and then released by the liver and other tissues into circulation (19). The liver also has the ability to oxidize PLP to 4-pyridoxic acid, where it is then released into the system and excreted (19). Muscle, plasma and erythrocytes have the highest PLP-binding capacity; when other tissues are saturated they are able to accumulate high levels of PLP (204).

2.2.1.3 Biochemical Functions

As mentioned earlier, vitamin B_6 is one of the key vitamins involved in the one-carbon metabolism pathway, and works in conjunction with folate, vitamin B_12 and choline within the different pathways that participate in one-carbon metabolism. In the two-step transsulfuration pathway, vitamin B_6 acts as a cofactor in the degradation of Hcy to form cystathionine and eventually cysteine (21). Vitamin B_6 also helps to facilitate the conversion of serine to glycine through its action as a coenzyme for serine hydroxymethyl transferase, which is responsible for catalyzing the conversion of THF to 5,10-methyleneTHF (21).

2.2.1.4 Vitamin B_6 Requirements

The EAR and RDA for adults aged 19-50 years is 1.1 and 1.3 mg/day, respectively. For adults aged 51-70 years, the EAR and RDA are further stratified in gender, where female requirements are 1.3 mg (EAR) and 1.5 mg (RDA), while males are 1.4 mg (EAR) and 1.7 mg (RDA) (19). PLP concentrations of 20 nmol/L or greater is the most significant indicator of vitamin B_6 adequacy, and considered to be reflective of tissue stores (205).

The UL for vitamin B_6 has been established at 100 mg/day, representing total intake from both food and supplementation. It is based on case reports identifying the development of sensory neuropathy using supraphysiologic doses (19). To date, high dietary intakes from food alone have not been associated with any adverse effects (19).
2.2.1.5 Biomarkers of Vitamin B₆

PLP is the most commonly used biomarker to assess vitamin B₆ status, as it has been shown to be a good indicator of both tissue stores and long-term status. A plasma PLP cut-off of ≥30 nmol/L is considered to reflect vitamin B₆ adequacy in adults (206), however a plasma PLP concentration of ≥20 nmol/L has also been proposed (207).

Microbiologic assays, enzymatic assays, liquid chromatography with fluorescence detection, and LC-MS/MS can be used to determine plasma PLP. Similar to folate assays, inter-laboratory variation and methodological differences can make it difficult to compare concentrations from a population health standpoint (208).

2.2.1.6 Vitamin B₆ and Health

Vitamin B₆ deficiency is rare in the general population, likely owing to the broad range of products available in both its natural and fortified vitamin forms. Certain populations pose a higher risk of deficiency, including those with gastrointestinal disorders (malabsorption), autoimmune diseases, renal dysfunction (dialysis) and alcohol dependence. Clinical signs of vitamin B₆ deficiency include microcytic anemia, depression and confusion, amongst others (209,210).

Vitamin B₆ status has been associated with several diseases; most notably, coronary artery disease and stroke (19). Vitamin B₆, in conjunction with folate and vitamin B₁₂, intersect at the Hcy pathway, and it has been postulated these vitamins may play a role in reducing cardiovascular risk through its reduction of hyperhomocysteinemia. As such, there have been a number of clinical trials investigating the effect vitamins B₆, B₁₂, and folate may have in mitigating this risk in both the general population and in the chronic kidney disease population (3,107). To date, results have failed to demonstrate that vitamin B₆ supplementation alone, or in combination with folate and vitamin B₁₂, reduces the risk of coronary artery disease or stroke (107).
2.2.2 Vitamin B₁₂

2.2.2.1 Definition, Chemical Structure and Dietary Sources

Vitamin B₁₂, also known as cobalamin, is a complex water-soluble compound that is characterized by a cobalt atom which is positioned in the center of a corrin ring. Vitamin B₁₂ comes in many forms, depending upon the moiety attached to cobalt, including methylcobalamin, 5-deoxyadenosylcobalamin, cyanocobalamin, and hydroxycobalamin (211). The two biologically active vitamin forms are methylcobalamin and 5-deoxyadenosylcobalamin (211). Cyanocobalamin is the most stable form of this vitamin and is found in both supplements and fortified foods. Vitamin B₁₂-rich food sources include red meat, chicken, fish, shellfish, organ meats and dairy products, and have an average bioavailability of fifty percent (19). Adequate vitamin B₁₂ is essential for neurological function and the maintenance of optimal red blood cell production (19).

2.2.2.2 Absorption and Metabolism

Upon ingestion, vitamin B₁₂ binds with haptocorrin, a salivary vitamin B₁₂-binding protein. It is then proteolyzed by pancreatic proteases in the duodenum and where it attaches to intrinsic factor, a gastric vitamin B₁₂-binding protein which is secreted from parietal cells of the stomach (19). This complex enters mucosal cells in the distal ileum via receptor-mediated endocytosis; it first binds to cubilin on the enterocyte and is then endocytosed. Inside the enterocyte, cobalamin is separated from intrinsic factor and is transferred to a plasma transport protein, transcobalamin II (211). Vitamin B₁₂ in circulation is attached to one of three plasma proteins: transcobalamin I, II, or III. While approximately 80% of vitamin B₁₂ circulating in the blood is bound to transcobalamin I, it is transcobalamin II which is involved in the delivery of vitamin B₁₂ to the tissues (19,211).
2.2.2.3 Biochemical Functions

Vitamin B\textsubscript{12} plays an important role in one-carbon metabolism; its methylcobolamin form acts as a cofactor to methionine synthase, an enzyme which facilitates the transfer of a donated methyl group from methylnethyldrafolate to the sulphur-containing amino acid homocysteine, which then forms methionine and tetrahydrafolate (19). This is a critical component of the one-carbon metabolism pathway, as methionine is then condensed with adenosine triphosphate to form SAM, universal methyl donor in \textgreater 100 methylation reactions (25,39). Adenosylcobalamin, the other metabolically active form of vitamin B\textsubscript{12}, is a cofactor for methylmalonyl-CoA-mutase, which is required for the conversion of methylmalonyl-CoA into succinyl CoA. This reaction is a critical component in the catabolism of various amino acids and fatty acids (19).

2.2.2.4 Vitamin B\textsubscript{12} Requirements

The EAR and RDA for vitamin B\textsubscript{12} in a healthy adult population is 2.0 and 2.4 µg, respectively. These targets are based on the amount required to maintain both hematological status and serum vitamin B\textsubscript{12} concentrations (19). In pregnancy, requirements increase to 2.2 µg (EAR) and 2.6 µg (RDA), based on fetal deposition of the vitamin and evidence suggesting absorption is more efficient during this timeframe (19). Lactation needs have been set at 2.4 µg (EAR) and 2.8 µg (RDA) (19). In persons over 50 years of age, the Institute of Medicine recommends meeting needs through ingestion of foods fortified with vitamin B\textsubscript{12} or supplementation, as absorption of natural food-bound vitamin B\textsubscript{12} can be reduced in up to thirty percent. There has not been a UL established due to a lack of evidence (19).

2.2.2.5 Biomarkers of Vitamin B\textsubscript{12}

Vitamin B\textsubscript{12} status can be assessed through the utilization of four biomarkers. Circulating biomarkers include serum vitamin B\textsubscript{12} and holotranscobalamin; the biologically active component of total circulating vitamin B\textsubscript{12}. At this time, serum vitamin B\textsubscript{12} is the most common biomarker used to determine adequacy, as studies examining the utility of holotranscobalamin as
a biomarker are limited (212). A commonly used deficiency cut-off for serum vitamin B\textsubscript{12} is <150 pmol/L, while sub-clinical or marginal deficiency is defined as being between 150-258 pmol/L (212). Functional biomarkers include tHcy and methylmalonic acid, both of which have an inverse relationship with vitamin B\textsubscript{12} concentrations (19). Methylmalonic acid is the preferred functional biomarker, as tHcy is affected by renal dysfunction as well as other methyl donor nutrients involved in one-carbon metabolism such as folate and vitamin B\textsubscript{6} (212). The most commonly used cut-off for methylmalonic acid is >271 nmol/L (213).

2.2.2.6 Vitamin B\textsubscript{12} and Health

Vitamin B\textsubscript{12} deficiency has been linked to neurologic, hematologic, and cognitive effects, including neurodegeneration, megaloblastic anemia and cognitive decline (19). As stated earlier, vitamin B\textsubscript{12} and folate are intricately linked, with high folic acid intake having the potential to mask symptoms of vitamin B\textsubscript{12} deficiency until neurological symptoms develop (24,150). No adverse health outcomes have been observed in individuals with excess vitamin B\textsubscript{12} intake (19).

2.3 DNA Methylation

2.3.1 Definition, Function and Biological Significance

The inheritance of information based on levels of gene expression is known as epigenetics (214), and include mechanisms such as DNA methylation, histone modifications, and RNA interference, all of which play significant roles in gene expression and function (12). In contrast to genetic changes in human diseases, epigenetic changes are gradual in onset and progressive; their effects are dose-dependent and potentially reversible by dietary and pharmacologic manipulations (215,216). DNA methylation is an important epigenetic determinant of gene expression, maintenance of DNA integrity and stability, and in chromatin modifications (11).
Aberrant patterns and dysregulation of DNA methylation have been mechanistically linked to the development of several chronic diseases including cardiovascular disease (217), diabetes (218) and cancer (219); all of which have an increased prevalence in ESKD (1,220,221). DNA methylation is mediated by DNA methyltransferase using SAM, the primary methyl group donor for most biological methylation reactions, as the methyl donor, and requires methyl group donor nutrients such as folate, vitamin B₆ and vitamin B₁₂ to support the provision of SAM (168,170). Folate is essential in DNA methylation; folate in the form of 5-methylTHF facilitates the remethylation of Hcy to methionine, the precursor of SAM (222). The role of folate in DNA methylation changes has been investigated in both animal and human studies. Collectively, results from animal and human studies suggest the effects of folate on DNA methylation patterns are gene and site-specific and appear to depend on many variables including the timing, dose and duration of folate intervention (168,170,216,223). In some human studies, folic acid supplementation has been found to increase genomic DNA methylation (224–226). For example, 0.4 mg folic acid/day given over ten weeks significantly increased genomic DNA methylation in lymphocytes (31%) and colonic mucosa (25%) in patients with colorectal adenomas compared with placebo (227). Observational studies have also identified positive associations between dietary and blood levels of folate and genomic DNA methylation in both lymphocytes and colonic tissues, particularly in those with a higher risk of health complications such as individuals with adenocarcinomas, colorectal adenomas, or previously resected neoplastic tumors (168,228,229). The possible significance of methylation abnormalities in patients with kidney disease is discussed below.

2.4 End-Stage Kidney Disease (ESKD)

ESKD is the last stage (Stage 5) of chronic kidney disease and defined by a glomerular filtration rate of 15 mls/min or less (230). In this stage, kidney function is considerably reduced, resulting in the accumulation of waste products and toxins. Eventually, renal replacement therapies such as dialysis or transplantation are required to sustain life.
In 2017, one in ten Canadians (4 million people) excluding Quebec were living with chronic kidney disease, of which 38,833 were being treated for ESKD with some form of dialysis (n=22,495 [57.5%]) or functioning transplant (n=16,338 [42.5%]) (1). The number of individuals with ESKD on renal replacement therapy (dialysis or transplant) has increased by 35% over the last ten years, indicating this chronic disease is a growing public health issue. **Table 2.4** highlights the prevalence of ESKD in Canada by primary diagnosis. Diabetes continues to be the leading cause of kidney failure in prevalent patients since 2002 (1,231).

**Table 2.4 Prevalence of ESKD in Canada by primary diagnosis, 2017**

<table>
<thead>
<tr>
<th>Diagnosis^</th>
<th>Percentage</th>
<th>Total Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>29.7</td>
<td>11,546</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>21.3</td>
<td>8,256</td>
</tr>
<tr>
<td>Other^</td>
<td>14.1</td>
<td>5,456</td>
</tr>
<tr>
<td>Renal vascular disease</td>
<td>11.0</td>
<td>4,290</td>
</tr>
<tr>
<td>Unknown/not reported</td>
<td>10.6</td>
<td>4,097</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>7.4</td>
<td>2,891</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>4.6</td>
<td>1,783</td>
</tr>
<tr>
<td>Drug induced</td>
<td>1.3</td>
<td>514</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0</strong></td>
<td><strong>38,833</strong></td>
</tr>
</tbody>
</table>

^Excludes Quebec
^See Appendix E for a list of all primary diagnoses.

In 2017, diabetes (39.2%; n=2197) and renal vascular disease (12.8%; n=718) were the leading causes of kidney failure in Canadian incident ESKD patients (total n=5599) (1). Globally, diabetes and hypertension make up 80% of incident ESKD occurrences, but this varies greatly between countries (232). The number of people starting renal replacement therapy (dialysis or transplantation) has also increased; up 27% since 2008 (1). Mortality is significantly higher in individuals with ESKD compared with the general population. Unadjusted 1, 3, 5 and 10-year
survival rates in Canadian ESKD patients receiving dialysis in 2006 were 83.6%, 61.2%, 42.0% and 15.9%, respectively (1).

2.4.1 Treatment of End-Stage Kidney Disease

Treatment options for ESKD include dialysis or renal transplantation, of which dialysis is the more common therapy. There are two types of dialysis therapies; hemodialysis and peritoneal dialysis. Hemodialysis is a mechanical process, utilizing a machine which contains a dialyzer that acts as a filter to artificially clean the blood. Blood is pumped from a patient’s access site to the dialyzer, where fluid, electrolytes, waste products and other solutes pass through microscopic pores in the dialyzer and into a surrounding dialysate solution through a process of diffusion and ultrafiltration. The cleaned blood is then returned to the patient’s body, while the used dialysate flows out of the machine and into a drain. This process is repeated many times depending on the individual’s treatment needs (233). Conventional, or in-centre hemodialysis (IHD), is carried out in a hospital or dialysis centre by a trained healthcare professional, where patients receive treatment three times per week for approximately four hours in duration. Removal of fluid, electrolytes and waste products is significantly less than in people with normal kidney function (233); therefore, dietary and fluid restrictions are often required. Studies indicate the IHD population has a high symptom burden including fatigue, pruritis, anorexia, pain, sleep disturbance, nausea and restless legs (234). Despite this, IHD is the most common dialysis modality used in Canada, with 75% of ESKD patients (n=16,338) receiving this form of treatment in 2017 (1).

An alternative to receiving hemodialysis in the hospital is to carry out the hemodialysis treatments at home. Home hemodialysis (HHD) is often a more intensive treatment, where patients typically dialyze more frequently and/or for longer duration (233). The intensive treatment usually allows for diet liberalization and decreased medication use. Reduced symptom burden and improved quality of life has also been reported in this population (235). Extensive training is required to learn the mechanics of the machine, and patients are assessed at the end of their training to determine if they are able to carry out their treatments independently. Due to the
extensive training and abilities required to independently manage one’s hemodialysis treatment, only a small percentage of ESKD patients receive this type of dialysis therapy. For example, in 2017, only 4.7% (n=1058) of Canadian ESKD patients were on HHD (1).

Peritoneal dialysis (PD) is a home dialysis therapy where the lining of the abdomen, or peritoneal membrane, forms a sac known as the peritoneal cavity and is used to remove fluid, electrolytes, waste products and other solutes from the body. A catheter is inserted into the peritoneal cavity, which is then used to fill the cavity with a dialysate solution. The dialysate is held for a set period of time to allow fluid, electrolytes, waste products and other solutes from surrounding blood vessels to move across the membrane through a process of diffusion and osmosis, after which the used dialysate, or effluent, is discarded (233). This exchange is done manually by the individual several times per day (continuous ambulatory peritoneal dialysis [CAPD]), or by a machine during the night (continuous cycling peritoneal dialysis [CCPD]), depending on the person’s treatment needs and/or preference. Patients on CCPD may or may not carry fluid in their peritoneal cavity through the day.

Contrary to IHD, PD is performed on a daily, more frequent, basis (233). And similar to HHD, it requires training, although it is much less intensive. The daily removal of fluid and solutes permits greater liberalization of fluid and dietary intake; however, appetite suppression has been reported in some patients due to the peritoneal dialysate volume pressing on the gastrointestinal tract (236). As well, the dialysate is comprised of either glucose or maltose-based solutions, which can contribute to a patients’ daily caloric load and may provoke a gain in fat mass. As well, individuals with diabetes may require medication adjustments to account for the increased carbohydrate load. PD treatment allows for greater flexibility with work and travel; patients can often fine tune their treatment in accordance with work hours and the portability of the PD machine provides opportunity to travel short or long distances. Another benefit of PD therapy is the preservation of residual renal function (RRF). Studies have observed the decline in RRF is slower in PD compared to hemodialysis (237,238), and a higher RRF has been associated with better patient survival in both dialysis modalities (239); likely due, in part, to better clearance of
middle molecular weight uremic toxins, improved fluid balance and blood pressure control (240).

2.4.2 End-Stage Kidney Disease and Nutritional Status

Prevalence of malnutrition in ESKD has been reported as being between 18-75% (241–243). The wide variation is due to many factors, including types of nutrition assessment tools utilized, mode of dialysis, as well as the country, region and types of patients being studied (241,244, 245). However, one of the largest confounders has been the marked variation in how malnutrition is classified in ESKD. While protein-energy malnutrition can occur through inadequacies in the diet, patients with ESKD have additional inflammatory processes contributing to loss of lean body mass and fat stores, irrespective of nutrient intake (246). In 2007, the International Society for Renal Nutrition and Metabolism coined the term protein-energy wasting (PEW), and established a defined set of criteria for the assessment and standardization of malnutrition to better reflect the unique and multi-faceted characteristics within the ESKD population (246). PEW therefore encompasses both nutritional and metabolic disturbances that are often simultaneously present in this population, including reduced nutrient intake, inflammatory processes, oxidative stress, and loss of nutrients through the dialysate (247). A comprehensive list of these disturbances is depicted in Figure 2.6.
Figure 2.6 A schematic overview of causes and consequences of PEW. *PEW is strongly associated with these adverse events, but the degree, if any, to which PEW actually causes these adverse events is not known. CVD, cardiovascular disease; DM, diabetes mellitus; HF, heart failure; IGF-I, insulin-like growth hormone-I; PEW, protein-energy wasting; PTH, parathyroid hormone. Adapted from Lodebo, 2018 (247) and reprinted with permission.

PEW has been associated with increased morbidity and mortality in ESKD (248,249) and can be treatable with early detection, nutritional intervention and close monitoring of nutritional status (250–253). Many assessment tools are available to determine nutritional status and presence of PEW in ESKD; however, no lone method can reliably detect malnutrition. Dietary review, biochemical markers and anthropometric measures are commonly used in the ESKD population, while bioelectrical impedance, handgrip strength, and mid-arm muscle circumference are less utilized, although clinically valuable tools (247). Subjective global assessment (SGA) is also a well-established, clinically useful tool to assess nutritional status (254). A modified version, the 7-point SGA, has been previously validated in the ESKD population and is used globally by the nephrology community to determine nutritional status. A multicenter study conducted in Canada and the United States investigated mortality and nutritional status in 680 CAPD patients using the 7-point SGA tool and found a 1-unit decrease in SGA was equivalent to a 25% increase in
A recent meta-analysis reviewed 90 studies (2000-2014) from 34 countries which used SGA (80 studies) or a modified version of SGA (10 studies) to assess PEW in 16,434 dialysis patients. Canada was not included in the study due to lack of data. Median PEW prevalence was 28-54% using the 25th-75th% range, and wide variation was observed globally (Figure 2.7). The global variation was able to be explained, in part, by geographic location, although this was only able to account for 23% of the heterogeneity (245). A greater understanding of the pathogenesis of PEW is needed in order for clinicians to tailor nutrition interventions accordingly.

Figure 2.7 Global prevalence of protein-energy wasting (PEW) in kidney disease. Prevalence of PEW among patients undergoing maintenance dialysis worldwide reported from studies published during 2000-2014. Color gradation reflects PEW prevalence in all included studies from each country (weighted averages within countries). PEW, protein-energy wasting. Reprinted with permission. Carrero, 2018 (245).
2.4.3 End-Stage Kidney Disease and Health

2.4.3.1 Diabetes

Diabetes mellitus is the leading cause of ESKD globally (255). In Canada, diabetes has remained the most common cause of renal failure for nearly twenty years; easily surpassing other causes of ESKD, such as renal vascular disease and glomerulonephritis (1,231). As mentioned previously, in 2017, diabetes accounted for 39% (n=2197) of all new diagnoses of ESKD, compared to 32% (n=1527) in 2000 (1). Epidemiological evidence has long established that ESKD is associated with high mortality and accelerated cardiovascular disease compared to the general population (256); a scenario analogous to those living with diabetes (257). Results from a meta-analysis, which included 102 prospective studies, found the presence of diabetes approximately doubles the risk of a broad range of vascular diseases and shortens life expectancy by roughly six years in adults aged 40-60 years (257). Additionally, individuals living with Type 1 or Type 2 diabetes were found to be at an increased risk of premature death from a wide range of other causes (258,259) (Figure 2.8); a risk that is further enhanced in the presence of kidney disease (232,260).
Figure 2.8 Hazard ratios for death from cancer, noncancer, nonvascular causes among participants with diabetes compared to those without diabetes at baseline. Panel A shows hazard ratios for deaths from cancer, and Panel B shows hazard ratios for deaths from noncancer, nonvascular causes. With the exception of the classifications “site unspecified or other” in Panel A and “other noncancer, nonvascular deaths” in Panel B, causes of death are presented in descending order according to their estimated hazard ratios. Analyses were stratified on the basis of study, sex, and trial group (where applicable) and adjusted for baseline age, smoking status (current smoker vs. any other status), and body-mass index. There was evidence of heterogeneity in hazard ratios among cancer sites and among the noncancer, nonvascular causes of death (P<0.001 for both comparisons). Participants with known preexisting cardiovascular disease at baseline were excluded from all analyses. The sizes of the data markers are proportional to the inverse of the variance of the loge hazard ratios. In Panel A, risk estimates for cancer of the colorectum were broadly similar to those for cancer at subsites. In Panel B, death from endocrine disorders does not include death coded as being due to diabetes. Other noncancer, nonvascular deaths are those that could not be attributed to a major organ or system. COPD denotes chronic obstructive pulmonary disease. Reproduced with permission from Emerging Risk Factors Collaboration, N Engl J Med 2011; 364:829-841, Copyright Massachusetts Medical Society (259).
### 2.4.3.2 Cardiovascular Disease

CVD is the principal cause of death in individuals with ESKD kidney disease receiving dialysis therapy (261,262). A 10 to 30-fold greater risk of cardiovascular death compared to the general population has been observed (256), with Hhcy historically being proposed as a potential risk factor for the increased cardiovascular incidence and mortality (263). Plasma tHcy concentration (Hcy; an accurate inverse indicator of folate status) is governed by several methyl donor nutrients involved in one-carbon metabolism including folate, vitamin B₆ and vitamin B₁₂ (24). Increased tHcy concentrations are commonly observed in patients on dialysis; this elevation has been attributed to impaired renal metabolism and clearance of homocysteine, as well as to altered absorption and metabolism and/or deficiencies in one or more of the aforementioned nutrients, with folate being implicated as one of the major contributors (264–266). As such, there has been intense interest in using folic acid, B₆ and B₁₂ supplementation to provide a cardioprotective benefit through reduction of Hcy levels in patients with ESKD. Folic acid supplementation (2-40 mg/day) has been shown to significantly lower plasma tHcy concentrations in people with kidney disease up to 30% (267–271), but no further reduction is observed with doses >15 mg/day (272). Additionally, results of several RCTs found that folic acid supplementation (1-15 mg/day for 1-3.6 yrs) did not affect risk of cardiovascular outcomes (fatal or non-fatal) in Stage 5 chronic kidney disease, IHD or PD (267,270,273,274). Similar findings were seen in studies using a combination of folic acid, vitamins B₆ and B₁₂-based Hcy-lowering therapy on risk of cardiovascular events, all-cause mortality or death from cardiovascular outcomes (263,267,275). In 2012, a systematic review of randomized clinical trials concluded that folic acid does not reduce cardiovascular events in patients with kidney disease and should not be used for prevention in this population (3). Yet supplementation of ≥1 mg/day folic acid in ESKD continues to be a recommended practice within North America (2).

### 2.4.3.3 Cancer

Increased cancer incidence has been well documented in ESKD. A multinational study in the
pre-fortification era (1980-1994) reviewed 831,804 dialysis patients to compare frequency of cancer with the general population. Average follow up of 2.5 years revealed a higher incidence of cancer development in the ESKD population (3%) compared to the general population (2.5%) (standardized incidence ratio 1.18 [95% CI 1.17-1.20]), with subjects under 35 years of age exhibiting the highest cancer risk (3.68%; standardized incidence ratio 3.68 [3.9-3.99]) (276). However, study results must be interpreted with caution due to possible inaccuracies with classification of incident versus prevalent cancers in the United States component. Despite this limitation, these findings have been echoed globally in other large cohort studies (277–282). An elevated risk of site-specific cancers has also been observed, albeit with mixed results, including cancers of the stomach, small intestine, colon, liver, biliary tract, lung, cervix, kidney and bladder (280,282–285).

Features specific to patients with ESKD may contribute to this observed increased risk. First of all, more than 50% of ESKD patients have diabetes (286); a comorbid condition known to increase the risk of colon and other cancers and therefore may harbor undiagnosed (pre)cancerous foci, which can be promoted to progress by folic acid supplementation (10). Second, it is purported that chronic inflammation, decreased immune function, and impaired DNA repair mechanisms, all of which are the consequence of reduced renal function, may promote cancer (287). Table 2.5 outlines potential mechanisms of malignant transformation in ESKD. Increased frequencies of micronuclei, an early biomarker for cancer risk in the general population and indicator of genomic damage (288), have been found in patients undergoing conventional hemodialysis therapy (289). These findings have been supported by several studies which identified extensive DNA damage (290–292) and reduced DNA repair capacity in patients with ESKD (283,284), particularly in those with increased dialysis vintage (222,295).
Table 2.5 Potential mechanisms of malignant transformation in ESKD

<table>
<thead>
<tr>
<th>Potential mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired function of the immune system</td>
</tr>
<tr>
<td>Parathyroid hormone excess and deficiency of 1,25-dihydroxycholecalciferol</td>
</tr>
<tr>
<td>Decreased antioxidant capacity and increased formation of reactive oxygen species</td>
</tr>
<tr>
<td>Chronic infections and inflammations</td>
</tr>
<tr>
<td>Drugs: prior immunosuppressive therapy, analgesic abuse</td>
</tr>
<tr>
<td>Accumulation of carcinogenic compounds</td>
</tr>
<tr>
<td>Hypomethylation</td>
</tr>
<tr>
<td>Tobacco</td>
</tr>
<tr>
<td>Dialysis-related factors</td>
</tr>
<tr>
<td>Disturbed DNA repair</td>
</tr>
</tbody>
</table>

Adapted from Vamvakos, 1996 and reprinted with permission (293).

2.4.4 DNA Methylation and DNA Repair in End-Stage Kidney Disease

Limited research has been done on DNA methylation and uremia. However, studies to date suggest metabolic disturbances within the uremic milieu such as Hhcy (14) and inflammation (15,296) may contribute to aberrant DNA methylation, while oxidative stress (16) has been associated with genomic damage and altered DNA repair mechanisms. Furthermore, inflammation and oxidative stress are key components of PEW (251) and purported to be enhanced, in part, by the dialysis treatment itself (297,298), suggesting nutrition and mode of dialysis may also exert an influence on DNA methylation.

2.4.4.1 Hyperhomocysteinemia

Hhcy, present in >85% of pts with ESKD (299), has been associated with the impairment of key methylation reactions through accumulation of the homocysteine precursor SAH (300). This accumulation inhibits DNA methyltransferases which can lead to DNA hypomethylation (300), causing it to become less stable, and thereby increasing its susceptibility to genomic damage (222). Global DNA hypomethylation has been observed in patients with Hhcy on thrice weekly
hemodialysis (14), and positive associations have also been found between plasma SAH and DNA hypomethylation in patients with vascular disease (301). In-vitro studies suggest alterations of DNA methylation may contribute to atherosclerosis through the down-regulation of atherosclerosis-protective genes and/or up-regulation of atherosclerosis-susceptible genes in smooth muscle cells (302). In one study involving incident patients with ESKD who were close to initiating dialysis, researchers found SAH, but not Hcy, was independently associated with cardiovascular disease (303). This correlation may, in part, explain why a reduction in Hcy levels through increased folic acid intake has not been associated with a decrease in cardiovascular incidence or mortality.

2.4.4.2 Inflammation and Oxidation

Inflammation may influence epigenetic changes in cells through the regulation of a DNA methyltransferase gene by inflammatory mediators such as cytokines (304). DNA hypermethylation was found to be correlated with inflammation and increased mortality in a small number of patients on thrice-weekly hemodialysis (305). Hypermethylation was also observed in 80 IHD patients compared to controls, with the highest levels observed in patients who were inflamed (306). As well, several studies have identified elevated levels of C-reactive protein (CRP), a marker of inflammation, as a predictor of poor dialysis outcome (307–310). A dose-response relationship between CRP and mortality has been demonstrated in both peritoneal and hemodialysis cohorts (311). Clinical conditions which are burdened by inflammation, as observed in ESKD, are also more prone to the development of oxidative stress (312,313). Oxidative stress, characterized by the overproduction of highly reactive molecules, can damage DNA through a variety of mechanisms (21). A significant, inverse relationship between oxidative stress and kidney function has been observed (314–316); one which becomes more pronounced in the presence of uremia and ESKD (317,318), independent of other contributory factors such as age, diabetes, and malnutrition (314). This was demonstrated in a study by Stoyanova et al (2009) who examined the levels of oxidative damage in DNA bases in 253 patients with varying stages of chronic kidney disease, including dialysis, and found overall
genetic and oxidative damage was higher in IHD patients than those in the earlier stages of chronic kidney disease (319).

2.4.4.3 Nutritional Considerations

Studies examining folic acid supplementation and its effects on DNA methylation and genomic damage in the ESKD population are scarce. In one study, supplementation of folic acid (15 mg; thrice weekly) in isolation or in conjunction with vitamin B_{12} (1000 μg; once weekly) was found to reduce genomic damage in a group of 27 IHD subjects, but did not alter DNA methylation status (320). In a separate study, reductions in DNA hypomethylation and normalization of patterns of gene expression were observed in a small number of IHD subjects after supplementation of 15 mg folic acid/day for a period of eight weeks (321). These results suggest folate status can play a role in the modulation of DNA methylation and genomic damage in patients with ESKD, but to what extent is still unclear. Low folate status has typically been associated with an increased risk of certain disease types in both the general and ESKD population (322,323). While folic acid is often used as a therapeutic intervention to reverse these conditions, evidence suggests excess supplementation of this micronutrient may alter DNA methylation patterns with consequent adverse functional outcomes (324). High folic acid intakes can cause an accumulation of DHF, thereby inhibiting thymidylate synthase and MTHFR, leading to decreased levels of thymidylate and 5-MethylTHF, and the impairment of DNA integrity and methylation, respectively (325,326). These alterations can impede folate metabolism through saturation of DHFR and paradoxically generate effects similar to that of folate deficiency (36).

Although folate is considered to be the key nutrient involved in DNA methylation, adequacy of SAM is dependent upon a myriad of other methyl donor nutrients including choline and vitamins B_{2}, B_{6} and B_{12} (327). It is important to consider their role when assessing folate and DNA methylation status, as these nutrients work collaboratively within a metabolic framework of one-carbon transfer reactions (24). Altered intake of one nutrient can influence the one-carbon metabolism cycle; modulating DNA methylation if other nutrient imbalances are present (327).
This disparity may also redirect the use of other methyl donor nutrients, leading to aberrant DNA methylation (24,327).

2.4.4.4 Dialysis Modality

The extent to which DNA methylation status is affected by dialysis modality has not been established. Folate is easily removed during the dialysis process due, in part, to its low molecular weight (441.4 g/mol) (328). The introduction of more frequent (PD) or intensive (HHD) dialysis to improve uremic toxin removal is thought to exacerbate this loss (329,330), although not all studies confirm this. Coveney et al (2011) found no significant difference between serum folate levels of those receiving HHD versus IHD; however, RBC folate (RBC; an accurate indicator of long-term folate status), total folate intake and nutritional status were not assessed (331). This enhanced removal of toxins, and possibly folate, could influence DNA methylation. To date, comparative studies have not been done on the effect of dialysis modality on DNA methylation; however, the effect of dialysis on levels of genomic damage, a functional ramification of aberrant DNA methylation, has been examined. HHD and PD have been associated with a lesser degree of genomic damage and lower levels of chromosome damage, respectively, when compared with patients on IHD (16,332,333). In the predialysis population, a direct relationship between the degree of kidney impairment and amount of genomic damage has been demonstrated (292). As PD is known to be associated with a better-preserved RRF for a significantly longer period of time compared to IHD, this may explain, in part, why less genomic damage has been observed in the PD population. However, other comparative studies have observed opposite results (295,334), the discrepancy possibly due to confounding factors such as diabetes or age; both of which can impact genomic damage (295).

2.4.5 End-Stage Kidney Disease and Nutrition

It has been well documented that the uremic environment present in ESKD is host to a variety of metabolic disturbances including endothelial dysfunction, inflammation and oxidative stress (335). As mentioned previously, PEW is often associated with these disturbances (246,335).
Collectively, these metabolic abnormalities alongside traditional risk factors such as diabetes and hypertension have been shown to predict CVD risk and poor outcomes in this population, including decreased quality of life and increased hospitalization and mortality (243,246). A number of factors also predisposes individuals with ESKD to vitamin deficiencies (Table 2.6); yet dietary intake and biomarker studies to assess micronutrition in this population has not been extensively studied (336). Reduced kidney function, presence of uremia and dialysis treatment in ESKD can alter the biochemistry, metabolism and/or requirements for several vitamins (337). Many vitamins, including folate and other methyl group donor nutrients, are essential cofactors or substrates in the regulation of metabolic pathways within the body. Adequate vitamin intake is essential to maintain concentrations within blood and tissue required for these biochemical processes (337). Whereas a well-balanced diet can on average meet the nutritional needs of an individual, alterations in diet, along with kidney dysfunction and the aforementioned parameters, can have a substantial impact on one’s ability to maintain adequacy of certain micronutrients. Water-soluble vitamins in particular are a concern due to their ease of dialyzability (266,338). For this reason, patients with ESKD on dialysis in Canada are prescribed a renal multivitamin (Replavite) which contains water-soluble vitamins in amounts greater than the RDA.

Table 2.6 Factors contributing to micronutrient inadequacies in ESKD

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultural</td>
<td>Consumption of different food types due to cultural diversity</td>
</tr>
<tr>
<td>Dialysis Modality</td>
<td>Vitamin losses through hemodialysis or peritoneal dialysis treatments</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Intestinal malabsorption; malabsorptive disorders</td>
</tr>
<tr>
<td>Non-physiologic</td>
<td>Dietary restrictions; reduced dietary intake due to poor appetite</td>
</tr>
<tr>
<td>Pharmacological</td>
<td>Medications affecting vitamin absorption or activity</td>
</tr>
<tr>
<td>Uremia</td>
<td>Impaired metabolism, absorption and utilization of vitamins</td>
</tr>
</tbody>
</table>

Adapted from Wolk, 1993 (336); Rocco, 1997 (256); Soohoo, 2017 (339) and reprinted with permission.
2.4.5.1 Intake Status of Folate, Vitamin B\textsubscript{6}, and Vitamin B\textsubscript{12} in Patients with ESKD

Adequacy of folate, vitamin B\textsubscript{6}, and vitamin B\textsubscript{12} in ESKD has garnered much attention over the years due to their role in anemia management and Hhcy (323). Despite this, studies examining dietary intake of these B vitamins in this population are scarce; many are older with small sample sizes and reflect outdated dietary intake patterns and/or fortification practices (339). Published data pertaining to the dietary intake of these B-vitamins in ESKD patients are conflicting; due, in part, to the broad range of assessment methods utilized (food frequency questionnaires (FFQ) (340); food records (341); 24-hour recalls (342) and variation in food practices and measurement precision (343).

B vitamin intakes in ESKD patients may be influenced by several factors. Protein and calorie consumption were strongly correlated with B vitamin intake status in a group of 25 IHD subjects (266), while inadequate intakes of vitamin B\textsubscript{6} and folate were significantly associated with loss of RRF and reduced urea clearance in a group of 242 PD patients (344). Ribeiro et al (2011) observed higher dietary intakes of vitamin B\textsubscript{6} and folate in IHD patients consuming dinner meals prepared at home compared to those choosing convenience (fast) foods, suggesting dietary habits may also exert influence over intake status of the aforementioned B vitamins (345). The majority of studies report dietary vitamin B\textsubscript{6} and folate intakes as being below recommendations (266,341,343,344), yet vitamin B\textsubscript{12} intakes exceed daily requirements in most studies (341,342,346); possibly a result of the importance placed on consuming a high protein diet in ESKD (347). Table 2.7 highlights the calculated vitamin content of vitamin B\textsubscript{6}, folate and vitamin B\textsubscript{12} typically consumed with differing levels of protein intake.
Table 2.7 Recommended dietary allowance (RDA) and calculated vitamin content of diets with different protein intake

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Units</th>
<th>RDA*</th>
<th>Daily Protein Intake (grams)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₆</td>
<td>mg</td>
<td>1.3-1.7</td>
<td>1.0</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Folate (DFE)</td>
<td>mcg</td>
<td>400</td>
<td>260</td>
<td>290</td>
<td>320</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>mcg</td>
<td>2.4</td>
<td>2.3</td>
<td>3.2</td>
<td>5.1</td>
</tr>
</tbody>
</table>

*Adult life stage groups only; excludes RDA values for pregnancy or lactation. Adapted from Descombes, 1993 (7) and Clase, 2013 (348) and reprinted with permission.

2.4.5.2 Biomarker Status of Folate, Vitamin B₆, and Vitamin B₁₂ in Patients with ESKD

A large number of vitamins have molecular weights (MW) that fall within a category between small (<300 Daltons [D]) and middle (300-12000 Daltons [D]) molecules, and are therefore susceptible to removal during dialysis treatment (349). Most water-soluble vitamins are lost through the dialysate (338,350), and both dialysis modality and dialyzer type are thought to impact the degree of vitamin removal, but findings have been inconsistent. PD treatment removes larger molecules and proteins than hemodialysis therapy (5,351,352), suggesting this population may be at an increased risk of vitamin losses; yet this has not been universally observed (265). High-flux dialyzers initially thought to exacerbate water-soluble vitamin losses were not found to be significantly different than their low-flux counterparts (349). The extended hours seen in HHD suggest a greater degree of vitamin loss, but little data exists on vitamin status in this population (331). Differences in protein-binding affinity, mechanisms of transmembrane transport and uremic effects are believed to exert an influence over water-soluble vitamin dialysis losses, and may play a role in the wide variation of results (343).

Folate is a relatively small water-soluble molecule (MW 441D) with low protein binding, and is easily cleared during dialysis (328). In CAPD, a mean 107 μg folic acid was recovered in the dialysate (353), while an estimated 10-250 g is lost each IHD treatment (344–347), with serum folate levels dropping between 26-37% post-dialysis (328,349). Other studies have observed
folic acid loss in IHD to be similar or slightly above 24-hr urinary excretion (>0.6 μg/day) in subjects with normal kidney function (358). RBC folate is dialyzed out in a limited amount (349). A series of biomarker studies pre- and post-fortification investigating folate status in the absence of folic acid supplementation are also contradictory; some have noted folate deficiencies in their findings (328,353,359,360), while others observed low, but sufficient levels (361–364). However, these deficiencies were primarily determined by results of serum folate; in studies which examined both serum and RBC folate, the deficiency was not observed (328,346,360,365). These findings are supported by Cunningham et al (1981), who found little evidence of RBC folate deficiency (1.4%) in IHD patients not on folic acid supplementation for a mean 49 (6-120) months (358). Studies investigating folate status in ESKD patients receiving folic acid supplementation have reported high concentrations in both serum and RBC folate (366). To date, the need for folic acid supplementation remains controversial.

Vitamin B₆ (pyridoxine) has the lowest MW (245D) compared to folate and vitamin B₁₂ (367), yet quantification of dialytic removal for this vitamin has not been firmly established. LeBlanc et al (2000) reported insignificant removal of pyridoxal 5'-phosphate (PLP; the biologically active form of vitamin B₆ and most appropriate indicator of long-term body stores) (368) through IHD (328); a finding supported by earlier research, where 70-35 μg PLP was recovered per hemodialysis treatment; well below a 24-hr urinary loss (>500 μg) seen in persons without kidney disease (369). Absence of PLP in hemodialysis ultrafiltrate was noted with the use of low-flux dialyzers (370); however, mixed results were seen with high flux dialyzers (328,349,371) and a 28-48% decrease in serum PLP levels has been seen depending on the dialyzer used (328,349,371). Peritoneal losses have been observed to be lower compared to IHD, as demonstrated in a study by Ross et al (1989) where PLP and total vitamin B₆ (sum of all vitamin B₆ metabolites) removal averaged 1.9 ±0.4 μg and 129.3 ± 14 μg/day respectively (372). A systematic review (2000-2010) found a wide prevalence (24–56%) of vitamin B₆ deficiency in IHD patients and a 28-48% decrease in serum PLP levels depending on the dialyzer used (367). These variances may be explained, in part, by the use of different vitamin B₆ forms to assess status. PLP is not always utilized, making the extrapolation of results difficult (7). Although
most studies suggest a pronounced B₆ deficiency in the dialysis population, which likely requires B₆ supplementation, the amount and frequency of vitamin B₆ supplementation remains unclear.

Vitamin B₁₂ (cobalamin) has the largest MW (1355D) in comparison to folate and vitamin B₆ (367). Contrary to the mixed results seen with folate and vitamin B₆, it is widely appreciated that vitamin B₁₂ is minimally cleared during dialysis treatment, regardless of modality (331,353,373) or type of dialyzer utilized (374). As such, serum vitamin B₁₂ levels are reported as being within or above the normal range in IHD, PD and HHD groups (6,7,328,331,346,353,362,363). Most studies acknowledge vitamin B₁₂ supplementation is likely unnecessary in dialysis populations, yet it continues to be a part of micronutrient therapy despite this.

2.4.5.3 Recommended Intakes of Folate, Vitamin B₆, and Vitamin B₁₂ in Patients with ESKD

The goal of water-soluble vitamin therapy in ESKD is to achieve normalization of levels in the circulation and tissue (266,338), yet optimal dosing regimens have not been clearly defined (323). There is a paucity of large, randomized, placebo-controlled trials examining the effects of water-soluble vitamins administered in various doses, and results to date have been conflicting; leading to limited, and often broad, evidence-based recommendations (375). As a result, large variations exist in the prescription of water-soluble vitamins both within and between countries. This was noted in the Dialysis Outcomes and Practice Patterns Study; a prospective, observational cohort study of adult IHD patients (n=16,345) from 308 dialysis facilities across seven countries. It identified a statistically significant variation (p<0.001) in the use of water-soluble vitamin supplements (3.7-71.9%) as well as a significantly lower risk of mortality (RR, 0.84; P=0.001) with water-soluble vitamins use. However, results must be interpreted with caution due to possible misclassification bias (366).

Table 2.8 lists current ESKD recommendations for vitamin B₆, folic acid and vitamin B₁₂ in North America. Many researchers agree routine supplementation of vitamin B₆ is likely needed, owing in part to its high prevalence of deficiency (7,353,370,376), as well as a short biological

61
half-life and lack of long-term storage in the body (7) (Table 2.9). However, current recommendations are not based on dose-response studies, leaving the amount and frequency of B₆ supplementation still unclear. Vitamin B₁₂ stores exceed the RDA by approximately 1000-fold and have a half-life of more than one year (Table 2.9), allowing utilization over an extended period of time (377). There is a general consensus that B₁₂ supplementation is likely unnecessary; yet is recommended due to its low cost and safety profile. The need for folic acid supplementation remains an ongoing debate. To date, there is a paucity of evidence supporting the need for folic acid supplementation at or above the UL in ESKD, leaving the debate around recommended intakes of this water-soluble vitamin rife with conflicting opinions.

Table 2.8 Current recommendations on daily dose of water-soluble vitamin supplements in ESKD compared to the RDA and UL in the general population

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>RDA (IOM)</th>
<th>UL (IOM)</th>
<th>KDOQI (U.S)</th>
<th>IHD/PD (Canada/US)</th>
<th>HHD (Canada/US)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₆ (Pyridoxine)</td>
<td>1.3-1.7 mg</td>
<td>50-100 mg</td>
<td>as needed to prevent deficiency</td>
<td>10 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Vitamin B₉ (folate: DFE)</td>
<td>400µg</td>
<td>1 mg</td>
<td>as needed to prevent deficiency</td>
<td>1 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td>Vitamin B₁₂ (Cobalamin)</td>
<td>2.4 µg</td>
<td>not established</td>
<td>as needed to prevent deficiency</td>
<td>6.0 mcg</td>
<td>12.0 mcg</td>
</tr>
</tbody>
</table>

*a* Institutes of Medicine, 1998; *b* NKF (19); *c* Kopple 2013 (2). Adapted from Jankowska, 2017 (379) and reprinted with permission.

Table 2.9 Biological half-life and vitamin stores in humans

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Biological Half-life</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B₆</td>
<td>15-20 days</td>
<td>3-4 months</td>
</tr>
<tr>
<td>Folate</td>
<td>&gt;100 days</td>
<td>1-1.5 years</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>&gt;1 year</td>
<td>3-5 years</td>
</tr>
</tbody>
</table>

Adapted from Schaefer, 1977 (13); Descombes 1993 (7) and reprinted with permission.
High intakes and blood levels of folate and folic acid have been associated with several adverse health outcomes in the general population (10). In the chronic kidney disease population, previous trials have demonstrated a null or harmful effect of supplementation with folic acid in combination with vitamins B₆ and B₁₂. Jamison et al (HOST trial, 2007) carried out a double-blind randomized controlled trial in patients with advanced kidney disease (glomerular filtration rate <30%) or ESKD (on IHD), where participants received a daily capsule containing 40 mg folic acid, 100 mg vitamin B₆ and 2 mg vitamin B₁₂ or a placebo. No differences were observed between the two groups in survival (hazard ratio [HR], 1.04; 95% CI, 0.91-1.18), incidence of myocardial infarction (HR, 0.86; 95% CI, 0.67-1.08), stroke (HR, 0.90; 95% CI, 0.58-1.40), or time to initiation of dialysis (P=0.38) (269). In a multicenter, randomized, double-blind, placebo controlled trial by House et al (DIVINE trial, 2010), patients with diabetic nephropathy receiving 2.5 mg/day folic acid, 25 mg/day vitamin B₆ and 1 mg/day vitamin B₁₂ had a reduction in plasma tHcy concentrations, but also saw a greater decline in RRF (mean [SE] of 16.5 [1.7] mL/min/1.73 m²) compared to those taking placebo. Vascular events were also increased in the supplemented group (HR, 2.0; 95% CI, 1.0-4.0; P=.04) (18). Folic acid is known to bind tightly to the FRα, which is expressed in the kidney (24), making it plausible that excess folic acid may be harmful in those with reduced kidney function. Nephrotoxic effects have been demonstrated in mouse models using high doses of folic acid supplementation (380–382). It is therefore prudent for clinicians to weigh the risks of both inadequacy and excess in the ESKD population, who are already characterized by a high co-morbid burden.

2.4.5.4. Nutritional Status and Dialysis Modality

Nutritional imbalances can occur in the presence of undernutrition (malnutrition) or overnutrition (excess intake/supplementation). The introduction of intensive (HHD) and more frequent (PD) dialysis, where patients are dialyzed more often and/or for longer duration, allows for enhanced clearance of middle-molecular uremic toxins (329,383). This enhanced toxin removal is associated with improvement of quality of life and survival (384–388). Appetite stimulation (389) and liberalization of the renal diet (2,389) have been observed in these populations, both of which may contribute to an improved nutritional state (390). A study by Sikkes et al (2009)
found individuals on HHD therapy saw improvement in appetite, food group and macro- and micronutrient intake, as well as body weight and biochemical parameters (389). Despite these benefits, the vast majority of patients with ESKD receive IHD, characterized by three to four-hour sessions thrice-weekly (1).
Chapter 3: Rationale, Research Questions, Hypotheses and Objectives

3.1 Rationale

Dialysis patients in Canada are supplemented with ≥1 mg folic acid/day; initially implemented for prevention of folate deficiency and later, to reduce cardiovascular events through homocysteine-lowering (2), although the role of folic acid in cardiovascular risk reduction has not been demonstrated in subsequent clinical research (3). The intakes and blood levels of folate and folic acid in the general Canadian population have significantly increased due primarily to mandatory folic acid fortification and widespread supplemental use (88,391). A similar trend is expected in ESKD patients receiving dialysis. Therefore, current folic acid recommendations in dialysis patients may no longer be appropriate. However, there is a paucity of published literature examining intake and blood levels of folate and folic acid in the Canadian dialysis population post-mandatory folic acid fortification of the Canadian food supply in 1998, particularly in the HHD and PD cohorts. Furthermore, it is unclear the extent to which nutritional status and mode of dialysis may influence folate status.

Folate is essential for one-carbon transfer reactions involved in biological methylation reactions including DNA methylation. Altered intakes (inadequacy/excess) of folate and folic acid can potentially modify, albeit in a highly complex manner, DNA methylation, leading to possible adverse biological and health outcomes such as coronary heart disease and cancer (13,302). As dialysis patients are characterized as being a high-risk population for all-cause mortality, coronary heart disease, stroke and some forms of cancer, they may be particularly susceptible to the adverse effects of abnormal DNA methylation resulting from high folate and folic acid intake. This study aims to determine dietary intake and blood levels of folate and folic acid in a Canadian dialysis population 20 years post-fortification. It will also examine important relationships among nutritional status, dialysis modality, intake and blood levels of corresponding methyl donor nutrients involved in one-carbon metabolism and biological methylations.
3.2 Research Questions

1. What are the current intake and blood levels of folate and folic acid in a Canadian dialysis population 20 years after the advent of a national folic acid fortification program?

2. Does nutritional status influence blood levels of folate and methyl group donor nutrients?

3. Does the type of dialysis modality influence blood levels of folate and methyl group donor nutrients?

3.3 Hypothesis

We hypothesize that:

- folic acid intake derived from fortified foods and daily vitamin supplementation will result in high folate/folic acid intake and blood levels in the dialysis population.

- nutritional status and dialysis modality will influence blood levels of folate and other methyl group donor nutrients.

3.4 Objectives

1. To estimate total folate and folic acid intake consumed through food and dietary supplementation in the dialysis population.

2. To describe associations between folate and folic acid intake and blood levels of folate and other methyl donor nutrients.

3. To examine relationships between nutritional status and intake and blood levels of folate and other methyl donor nutrients.

4. To examine relationships between dialysis modalities and intake and blood levels of folate and other methyl donor nutrients.
Chapter 4: Nutritional Status and Dialysis Modality: Their Effect on Folate Status 20 Years After the Institution of a National Folic Acid Fortification Program

4.1 Subjects and Methods

4.1.1 Subjects and Study Design

This was a cross-sectional, descriptive study performed in the dialysis population. Patients with stage 5 chronic kidney disease who were receiving intensive (HHD), frequent (PD) or conventional (IHD) dialysis at the Toronto General Hospital (TGH) Nephrology Program, and who met the selection criteria, were considered eligible for recruitment into the study. TGH is part of the University Health Network (UHN) organization and fully affiliated with the University of Toronto. The TGH Nephrology Program is considered to be one of the largest nephrology programs in Ontario; it provides care for more than 1000 patients, of which approximately 523 receive dialysis treatment. This study centre is located in downtown Toronto and as such, serves a population that is both socioeconomically and ethnically diverse. The recruitment of dialysis subjects from all three modalities at our study centre enabled us to include a broad range of both dietary folate and supplementary folic acid practices.

This study protocol received ethical approval by the Research Ethics Board at UHN (REB 15-9239.2). All subjects provided written informed consent (Appendix B). Patients in the TGH Nephrology Program were eligible to participate if they were 18 years or older and had received regular dialysis treatments for at least three months prior to enrolment into the study. Patients were excluded from the study if they had a documented gastrointestinal disorder affecting folate metabolism and absorption (e.g., Crohn’s disease, celiac disease), an active infection including peritonitis, or had been hospitalized within the previous 30 days. Those on IHD who were dialyzing more or less than three times per week were excluded to maintain consistency of treatment. Patients deemed palliative or identified as being non-adherent to treatment were considered ineligible for the study. Patients who were unable to complete the food records due
to cognitive or language barriers were also excluded if they did not have supports available to assist them with the necessary documentation.

4.1.1.1 Recruitment

All patients who were part of the TGH Nephrology Program and receiving dialysis ≥3 months were screened for eligibility. Between November 2017 and March 2019, a total of 357 patients met eligibility criteria and 215 (n=60.2%) were approached by a research volunteer, nurse or administrative staff using a standardized script (Figure 4.0). Those interested in hearing about the study were then followed up by the study dietitian, who provided study logistics and answered questions pertaining to the study. Of these, 83 (38.6%) agreed to participate. Reasons for declining the study are highlighted in Figure 4.0. Written informed consent was obtained and a total of 70 participants completed the study. A small number of subjects (n=13 [15.6%]) withdrew from the study (Figure 4.0). Flow charts detailing the study recruitment process for each modality can be found in Appendix A. Study visits and data collection began in March 2018 and were completed in April 2019.
Figure 4.0: Flowchart of study recruitment, all modalities
4.1.1.2 Study Visits

Participants completed 2 study visits in total. Details of the study visits are listed in Table 4.0 below:

Table 4.0: Breakdown of procedures and tests during study visits 1 and 2

<table>
<thead>
<tr>
<th>Study Visit</th>
<th>Procedures and Tests</th>
</tr>
</thead>
</table>
| Visit 1 (~30 minutes) | • Participants met with study investigator to review and sign the consent form.  
• Participants provided with a food scale and 3 day weighed food record collection sheets (Appendix C; Appendix D).  
• Instructions given on scale use and how to record food and fluid intake. |
| Visit 2 (~90 minutes) | • Participants met with study investigator prior to starting dialysis treatment OR dialysis clinic visit to return 3 day weighed food records and the scale.  
• Food records reviewed to ensure all food/fluid entries are understood.  
• Nutritional assessment completed by the study investigator including height, current dry weight, BMI (Appendix E) and SGA (Appendix F).  
• Participants interviewed on the following: highest level of education, employment status, smoking and alcohol use (current and previous), medications, use of vitamins/minerals, herbals and oral nutritional supplement (Appendix E).  
• Additional demographic information including cause of kidney failure, dialysis vintage and other co-morbidities collected from participant’s medical chart (Appendix E).  
• Blood samples obtained by a nurse or laboratory technician. |
4.1.2 Blood Sample Collection and Analysis

**Intensive/frequent dialysis:** Fasting blood samples were collected from subjects receiving intensive (HHD) and frequent (PD) dialysis between Monday to Friday in the morning during their scheduled appointment with researchers. Appointments were coordinated with subjects’ regular clinic visits as much as possible to eliminate the need for additional travel.

**Conventional dialysis:** Fasting blood samples were collected from IHD subjects mid-week in the morning (Wednesday or Thursday) through their venous access prior to the commencement of dialysis treatment. This ensured all IHD subjects had not dialyzed for one day prior to the collection of blood samples. Participants who dialyzed on an afternoon or evening hemodialysis shift were switched to the morning session to control for diurnal variations in bloodwork.

**Blood biomarkers:** Participants were asked to refrain from eating at least eight hours before their bloodwork was drawn. Fasting blood samples were collected in six evacuated tubes for the following assays: serum folate, plasma vitamin B₆, serum vitamin B₁₂, plasma tHcy, complete blood count (CBC), CRP, serum albumin, urea and creatinine. Blood samples collected in four of the evacuated tubes were immediately brought to the Laboratory Medicine Program (LMP) Core Lab at TGH for analysis and in accordance with standardized LMP protocols.

Blood samples collected in two of the evacuated tubes containing ethylenediamine tetraacetic acid (EDTA) were then transported to the Kim laboratory in the Keenan Research Centre for Biological Sciences of St. Michaels Hospital, where plasma was isolated from whole blood within two hours of collection and both plasma and whole blood were stored at -80°C until further analyses for serum folate concentrations.
4.1.2.1 Serum Folate and Plasma Vitamin B₆

The blood samples for serum folate and plasma vitamin B₆ were kept on ice and protected from light until processing. For whole blood folate, 900 µL of sterile-filtered 0.5% ascorbic acid solution was mixed with 100 µL whole blood in a 1.5 mL labeled cryovial and gently vortexed to lyse the RBCs and release the cell folates into the mixture. The vortexed vial was then incubated at 37°C for thirty minutes. For serum folate and plasma vitamin B₆, the remaining test tubes of whole blood were centrifuged at 1500xg at 4°C for twenty minutes. Plasma was then separated and aliquoted into two labeled cryovials: 475 µL plasma into 25 µL of 10% ascorbic acid solution (plasma folate) and 1.5 mL plasma (vitamin B₆). All three cryovials (whole blood folate, serum folate and plasma vitamin B₆) were quickly frozen and stored at –80°C until analysis (392).

**Serum folate** samples were analyzed at the Kim laboratory in the Keenan Research Centre for Biological Sciences at St. Michael’s Hospital in Toronto, Ontario, Canada. Serum folate samples were determined using the microbiologic assay (393,394). Diluted serum or whole blood hemolysate was added to an assay medium containing *Lactobacillus rhamnosus* (*L. rhamnosus*) (ATCC® 7469™) and all of the nutrients necessary for the growth of *L. rhamnosus* except folate. The inoculated medium was incubated for 45 hours at 37°C. The growth of *L. rhamnosus* is proportional to the amount of total folate present in serum or whole blood samples; therefore, total folate level was assessed by measuring the turbidity of the inoculated medium at 590 nm in a microplate reader. 5-methyltetrahydrofolic acid (5MeTHF) was used to generate the standard cure (393,394), with an inter-assay coefficient of variation of 5%. Folate deficiency was defined as a serum folate <7.0 nmol/L and ≥45nmol/L was considered elevated (77,395).

**Plasma vitamin B₆** (pyridoxal 5'-phosphate [PLP]) samples were analyzed at the In-Common Laboratory in the London Health Sciences Center in London, Ontario, Canada. Cold 1.2 mmol/L perchloric acid was added to aliquots of standards, controls and plasma samples (EDTA) and vigorously mixed using a vortex. Tubes were placed in the refrigerator (4°C) for twenty minutes to facilitate the precipitation process and then centrifuged to remove precipitated material. The
supernatants were transferred to vials for HPLC analysis. A Hewlett-Packard HPLC Model 1100 equipped with a fluorescence detector was set at an excitation wavelength of 300nm. An emission wavelength of 400 nm was equipped with a Thermo-Fisher Aquasil C18, 15cm x 4.6mm, 5-micron particle-size column and fitted with an Aquasil C18 guard cartridge. The mobile phase was a pH 3 potassium dihydrogen phosphate buffer containing sodium perchlorate and sodium bisulfite, which was pumped through the column at 30°C. Concentrations were calculated from peak heights by external standardization and quadratic curve fitting with 1/x weighting (396,397). The inter-assay coefficient of variation was 3.8%. Vitamin B₆ deficiency was defined as PLP <20 nmol/L (19,398).

4.1.2.2 Serum Vitamin B₁₂ and Plasma Total Homocysteine

Serum Vitamin B₁₂

Serum vitamin B₁₂ samples were determined by competitive immunoassay with intrinsic factor and labeled-B₁₂ conjugate using standardized methods in the LMP at TGH (Chemiluminescent Microparticle Immunoassay). The inter-assay coefficient of variation was 7.1%. Reference ranges for vitamin B₁₂ are categorized as follows: deficient (<150 pmol/L) (62); marginal or subclinical deficiency (150-258 pmol/L) and normal (>258 pmol/L) (399,400). As there is no set cut-off for adequacy, a value above deficiency is typically used (198).

Plasma Total Homocysteine

Plasma tHcy is an inverse indicator of folate status (401) and was analyzed in the LMP at TGH. Samples were immediately placed on ice after collection and centrifuged within twenty minutes. The Immulite 2000® analyzer (Diagnostic Products Corporation) was used to determine plasma tHcy concentrations by competitive immunoassay. Hcy was first reduced and then enzymatically converted to s-adenosylhomocysteine according to TGH protocol. Coefficient of variation (CV) of this assay has been found to be 7.1% (402) to 9.3% (403). Hhcy was defined as a plasma tHcy >13 μmol/L (65).
4.1.2.3 CBC, C-Reactive Protein, Serum Albumin, Urea and Creatinine

Serum albumin levels are commonly used to aid in the assessment of nutritional status in those with end-stage renal disease on dialysis therapy. The clinical utility of serum albumin is limited, however, as it can be influenced by non-nutritional factors such as presence of inflammation; thereby making it an insensitive marker of nutritional status. To determine presence of inflammation, concentrations of CRP were assessed and a CRP of >10 mg/L was indicative of a marked inflammatory status (404). Analyses of CBC, CRP, serum albumin, urea and creatinine were performed using standardized methods in the LMP at TGH.

Table 4.1. Biomarker measurements

<table>
<thead>
<tr>
<th>Blood Sample Type</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate</td>
<td>Microbiologic assay</td>
</tr>
<tr>
<td>RBC folate</td>
<td>Microbiologic assay</td>
</tr>
<tr>
<td>Plasma B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>HPLC and fluorescence detector (Hewlett-Packard HPLC Model 1100)</td>
</tr>
<tr>
<td>Serum B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Chemiluminescent microparticle immunoassay (Abbott Architect i2000 Analyzer)</td>
</tr>
<tr>
<td>Plasma tHcy</td>
<td>Competitive immunoassay (Immulite 2000 Analyzer)</td>
</tr>
</tbody>
</table>

4.1.3 Nutrient Intake Data

Estimated intake of total folate, folic acid, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> from food and dietary supplements were evaluated using three-day weighed food records. Subjects were instructed on weighing and recording food items with specific attention towards food type, brand identification and method of preparation. Proper use of Ozeri Pronto Digital Multifunction food scales was also reviewed in length. Subjects on IHD were instructed to collect intake data from one dialysis day and two non-dialysis days. The two non-dialysis days consisted of one weekday and one
weekend day. Subjects receiving intensive (HHD) or frequent (PD) dialysis were instructed to collect intake data from two weekdays and one weekend day to ensure food records adequately reflect variation in meal patterns. Nutrient intake was analyzed using the Food Processor Nutrition Analysis Software by ESHA Research 2018, Version 11.6.522, and is based on the 2015 Canadian Nutrient File.

4.1.4 Assessment of Global Nutritional Status

Evaluation of patients’ global nutritional status was performed using anthropometrics and subjective global assessment (SGA). Anthropometric measurements consisted of height, weight and body mass index (BMI). Height was collected from subjects’ charts or through use of a stadiometer, and a calibrated standing scale was used to measure weight. Subjects receiving conventional hemodialysis treatment (IHD) were weighed after their dialysis session to ensure an accurate reflection of dry weight. Subjects receiving intensive (HHD) or frequent (PD) dialysis were weighed in the morning at the beginning of their scheduled appointment with the study dietitian. Subjects who are on PD were asked to verify the amount of fluid (litres) being held in their peritoneal cavity, and this quantity was subtracted to reflect subjects’ dry weight. BMI values were calculated using measured weight (kilograms) divided by height (centimetres$^2$). A 7-point modification of the 3-point SGA scale designed by Baker and Dexter (254) was used. This scale has been validated in the dialysis population and correlates well with objective nutritional parameters including BMI and biochemical measures (239). This modified version is also able to identify smaller differences in nutritional status (239).

4.1.5 Statistical Analysis

Descriptive statistics were reported as mean ±standard deviation (SD) and median interquartile range (IQR) for normally distributed and transformed variables, respectively. Categorical variables were reported as n (%). To compare the characteristics of patients receiving each of the dialysis modalities, one-way ANOVA tests were used for continuous variables found to be
symmetrical and Kruskal-Wallis (non-parametric) tests were used for asymmetrical variables. Chi square and Fisher’s exact tests were used for categorical variables to determine differences among the three dialysis groups. When an ANOVA or Kruskal-Wallis found significant differences among the dialysis groups, post-hoc analyses were run using Tukey’s Honest Significance Difference or Mann-Whitney U tests to identify where the significance was between the three groups. Correlations between intake and biomarker parameters were assessed using Spearman’s rank correlation coefficients.

The primary research objectives were to ascertain folate status in the dialysis population, as well as investigate whether dialysis modality and nutritional status affected blood concentrations of folate, vitamin B₆ and vitamin B₁₂. The latter was addressed using ordinary least squares regression analysis to identify the effects of nutritional status and dialysis modality on these three concentrations, after demographic and clinical variables were controlled for. Cook’s Distance test was used in regression analysis to identify influential outliers. An interaction between intake and dialysis modality was included in each regression model to test for a possible interaction. A significant interaction would mean the effect of dietary intake on blood concentrations differed by modality.

Because plasma vitamin B₆ and serum vitamin B₁₂ distributions were highly skewed, they were log-transformed to meet the assumptions of normality prior to the regression analysis. For the same reason, total intake of both vitamin B₆ and vitamin B₁₂ were also log-transformed. For bivariate regression analysis, intake of the relevant nutrient (total folate [DFE], vitamin B₆ or vitamin B₁₂) was included as an independent variable. This was followed by multiple regression analysis where other prognostic variables were considered including age, gender, smoking, ethnicity, education level, diabetes, dialysis vintage, tHcy, BMI and total energy intake. Backward selection was used to identify significant predictors of serum levels of each of the three nutrients; covariates were retained if they were significant at the p<0.05 level.
All tests were two-tailed and an alpha of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics, Version 25.0 software (IBM Corp., Armonk, NY; USA). All available data were included in the analyses and sample size for each analysis was reported.

4.2 Results

4.2.1 Subject Characteristics

Subject characteristics are listed in Table 4.2. Mean (±SD) age of the study subjects was 52.4 ±14.5 years. The HHD cohort was significantly younger than the other two dialysis groups (p=0.008). Mean BMI and mean weight of the HHD group were lower than those of the IHD and PD groups, but the differences did not reach statistical significance. Nearly two-thirds of all study participants were male (62.9%); a proportion which was observed uniformly across all three groups. Over half of the participants were Caucasian (51.4%). A large percentage of the study subjects (61.5%) had completed some form of post-secondary education, including a college or vocational diploma (22.9%), university degree, or higher (38.6%). Significant differences in employment status were observed among the three groups (p=0.021). The majority of the study subjects described their employment status as either being part-time (21.4%) or on disability (38.6%), while only a small number of the participants were working full-time (15.7%). Only 11.4% of the study subjects were current smokers. Alcohol consumption was noted in 42.9% of total subjects; however, only a few (5.7%) reported consuming alcohol on a regular basis.
Table 4.2 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=70)</th>
<th>IHD (n=26)</th>
<th>PD (n=23)</th>
<th>HHD (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs) [mean (SD)]</td>
<td>52.4 (14.5)</td>
<td>57.4 (13.8)(^a)</td>
<td>53.9 (15.4)(^{a,b})</td>
<td>44.7 (11.4)(^b)</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>Weight (kg) [mean (SD)]</td>
<td>76.9 (20.4)</td>
<td>79.0 (24.0)</td>
<td>79.2 (20.4)</td>
<td>71.7 (14.8)</td>
<td>0.39</td>
</tr>
<tr>
<td>BMI (kg/m(^2)) [mean (SD)]</td>
<td>26.8 (6.9)</td>
<td>27.5 (8.9)</td>
<td>27.5 (6.3)</td>
<td>25.0 (4.7)</td>
<td>0.40</td>
</tr>
<tr>
<td>Gender (M) [n (%)]</td>
<td>44 (62.9)</td>
<td>18 (69.2)</td>
<td>13 (56.5)</td>
<td>13 (61.9)</td>
<td>0.64</td>
</tr>
<tr>
<td>Ethnicity [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.49</td>
</tr>
<tr>
<td>African/Black</td>
<td>11 (15.7)</td>
<td>6 (23.1)</td>
<td>4 (17.4)</td>
<td>1 (4.8)</td>
<td>--</td>
</tr>
<tr>
<td>Asian/Oriental</td>
<td>8 (11.4)</td>
<td>2 (7.7)</td>
<td>2 (8.7)</td>
<td>4 (19.0)</td>
<td>--</td>
</tr>
<tr>
<td>Caucasian</td>
<td>36 (51.4)</td>
<td>13 (50.0)</td>
<td>13 (56.5)</td>
<td>10 (47.6)</td>
<td>--</td>
</tr>
<tr>
<td>Indian/Subcontinent</td>
<td>14 (20.0)</td>
<td>5 (19.2)</td>
<td>3 (13.0)</td>
<td>6 (28.6)</td>
<td>--</td>
</tr>
<tr>
<td>Mid-East Arabian</td>
<td>1 (1.4)</td>
<td>0</td>
<td>1 (4.3)</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Educational Attainment [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>None</td>
<td>2 (2.9)</td>
<td>1 (3.8)</td>
<td>1 (4.3)</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>High school diploma</td>
<td>25 (35.7)</td>
<td>11 (42.3)</td>
<td>8 (34.7)</td>
<td>6 (28.6)</td>
<td>--</td>
</tr>
<tr>
<td>College/trade</td>
<td>16 (22.9)</td>
<td>4 (15.4)</td>
<td>5 (21.7)</td>
<td>7 (33.3)</td>
<td>--</td>
</tr>
<tr>
<td>University degree</td>
<td>27 (38.6)</td>
<td>10 (38.5)</td>
<td>9 (39.1)</td>
<td>8 (38.1)</td>
<td>--</td>
</tr>
<tr>
<td>Employment [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>0.021</strong></td>
</tr>
<tr>
<td>Full-time</td>
<td>11 (15.7)</td>
<td>2 (7.7)</td>
<td>5 (21.7)</td>
<td>4 (19.0)</td>
<td>--</td>
</tr>
<tr>
<td>Part-time</td>
<td>15 (21.4)</td>
<td>2 (7.7)</td>
<td>4 (17.4)</td>
<td>9 (42.9)</td>
<td>--</td>
</tr>
<tr>
<td>Disability*</td>
<td>27 (38.6)</td>
<td>15 (57.7)</td>
<td>6 (26.1)</td>
<td>6 (28.6)</td>
<td>--</td>
</tr>
<tr>
<td>Retired</td>
<td>12 (17.1)</td>
<td>4 (15.4)</td>
<td>7 (30.4)</td>
<td>1 (4.8)</td>
<td>--</td>
</tr>
<tr>
<td>Not working†</td>
<td>5 (7.1)</td>
<td>3 (11.5)</td>
<td>1 (4.3)</td>
<td>1 (4.8)</td>
<td>--</td>
</tr>
<tr>
<td>Smoking Status (Y) [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>8 (11.4)</td>
<td>1 (3.8)</td>
<td>2 (8.7)</td>
<td>5 (23.8)</td>
<td>--</td>
</tr>
<tr>
<td>Previous smoker</td>
<td>17 (24.3)</td>
<td>7 (26.9)</td>
<td>7 (30.4)</td>
<td>3 (14.3)</td>
<td>--</td>
</tr>
<tr>
<td>Never smoked</td>
<td>45 (64.3)</td>
<td>18 (69.2)</td>
<td>14 (60.9)</td>
<td>13 (61.9)</td>
<td>--</td>
</tr>
<tr>
<td>Alcohol Status (Y) [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>Current drinker</td>
<td>30 (42.9)</td>
<td>11 (42.3)</td>
<td>10 (43.4)</td>
<td>9 (42.9)</td>
<td>--</td>
</tr>
<tr>
<td>- Regular</td>
<td>4 (5.7)</td>
<td>2 (7.7)</td>
<td>0</td>
<td>2 (9.5)</td>
<td>--</td>
</tr>
<tr>
<td>- Social^</td>
<td>26 (37.1)</td>
<td>9 (34.6)</td>
<td>10 (43.5)</td>
<td>7 (33.3)</td>
<td>--</td>
</tr>
<tr>
<td>Previous drinker</td>
<td>13 (18.6)</td>
<td>7 (26.9)</td>
<td>4 (17.4)</td>
<td>2 (9.5)</td>
<td>--</td>
</tr>
<tr>
<td>Never drank</td>
<td>27 (38.6)</td>
<td>8 (30.8)</td>
<td>9 (39.1)</td>
<td>10 (47.6)</td>
<td>--</td>
</tr>
</tbody>
</table>

Labeled means in a row without a common letter differ
*Disability: includes ODSP; work-related
†Not working: does not receive income; includes study subjects not currently working or have never worked
^Social: consumes less than 1 drink per month

78
4.2.2 Dialysis Characteristics

Dialysis characteristics are listed in Table 4.3. Study participants in the HHD group were observed to have a significantly longer dialysis vintage compared to those in the IHD and PD groups ($p<0.001$). Over one-third (38.6%) of the study participants had glomerulonephritis/autoimmune disease identified as their primary cause of renal failure. Interestingly, diabetes accounted for only 8.6% of the primary causes of ESKD in this study population, compared to 29.7% prevalence of diabetes as a primary diagnosis for ESKD in Canada in 2017 (1). The primary cause of ESKD was not significantly different among the three dialysis groups ($p=0.36$). Presence of each co-morbidity was individually analyzed and there were no significant differences found among the three dialysis groups. The majority of subjects (97.5%) were diagnosed as having hypertension. Only 22.9% of study subjects were diagnosed with diabetes; less than half of what is typically observed in the ESKD population (1). Almost 23% of the study subjects had prior kidney transplant; the PD group had a lower prevalence of prior kidney transplant than the other two dialysis groups, but this did not reach statistical significance ($p=0.08$). Eleven out of seventy subjects (15.7%) had documentation of having at least one previous cancer. Of these, nearly half (46%) had two or more cancers including the skin (5), kidney (3), breast (2), lung (2) and thyroid (2), amongst others. A full list of cancer diagnoses can be found in Table A.1, Appendix A.

Statistically significant differences were noted in certain types of medications prescribed [anemia ($p=0.04$), blood pressure ($p<0.001$), diuretic ($p<0.001$), bowel ($p<0.001$)], and other ($p<0.001$)]. Less than half (42.9%) of the HHD subjects were prescribed anti-hypertensives; much lower than what was seen in the IHD (69.2%) and PD (95.7%) groups. Minimal diuretic use was observed in both the IHD (7.7%) and HHD (4.7%) groups. The HHD group also used less bowel medications (4.8%) than the IHD (46.2) and PD (73.9%) groups. The total number of medications prescribed was significantly different among the three groups ($p<0.001$).
Table 4.3 Dialysis characteristics

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=70)</th>
<th>IHD (n=26)</th>
<th>PD (n=23)</th>
<th>HHD (n=21)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dialysis vintage [mean (SD)]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(mos.)</td>
<td>63.5 (59.0)</td>
<td>58.1 (54.5)</td>
<td>35.0 (27.0)</td>
<td>101.3 (70.9)</td>
<td></td>
</tr>
<tr>
<td>ESKD cause (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>GN*/Autoimmune</td>
<td>27 (38.6)</td>
<td>9 (34.6)</td>
<td>6 (26.1)</td>
<td>12 (57.1)</td>
<td>--</td>
</tr>
<tr>
<td>Nephropathy, DI*</td>
<td>5 (7.1)</td>
<td>1 (3.8)</td>
<td>3 (13.0)</td>
<td>1 (4.8)</td>
<td>--</td>
</tr>
<tr>
<td>Polycystic kidney</td>
<td>7 (10.0)</td>
<td>3 (11.5)</td>
<td>2 (8.7)</td>
<td>2 (9.5)</td>
<td>--</td>
</tr>
<tr>
<td>Congenital/Hereditary</td>
<td>9 (12.9)</td>
<td>3 (11.5)</td>
<td>2 (8.7)</td>
<td>4 (19.0)</td>
<td>--</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6 (8.6)</td>
<td>3 (11.5)</td>
<td>3 (13.0)</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Renal vascular disease</td>
<td>10 (14.3)</td>
<td>3 (11.5)</td>
<td>5 (21.7)</td>
<td>2 (9.5)</td>
<td>--</td>
</tr>
<tr>
<td>Other†, Unknown</td>
<td>6 (8.6)</td>
<td>4 (15.4)</td>
<td>2 (8.7)</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Comorbidities (Y) [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>16 (22.9)</td>
<td>7 (26.9)</td>
<td>6 (26.0)</td>
<td>3 (14.2)</td>
<td>0.53</td>
</tr>
<tr>
<td>HTN</td>
<td>67 (95.7)</td>
<td>25 (96.1)</td>
<td>23 (100)</td>
<td>19 (90.5)</td>
<td>0.29</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>49 (70.0)</td>
<td>21 (80.1)</td>
<td>16 (69.6)</td>
<td>12 (57.1)</td>
<td>0.21</td>
</tr>
<tr>
<td>CVD</td>
<td>20 (28.6)</td>
<td>11 (42.3)</td>
<td>5 (21.7)</td>
<td>4 (19.0)</td>
<td>0.15</td>
</tr>
<tr>
<td>PVD</td>
<td>5 (7.1)</td>
<td>2 (8.0)</td>
<td>3 (13.0)</td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>Previous cancer</td>
<td>11 (15.7)</td>
<td>2 (7.7)</td>
<td>4 (17.4)</td>
<td>5 (23.8)</td>
<td>0.31</td>
</tr>
<tr>
<td>Previous transplant</td>
<td>16 (22.9)</td>
<td>7 (26.9)</td>
<td>4 (17.4)</td>
<td>5 (23.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Medications (Y) [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>67 (95.7)</td>
<td>26 (100)</td>
<td>20 (70.0)</td>
<td>21 (100)</td>
<td>0.04</td>
</tr>
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<td>Anxiety</td>
<td>9 (12.9)</td>
<td>5 (19.2)</td>
<td>3 (13.0)</td>
<td>1 (4.8)</td>
<td>0.34</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>49 (70.0)</td>
<td>18 (69.2)</td>
<td>22 (95.7)</td>
<td>9 (42.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diuretic</td>
<td>18 (25.7)</td>
<td>2 (7.7)</td>
<td>15 (65.2)</td>
<td>1 (4.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone^</td>
<td>61 (87.1)</td>
<td>23 (88.5)</td>
<td>21 (91.3)</td>
<td>17 (81.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>Bowel</td>
<td>30 (42.9)</td>
<td>12 (46.2)</td>
<td>17 (73.9)</td>
<td>1 (4.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12 (17.1)</td>
<td>6 (23.1)</td>
<td>4 (17.4)</td>
<td>2 (9.5)</td>
<td>0.47</td>
</tr>
<tr>
<td>GERD</td>
<td>31 (44.3)</td>
<td>12 (46.2)</td>
<td>10 (43.4)</td>
<td>9 (42.9)</td>
<td>0.97</td>
</tr>
<tr>
<td>Immunosuppressive</td>
<td>12 (17.1)</td>
<td>4 (15.4)</td>
<td>4 (17.4)</td>
<td>4 (19.0)</td>
<td>0.95</td>
</tr>
<tr>
<td>Lipid</td>
<td>29 (41.4)</td>
<td>11 (42.3)</td>
<td>11 (47.8)</td>
<td>7 (33.3)</td>
<td>0.62</td>
</tr>
<tr>
<td>Other</td>
<td>52 (74.3)</td>
<td>24 (92.3)</td>
<td>18 (78.3)</td>
<td>10 (47.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Number of Medications Prescribed (n) [mean (SD)]

|                                | 8.7 (4.0) | 9.6 (3.7) | 10.5 (3.8) | 5.8 (2.9) | <0.001 |

Labeled means in a row without a common letter differ
*GN: glomerulonephritis; DI: drug induced
†Other: sepsis; amyloid
^Bone: bone mineral metabolism
Figure 4.1 illustrates the total number of prescribed medications, highlighting the level of pill burden within each of the three groups. The majority of study subjects (74.3%) were prescribed between 5-9 (45.7%) or 10-14 (28.6%) medications, respectively, but the proportion significantly differed among the three groups \((p=0.039)\).

Figure 4.1 Pill burden; stratified by dialysis modality
4.2.3 Dietary and Supplemental Intakes of Folate and Folic Acid, Vitamin B₆ and Vitamin B₁₂

**Total Dietary Intakes: 3-day Food Record**

Mean dietary intakes of calories (kcal), protein (g), folate (natural folate and folic acid fortified in foods [μg DFE]), vitamin B₆ (mg) and vitamin B₁₂ (μg) are summarized in Table 4.4. Overall, dietary intakes did not differ among the three groups for any of the macro- or micronutrients, although there appeared to be a trend toward mean calorie and protein consumption being higher in the HHD group than the other two dialysis groups (p=0.073). Mean folate (μg DFE) intake of subjects in each group was below the EAR (EAR; meets 50% of the populations’ needs), while mean vitamin B₁₂ (μg) intakes were above the EAR. Vitamin B₆ requirements for adults vary between age and gender. Total mean vitamin B₆ (mg) intake was near, at or above the EAR for all three dialysis modalities.

### Table 4.4 Total dietary intakes: 3-day food record

<table>
<thead>
<tr>
<th>Dietary Intakes</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD* [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
<th>EAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>1840 (632)</td>
<td>1840 (677)</td>
<td>1633 (533)</td>
<td>2067 (627)</td>
<td>0.073</td>
<td>-</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>76.6 (26.1)</td>
<td>72.3 (28.7)</td>
<td>72.2 (24.1)</td>
<td>86.6 (23.2)</td>
<td>0.11</td>
<td>-</td>
</tr>
<tr>
<td>Folate (μg DFE*)</td>
<td>286.2 (153.0)</td>
<td>301.5 (170.7)</td>
<td>290.6 (181.5)</td>
<td>263.0 (86.9)</td>
<td>0.69</td>
<td>320</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.3 (0.6)</td>
<td>1.2 (0.7)</td>
<td>1.3 (0.5)</td>
<td>1.3 (0.5)</td>
<td>0.85</td>
<td>1.1-1.4^</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>2.9 (1.9)</td>
<td>2.6 (1.5)</td>
<td>3.1 (2.3)</td>
<td>2.9 (2.0)</td>
<td>0.66</td>
<td>2.0</td>
</tr>
</tbody>
</table>

---

* IHD sample size 25 due to missing food record (1)
* DFE: dietary folate equivalent
^ Adults 19-50yrs: 1.1mg; women 51+ yrs: 1.3mg; men 51+ yrs: 1.4mg
Table 4.5 illustrates the mean dietary intakes of folate (μg DFE), vitamin B₆ and vitamin B₁₂ per day in comparison to the EAR and current renal recommendations. The prevalence of inadequacy (intakes less than the EAR) in all subjects for folate and vitamin B₁₂ was 67% and 46%, respectively. For vitamin B₆, subjects were divided by gender and age to align with EAR cut-off points. The prevalence of inadequacy for all males and females (IHD, PD, HHD) ages 19-50 years was 42.4%. For males and females 51+ years (IHD, PD, HHD), the prevalence of inadequacy was 42.8% and 66.7%, correspondingly. Mean dietary intakes of folate (DFE), vitamin B₆ and vitamin B₁₂ were all below current renal recommended daily targets.

<table>
<thead>
<tr>
<th>Dietary Intakes</th>
<th>All Subjects[^a] [Mean (SD)]</th>
<th>EAR</th>
<th>Prevalence of Inadequate Intakes (%)</th>
<th>Renal Recommendations[^b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (μg DFE)</td>
<td>286.2 (153.0)</td>
<td>320</td>
<td>67</td>
<td>1000[^c] – 2000[^d]</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td></td>
<td></td>
<td></td>
<td>10[^c] – 20[^d]</td>
</tr>
<tr>
<td>- (19-50yrs)</td>
<td>1.3 (0.50)</td>
<td>1.1</td>
<td>42.4</td>
<td>–</td>
</tr>
<tr>
<td>- Males (51+yrs)</td>
<td>1.5 (0.56)</td>
<td>1.4</td>
<td>42.8</td>
<td>–</td>
</tr>
<tr>
<td>- Females (51+yrs)</td>
<td>0.97 (0.45)</td>
<td>1.3</td>
<td>66.7</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>2.9 (1.9)</td>
<td>2.0</td>
<td>46</td>
<td>2.4[^c] – 4.8[^d]</td>
</tr>
</tbody>
</table>

[^a]: IHD sample size 69 due to missing food record (1); DFE: dietary folate equivalent; EAR: estimated average requirement
[^b]: listed as mcg folic acid; equal to 1667-3333μg DFE using conversion factor (1 μg DFE = 0.6 mcg folic acid)
[^c]: Recommended daily amount for individuals receiving IHD or PD
[^d]: Recommended daily amount for individuals receiving HHD

**Total Supplemental Intakes of Folic Acid, Vitamin B₆ and Vitamin B₁₂**

Overall, 88%, 96% and 100% of the IHD, PD and HHD subjects reported taking Replavite on a daily basis, respectively (Figure 4.2). A small number of the study subjects (12.9%) reported taking additional supplementation of folic acid (μg), vitamin B₆ (mg), and/or vitamin B₁₂ (μg); either in a high-dose individual or multi-vitamin form. Seventeen percent of the study subjects reported using oral supplements to optimize their macro- and micronutrition. The median [interquartile range (IQR)] supplemental intakes of folic acid (μg), vitamin B₆ (mg) and vitamin B₁₂ (μg) consumed from Replavite (renal multi-vitamin), individual vitamin supplements and/or oral nutrition supplements was 1000 (1000,2000), 10 (10,20), and 6 (6,12), respectively for all
subjects (Table 4.6). A statistically significant difference was observed among the three groups for all three vitamins (p<0.001), with the HHD group being significantly different than the IHD and PD groups.

Table 4.6 Total supplemental intakes: Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements

<table>
<thead>
<tr>
<th>Supplemental Intakes</th>
<th>All Subjects [Median (IQR)]</th>
<th>IHD [Median (IQR)]</th>
<th>PD [Median (IQR)]</th>
<th>HHD [Median (IQR)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid (μg)</td>
<td>1000 (1000,2000)</td>
<td>1000 (1000,1016)</td>
<td>1000 (1000,1000)</td>
<td>2000 (2000,2000)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>10 (10,20)</td>
<td>10 (10,10.1)</td>
<td>10 (10,10)</td>
<td>20 (20,20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>6 (6,12)</td>
<td>6 (6,6.6)</td>
<td>6 (6,6.1)</td>
<td>12 (12,12)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Labeled means in a row without a common letter differ

Figure 4.2 Percentage of subjects using Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements
Total Dietary and Supplemental Intakes

Mean dietary and supplemental intakes of calories (kcal), protein (g), folate (μg DFE), vitamin B₆ (mg) and vitamin B₁₂ (μg) are summarized in Table 4.7. Results did not differ between groups for either calories or protein consumption, despite the addition of oral nutrition supplements. There appeared to be a trend toward total mean caloric and protein intake being higher in the HHD group than in the other two groups, but this was not significant (kcal [p=0.77]; protein [p=0.16]). Total mean intake of folate (natural folate in diets + folic acid in fortified food and nutrition supplements + supplemental folic acid) (μg DFE) was high; nearly 6.5x higher than the EAR. Total (dietary + supplemental) mean intakes of vitamin B₆ (mg) and vitamin B₁₂ (μg) were more than 8- and 4x higher than the EAR for each vitamin, respectively. Statistically significant differences were observed among the three groups for folate (p<0.001), vitamin B₆ (p<0.001) and vitamin B₁₂ (p<0.001), with the HHD group being significantly different than the IHD and PD groups.

Table 4.7 Total dietary and supplemental intakes: 3-day food records, Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements

<table>
<thead>
<tr>
<th>Total Intakes</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD* [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories (kcal)</td>
<td>1837 (599)</td>
<td>1803 (598)</td>
<td>1665 (530)</td>
<td>2067 (627)</td>
<td>0.77</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>77.8 (25.7)</td>
<td>74.5 (28.2)</td>
<td>73.3 (24.1)</td>
<td>86.6 (23.3)</td>
<td>0.16</td>
</tr>
<tr>
<td>Folate† (μg DFE)*</td>
<td>2050 (1899,3312)</td>
<td>1944 (1856,2080)</td>
<td>1951 (1794,2125)</td>
<td>3543 (3433,3629)b</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Vitamin B₆† (mg)</td>
<td>11.7 (11.0, 20.9)</td>
<td>11.2 (10.9,12.0)</td>
<td>11.5 (10.8,11.9)</td>
<td>21.2 (20.8,21.7)b</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Vitamin B₁₂† (μg)</td>
<td>9.8 (7.8,12.9)</td>
<td>8.4 (7.6,9.7)</td>
<td>8.9 (7.7,11.6)</td>
<td>14.1 (13.1,15.9)b</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Labeled means in a row without a common letter differ
*IHD sample size 25 due to missing food record (1)
†Supplemental folic acid converted to DFE (DFE: dietary folate equivalent)
‡Median (IQR) used
4.2.4 Blood Biomarkers of Folate, tHcy, Vitamin B₆ and Vitamin B₁₂

Mean concentrations of blood biomarkers of folate, Hcy, vitamin B₆ and vitamin B₁₂ are summarized in Table 4.8. Mean serum folate concentrations were 62.5 (±43.1) nmol/L in the study subjects and well above the suggested cut-off value (>45.3 nmol/L) for elevated serum folate concentrations (77). Over 61% of subjects were in the range generally considered to be high and only 5.7% were classified as deficient. A statistically significant difference was observed among the three groups (p=0.003) for serum folate concentrations; post-hoc analysis demonstrates that the HHD group had significantly higher serum folate concentrations compared with the HHD and PD groups. Mean plasma tHcy concentrations (21.8 ±9.2), an inverse indicator of folate, were in the hyperhomocysteinemic range (>13 μmol/L) but were not statistically different among the three groups (p=0.32). Despite folic acid supplementation in 94.3% of total subjects, prevalence of Hhcy was 87.1%. Median plasma vitamin B₆ concentrations [121 (IQR: 66,179)] were considerably elevated and were not statistically different among the three groups (p=0.67), with clinical deficiency (PLP <20 nmol/L) being observed in only five subjects (7.1%). Median serum vitamin B₁₂ concentrations (532 [IQR: 355,720]) were within the normal range. Nearly two-thirds (65.2%) of the study subjects had serum vitamin B₁₂ concentrations within the normal range (222-652 pmol/L), while one-third (33.3%) were above the normal range. Only 1.5% of the study subjects were classified as being marginally deficient (150-258 pmol/L) and no subjects had a vitamin B₁₂ deficiency (<150 pmol/L). No significant differences in serum vitamin B₁₂ concentrations were observed among the three groups.
Table 4.8 Serum and plasma biomarkers: folate, tHcy, vitamin B₆, and vitamin B₁₂; stratified by dialysis modality

<table>
<thead>
<tr>
<th>Serum/Plasma</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate (nmol/L)</td>
<td>62.5 (43.1)</td>
<td>50.5 (34.0) a</td>
<td>52.4 (28.9) a</td>
<td>88.4 (55.3) b</td>
<td>0.003</td>
</tr>
<tr>
<td>Plasma tHcy (µmol/L)</td>
<td>21.8 (9.2)</td>
<td>22.7 (8.1)</td>
<td>24.7 (10.8)</td>
<td>17.7 (7.1)</td>
<td>0.32</td>
</tr>
<tr>
<td>Plasma B₆† (nmol/L)</td>
<td>121 (66,179)</td>
<td>105 (64,167)</td>
<td>128 (72,190)</td>
<td>112 (43,167)</td>
<td>0.67</td>
</tr>
<tr>
<td>Serum B₁₂† (pmol/L)</td>
<td>532 (355,720)</td>
<td>543 (334,829)</td>
<td>526 (394,751)</td>
<td>532 (367,676)</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Labeled means in a row without a common letter differ
†Median (IQR) used
4.2.5 Ancillary Nutrition and Inflammatory Biomarkers: Creatinine, Urea, Albumin and CRP

Mean serum levels of creatinine, urea, albumin and CRP are summarized in Table 4.9. Statistically significant differences among the three groups were observed for both creatinine (p<0.001) and urea (p<0.001). Post-hoc analysis reported the HHD group as having significantly lower serum urea and creatinine concentrations compared with the IHD and PD groups. The IHD group also reported a significantly lower serum urea concentration compared with the PD group. Mean serum albumin concentrations (38 g/L) were observed to be within recommended targets for the dialysis population (35-50 g/L), with no statistical significance observed among the three groups (p=0.57). Mean serum CRP levels (5 [IQR: 2,11] mg/dL) were within the upper normal range cut-off (<10 mg/dL), and were not significantly different among the three groups (p=0.31).

Table 4.9 Ancillary nutrition biomarkers: creatinine, urea, albumin, CRP*; stratified by dialysis modality

<table>
<thead>
<tr>
<th>Serum/Plasma</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine (mmol/L)</td>
<td>774 (301.5)</td>
<td>827 (235)</td>
<td>890 (363)</td>
<td>580 (202)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum urea (mmol/L)</td>
<td>18.0 (6.8)</td>
<td>19.1 (5.0)</td>
<td>23.0 (5.8)</td>
<td>11.1 (3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>38 (3.7)</td>
<td>38 (3.2)</td>
<td>36 (4.2)</td>
<td>39 (3.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>CRP (mg/dL)†</td>
<td>5 (2, 11)</td>
<td>5 (3,16)</td>
<td>5 (1,10)</td>
<td>3 (2,9)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Inflammatory marker
Labeled means in a row without a common letter differ
†Median (IQR) used
4.2.6 Global Nutritional Status and Dialysis Modality

Almost 73% of the study subjects fell within the mild risk to well-nourished category and only one individual was considered severely malnourished (3.8%). A statistically significant difference was observed ($p<0.022$) when nutritional status (SGA) was compared across the three dialysis modality groups (Table 4.10). Mean BMI was not significantly different among the three groups ($p=0.40$), and no differences were seen when BMI was further stratified into six classes according to renal guidelines ($p=0.90$). A detailed breakdown of BMI ranges in accordance with renal guidelines can be found in Table A.8, Appendix A.

<table>
<thead>
<tr>
<th>Nutritional Status</th>
<th>IHD [n (%)]</th>
<th>PD [n (%)]</th>
<th>HHD [n (%)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGA</td>
<td></td>
<td></td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>Mild risk to well-nourished</td>
<td>16 (61.5)</td>
<td>15 (65.2)</td>
<td>20 (95.2)</td>
<td></td>
</tr>
<tr>
<td>Mild to moderate malnutrition</td>
<td>9 (34.6)</td>
<td>8 (34.8)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>Severely malnourished</td>
<td>1 (3.8)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m$^2$) [mean (SD)]</td>
<td>27.5 (8.9)</td>
<td>27.5 (6.3)</td>
<td>25.0 (4.7)</td>
<td>0.40</td>
</tr>
<tr>
<td>BMI Class*</td>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>Underweight</td>
<td>4 (15.4)</td>
<td>2 (8.7)</td>
<td>2 (9.5)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>11 (42.3)</td>
<td>9 (39.1)</td>
<td>12 (57.1)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>5 (19.2)</td>
<td>5 (21.7)</td>
<td>3 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Obese 1,2,3</td>
<td>6 (23.0)</td>
<td>7 (30.4)</td>
<td>4 (19.0)</td>
<td></td>
</tr>
</tbody>
</table>

*BMI classes stratified according to renal guidelines
4.2.7 Regression Analyses: Relationships between Serum Folate, Plasma Vitamin B₆, Serum Vitamin B₁₂ and Nutritional Status and Dialysis Modality in ESKD

In unadjusted bi-variable linear regression models, total mean folate (μg DFE) (dietary folate + folic acid from fortified food and nutrition supplements + supplemental folic acid) intake and dialysis modality each separately predicted serum folate concentrations; however, after adjusting for covariates, only total mean folate (μg DFE) intake retained its significance and was the lone predictor of serum folate concentrations (p<0.0001). Nutritional status was not a predictor of serum folate levels (p=0.89) (Table 4.11.a).

Table 4.11.a Bivariate regression analysis: relationship between concentrations of serum folate and independent variables in ESKD

<table>
<thead>
<tr>
<th>Predictors of serum folate</th>
<th>Parameter estimate</th>
<th>95% Confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total folate (DFE) intake</td>
<td>0.025</td>
<td>0.016 – 0.035</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

R-squared = 0.29
Nutritional status: ns; p-value = 0.89
Dialysis modality: ns; p-value = 0.85
In unadjusted linear models, total vitamin B₆ intake and dialysis modality, ethnicity, and dialysis vintage predicted plasma vitamin B₆ (PLP) concentrations. After adjusting for covariates, total vitamin B₆ intake \((p=0.001)\), ethnicity \((p=0.022)\) and dialysis vintage \((p=0.006)\) remained significant. Nutritional status (SGA) was not a predictor of serum vitamin B₆ concentrations \((p=0.22)\). (Table 4.11.b)

<table>
<thead>
<tr>
<th>Predictors of log(plasma B₆)</th>
<th>Parameter estimate</th>
<th>95% Confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total vitamin B₆ intake</td>
<td>0.487</td>
<td>0.206 – 0.769</td>
<td>0.001</td>
</tr>
<tr>
<td>Months on dialysis</td>
<td>-0.004</td>
<td>-0.008 – 0.001</td>
<td>0.006</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African/Black</td>
<td>1.514</td>
<td>-0.027 – 3.055</td>
<td>0.054</td>
</tr>
<tr>
<td>Asian/Oriental</td>
<td>1.875</td>
<td>0.318 – 3.433</td>
<td>0.019</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2.083</td>
<td>0.594 – 3.571</td>
<td>0.007</td>
</tr>
<tr>
<td>Indian</td>
<td>1.728</td>
<td>0.205 – 3.251</td>
<td>0.027</td>
</tr>
<tr>
<td>Other (reference)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-squared = 0.315
Nutritional status was not significant: p-value = 0.22
Dialysis modality was not significant: p-value = 0.35
In unadjusted linear models, only nutritional status (SGA) \((p=0.006)\) and education \((p=0.018)\) predicted serum vitamin \(B_{12}\) concentrations; both remained significant after adjusting for additional covariates. Neither dialysis modality (ns; \(p=0.82)\) nor total vitamin \(B_{12}\) intake (0.076) was a predictor of serum vitamin \(B_{12}\) concentrations. \((Table 4.11.c)\)

**Table 4.11.c Regression analysis: relationship between concentrations of serum vitamin \(B_{12}\) and independent variables in ESKD**

<table>
<thead>
<tr>
<th>Predictors of log(serum (B_{12}))</th>
<th>Parameter estimate</th>
<th>95% Confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional Status (SGA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild-risk to well-nourished</td>
<td>-1.089</td>
<td>-1.944 – -0.234</td>
<td>0.006</td>
</tr>
<tr>
<td>Mild to moderately malnourished</td>
<td>-0.807</td>
<td>-1.670 – 0.056</td>
<td>0.013</td>
</tr>
<tr>
<td>Moderate to severely malnourished (reference)</td>
<td>0.000</td>
<td>-0.237 – 0.302</td>
<td>0.809</td>
</tr>
<tr>
<td>Education Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>-0.982</td>
<td>-1.596 – -0.368</td>
<td>0.002</td>
</tr>
<tr>
<td>High school diploma</td>
<td>-0.001</td>
<td>-0.238 – 0.235</td>
<td>0.991</td>
</tr>
<tr>
<td>College/trade</td>
<td>0.033</td>
<td>-0.237 – 0.302</td>
<td>0.809</td>
</tr>
<tr>
<td>University (reference)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-squared = 0.253  
Dialysis modality had a p-value of 0.823  
Total \(B_{6}\) intake had a p-value of 0.076
4.3 Discussion

To our knowledge, this is the first study to examine intakes and blood levels of folate, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> in a subgroup of Canadian patients with ESKD treated with three different modalities of dialysis in the post-fortification era.

4.3.1 Current Intake and Blood Folate Levels

A key finding of this study was that a large proportion of our subjects failed to achieve folate adequacy through dietary intake alone even with mandatory folic acid fortification. Prevalence of folate inadequacy (intakes less than the EAR) was 67% in all subjects; when stratified by modality, surprisingly those dialyzing on home therapies had a higher prevalence of inadequacy (PD: 73.9%; HHD: 71.4%) than those doing infrequent dialysis (IHD: 60%). Mean folate intakes were observed to be lower in the home modality groups (PD: 290.6 µg DFE; HHD: 263.0 µg DFE) compared to the IHD group (301.5 µg DFE). This result is unexpected, given that both PD and HHD offer greater flexibility in the diet compared to the IHD group. Liberalization of potassium in particular is commonly observed, and often characterized by a broader intake of potassium-rich fruits and juices (citrus, tropical), vegetables (dark green, orange), and legumes; all of which are abundant in folate and typically avoided when following a low potassium diet. One reason for the lower intakes may be that the dietary patterns of the home modalities are different than the IHD group. Patients who are on a home-based modality dialyze more frequently, and therefore experience a higher protein loss through the dialysate. While the importance of a high protein diet is emphasized by the dietitian in all three modalities, it is even more so in PD and HHD. Although not significant, the HHD group had a higher mean protein intake than the IHD group (HHD 86.6 g [23.2]; IHD 72.3 g [28.7]). It is plausible the higher amount of protein has naturally reduced the fruits and vegetables servings in the dietary pattern of the HHD group. This reasoning is unlikely for the PD group, whose mean protein intake was similar to the IHD group (PD 72.2g [24.1]; IHD 72.3g [28.7]). Analysis of other macronutrients is warranted to determine if variations in dietary patterns exist between the PD and IHD groups.
To date, no other studies have assessed dietary folate intake between the three modalities to which we can provide a comparative review. However, folate inadequacy in the diet has been observed in isolation within a small number of PD and IHD cohorts. In a study from Hong Kong by Wang et al (2002), a 7-day FFQ was employed to assess micronutrient status, including folate, and investigated whether dialysis adequacy (determined by urea clearance; \( \text{Kt/V} \)) and RRF predicted micronutrient intakes in 242 CAPD patients. Folate inadequacy (defined as being below the RDA) was observed in two-thirds of the subjects; however, the FFQ only incorporated food items containing >25% of the RDA for folate, which might have potentially underestimated total folate intake. Lower RRF was found to predict lower folate intakes (344); possibly due to appetite suppression, which is sometimes seen in patients requiring larger fill volumes and more frequent exchanges (236). In Mexico, micronutrient intakes of 73 PD patients were assessed using a 24-hour recall to determine its influence on nutritional (SGA) and inflammatory (CRP) status. No statistical significance was seen between median folate intakes and the aforementioned parameters, a finding which was paralleled in our study. Investigators also observed <15% of all subjects had adequate folate intakes as compared with DRI.s (nourished: median 190 [140,280] \( \mu \text{g} \) and malnourished: median 140 [60,220] \( \mu \text{g} \)) (342). Similar results have been reported in the IHD population; three studies analyzed folate intake using food records of four, five and nine days in length and found mean (±SD) intakes of 205.9 (± 68.3) \( \mu \text{g} \), 102 (±55) \( \mu \text{g} \) and 177 (±78) \( \mu \text{g} \), respectively (266,341,405). Reid et al (1992) used a modified FFQ to assess dietary and supplemental intakes of folate and zinc in a small Canadian hemodialysis cohort (n=21), stratified into four subgroups: no folate/zinc supplementation (n=7); no folate/22.5 mg zinc supplementation (n=4); 5 mg folic acid/no zinc supplementation (n=6); and 5 mg folic acid/22.5 mg zinc supplementation (n=6). A mean 260 (±43) \( \mu \text{g} \) dietary folate intake was observed for all subjects and no significant differences were found among the four groups (406). A more recent study in Poland (Jankowska et al, 2016) evaluated the vitamin intake of 202 patients with varying stages of kidney disease and treatment (CKD stages 3-5, IHD, PD and kidney transplant) using a 24-hour recall. Mean folate intakes were below the EAR in all groups: (260.4 [±132.7] \( \mu \text{g} \), 263.1 [±127.5] \( \mu \text{g} \), 206.1 [±112.0] \( \mu \text{g} \), and 225.1 [±158.7] \( \mu \text{g} \)) in CKD, IHD, PD, and kidney transplant, correspondingly (343). We are aware of only one study to date which has reviewed micronutrient status in the HHD population through the use of dietary intake assessment. Talemaítoga et al (1989) studied the nutritional status of 32 HHD patients dialyzing
in New Zealand by use of anthropometrics and 3-day food records. Mean (±SD) folate intake was found to be 162 (±56) µg, well below the EAR and mean folate intake of HHD subjects observed in our study (263 µg). No correlations were observed between nutritional intake and anthropometric parameters, although only macronutrients were included in the analysis (346).

Collectively, results from prior studies using dietary assessment solely as a means to determine folate status indicate the majority of dialysis patients, irrespective of modality or nutritional status, fall short of both the EAR (320 µg DFE) and RDA (400 µg DFE) guidelines (19). No dietary comparisons can be made with current renal recommendations, as they are specific to folic acid supplementation (1-2 mg/day) rather than DFE folate intake (µg DFE/day). These findings were substantiated in our present study where no significance was seen between mean dietary folate (DFE) and modality (p=0.69) or nutritional status (p=0.40, Appendix A), although mean intakes of our subjects both collectively, and within each dialysis modality, were higher than what has been previously reported (266,341–344,346,405,406). However, intake comparisons between studies can often be challenging. A variety of self-reporting tools were used to assess intakes; all of which are affected by their own types of measurement error and may therefore influence results (407). A positive correlation between dietary intake assessment methods has been previously reported in the dialysis population, but to our knowledge only macronutrient intakes (energy and protein) have been used as comparators (408). In addition, studies of folate intake in the ESKD population have occurred in both the pre- and post-fortification eras, and in countries which have mandatory, voluntary or no folic acid fortification policy in place, thereby making comparison of results difficult (266,342–344,346,405,406).

Mean total folate intakes of our study participants were higher than studies conducted in countries with similar fortification policies as Canada, suggesting other variables may be behind this discrepancy (341,342). The international diversity of the studies reflecting cultural influence, as well as socioeconomic status and geographic location, make comparison among these studies difficult, and could explain discrepancies observed in these studies (409,410). Another consideration is consumption patterns and how they can differ between seasons and across the lifespan (411). It is also reasonable to assume that one’s state of health and co-morbid
conditions may also alter the type of dietary pattern they may follow. This was observed in a recent study in Brazil by Santin et al (2019), which found dietary patterns differed significantly in all stages of chronic kidney disease, including dialysis, as well as individuals without chronic kidney disease (410).

Mandatory fortification of folic acid to enriched grains (U.S.) and flour, enriched pasta and cornmeal (Canada) have added 100-200 µg folic acid/day through this public health initiative (412,413). Results from population studies (Canadian Community Health Survey Cycle 2.2; NHANES 2015-16) show a mean folate (DFE) intake of 442 and 514 µg/day, respectively (414,415); well above the findings in our study (286.2 µg DFE/day). Although the ESKD population is characterized as having suboptimal intakes, 72.9% of our study participants were well-nourished or had a mild risk of malnutrition, as assessed by SGA. This finding is supported by our low prevalence of patients with diabetes, as they are known to have a much higher incidence of malnutrition and PEW (416). As well, mean CRP levels were not elevated in any of the three groups, suggesting study subjects were likely not inflamed as one may observe in PEW. Prevalence of malnutrition through assessment of SGA and other nutritional indices has been observed as being 18-75% (241–243), and more recently 25-56% with the introduction of a defined set of criteria for PEW (246). In our study, 27.1% of participants were classified as being moderately malnourished (n=18) and severely malnourished (n=1). This was higher than what was seen in a study by Wang et al (2002), who described the prevalence of moderate to several malnutrition as being only 18% in a group of 242 dialysis subjects from Korea (344), but lower than the 34% observed in 73 dialysis subjects from Mexico by Martin del-Campo in 2012 (342). As SGA has been validated as a rigorous nutritional assessment tool with a high degree of inter-observer agreement (254), the variation in results could be explained by geographical differences (e.g. ethnicity, food landscape, socioeconomic status) (247).

As mentioned previously, dietary folate intakes were not correlated with nutritional status in our study. One explanation is the possible underestimation of folate intake. Two potential sources of error for underestimating dietary folate intake include underreporting of folate on food records (417) and in food composition databases (200). In our subjects, we observed mean energy and
protein intakes of 1840 kcal (24.0 kcal/kg) and 76.6 g (1.0 g/kg), higher than in previous reports (266,342,343,405,408,418–421), but inadequate according to the Kidney Disease Outcomes Quality Initiatives (KDOQI) 2000 renal clinical practice guidelines of 30-35 kcal/kg and 1.2-1.3 g/kg, respectively (422). Upon stratification of our IHD, PD and HHD groups, mean calorie and protein intakes aligned more closely with findings from other studies (342,343,346,418,419). This is likely due to the higher mean energy (2067 [±627] kcal/day) and protein (86.6 [±23.2] g/day) intakes observed in our HHD group compared to the IHD and PD groups. Although it did not reach statistical significance, these results are not unexpected, as those on HHD have been observed to be younger, predominantly male and more nutritionally robust than their IHD and PD counterparts (1,346,390,423,424). This is corroborated by our finding that >95% of our HHD subjects were classified as well-nourished, as determined by SGA. Serum urea and creatinine concentrations are known to be useful ancillary markers for assessing protein adequacy and muscle mass, respectively, in ESKD patients. In this study, both mean serum urea and creatinine concentrations were significantly lower in the HHD group (urea, 11.1 [3.6] nmol/L; creatinine, 580 [202] nmol/L) compared to the IHD (urea, 19.1 [5.0] nmol/L; creatinine, 827 [235] nmol/L) and PD (urea, 23.0 [5.8] nmol/L; creatinine 890 [363] nmol/L) groups, owing to the more intensive dialysis regime received by the HHD group as opposed to poorer nutrition. When considering the IHD and PD groups separately, serum urea concentrations were within the target range for protein adequacy for each group (IHD, serum urea target 17-35 nmol/L; PD, serum urea target 15-30 nmol/L), suggesting possible underreporting of food records. Urea targets to assess adequacy of protein intake currently do not exist for the HHD population.

Despite dietary inadequacies, the mean serum folate concentrations for all subjects (n=70) was high (62.5 μmol/L), and strongly correlated to total folate (μg DFE) (dietary and suplemental) intakes (p<0.0001). Mean serum folate concentrations for the HHD group were found to be significantly higher (88.4 μmol/L) when compared with the IHD (50.5 μmol/L) and PD groups (52.4 μmol/L) (p=0.003). This difference is likely attributed to the renal vitamin (Replavite) dosing regimens, as individuals receiving HHD therapy are prescribed twice the amount of folic acid (2 mg) each day compared to their IHD and PD counterparts. High compliance rates for Replavite were observed at 88% (23/26), 96% (n=22/23) and 100% (n=21) in the IHD, PD and
HHD groups, respectively. Compliance was confirmed during the study visit, where patients were asked specifically about the frequency and timing of Replavite. Interestingly, of the 5.7% (n=4) participants classified as being deficient, three reported not taking their Replavite. A small number of patients were taking additional folic acid supplementation through the use of a multi-vitamin (5.7%) or oral nutritional supplement (17.1%).

Total median (IQR) intakes of folate (DFE) from both dietary and supplemental use for all subjects was 2050 (1899,3312) µg DFE/day; more than six times the recommended EAR for folate (19). Total median dietary and supplemental intake of folate (DFE) was even higher in the HHD group at 3543 (3433,3629) µg DFE/day; eleven times the EAR (19), and statistically significant when compared to the other modalities (p<0.0001). This is of concern, as studies have shown oral doses as little as 260 µg to overwhelm the capacity of DHFR (425,426), an enzyme that reduces folic acid, causing excess supplemental folic acid to appear in its unmetabolized form in the blood. To our knowledge, studies assessing the presence of circulating UMFA as a biomarker for excess folic acid are non-existent in the dialysis population. However, higher levels of UMFA in blood have been reported in the general population post-fortification (67) and are purported to be responsible for adverse health effects associated with high folate and folic acid intake, including masking of B₁₂ deficiency (24), decreased NK cell cytotoxicity (61), epigenetic dysregulation and cancer promotion (10), although this remains controversial. As the ESKD population is well-characterized by their reduced immune function and has been observed in some studies to exhibit impaired DNA repair mechanisms, it is prudent to reassess the large, supplemental doses of folic acid in this population.

Despite receiving large, supplemental doses of folic acid, mean plasma tHcy concentrations were elevated (21.8 [9.2] µmol/L), and present in 89% of our study subjects; a finding similar to previous reports (299). Hhcy has been linked with impairment of key methylation reactions through the accumulation of its precursor SAH (300). As thermodynamics favour SAH, excess SAH accumulates when plasma tHcy concentrations are elevated. This increase impedes methylation reactions through its inhibition of DNA methyltransferases, which can lead to DNA
hypomethylation (300). Although not statistically significant, mean tHcy concentrations in the HHD group were lower (17.7 [7.1] \(\mu\)mol/L) than the IHD (22.7 [8.1] \(\mu\)mol/L) and PD (24.7 [10.8] \(\mu\)mol/L) groups, suggesting increasing dialysis dose may be a more effective method to reduce plasma tHcy.

High serum and RBC folate concentrations in IHD and PD patients supplemented with large doses folic acid have been previously reported on (273,427–429). But there is a paucity of literature in the HHD population detailing supplementary practices of folic acid and folate status through biomarker assessment. A single study from Australia (331) compared the folate status of 52 HHD and IHD patients. Each patient was prescribed 5 mg folic acid on a weekly basis (~715 \(\mu\)g/day), despite the HHD receiving 50% more dialysis. Mean RBC folate concentrations were not significantly different between the two groups (HHD 1145 nmol/L; IHD 1522 nmol/L), suggesting folic acid requirements may not vary that greatly according to dialysis therapy. Folate is a low molecular weight molecule with moderate protein-binding ability and known to be removed by dialysis; however, losses have been reported as being slightly above typical urinary loss in a 24-hour period (430).

A number of studies have assessed folate status in unsupplemented IHD and PD populations using strictly biochemical markers. Studies from the pre-fortification era found folate deficiencies in approximately 11-46% of patients when serum or plasma folate was the sole biochemical indicator used (5,353,355,431). Other studies using RBC folate measures have found opposite results. A small study in New Zealand assessed folate status in a group of twelve patients receiving IHD or PD after stopping supplementation (361). Plasma and RBC folate concentrations were assessed at baseline, and again at six and twelve months; researchers found blood concentrations remained stable throughout the study period, with no deficiencies seen in any of the subjects (361). This finding was observed in two other studies, where investigators stopped folic acid supplementation for six and twelve months, respectively (358,362). Serum and RBC folate concentrations were measured at baseline and repeated on regular intervals until study completion. Both studies observed an initial, rapid decline in both plasma and RBC folate levels followed by a plateauing effect several months later. In both studies, subjects were able to
maintain adequate folate status at the low-normal range (358,362). The discrepancy in findings may in part be due to different assays employed among studies and the strengths and limitations that accompany them. As well, the uremic environment may alter metabolic processes within the body. Increased serum folate binding protein levels have been observed in patients with renal failure, and have suggested measurement by radioimmunoassay may falsely lower serum folate concentrations but not impair folate delivery to tissues (432). The choice of biomarker used can also produce very different results, especially in the absence of supplementation. Prevalence of deficiency was investigated by Lee et al (1999) in 34 CAPD and 60 IHD unsupplemented patients, respectively. Serum folate identified deficiencies in 3% (CAPD) and 71.7% (IHD) of patients, while RBC folate identified deficiencies in 0% (CAPD) and 10% (IHD) of patients. Similar results were seen by Bamonti et al (1999) who observed a 63% deficiency using serum folate results, but only a 1.8% deficiency when RBC folate measures were utilized (360,365).

In our study, we were only able to utilize serum folate as a biomarker to assess folate status. Despite this, the clinical utility of serum folate as a biomarker is still appropriate for assessment of total folate status in our population due to the high prevalence of supplemental folic acid intake (n=66 [94.3%]). Serum folate is known to reflect recent intake and is therefore sensitive to prandial variation. However, consistent intakes of large supplemental doses folic acid over an extended period of time, as seen in our population, is thought to mitigate this variation (433,434). As well, a strong correlation exists between serum and RBC folate concentrations, with several studies reporting serum folate as the more sensitive indicator to proportional changes in folic acid dosing (435–438). This aligns with our findings that demonstrated a statistically significant difference between the serum folate concentrations of HHD and the IHD and PD patients. However, one study did find RBC folate, which is an indicator of tissue stores and long term folate status, to be a more sensitive indicator once supplemental folic acid intakes exceeded 2 g/day (433). Crider et al (2019) recently examined the dose-response relationship between blood folate concentrations and folic acid intake under steady-state conditions, and estimated serum/plasma folate increased 11.6% for every 100 µg/day folic acid intake (439). Our study groups’ mean supplemental folic acid intakes were 1000 µg (IHD), 1000 µg (PD) and 2000 µg (HHD) per day with a mean dialysis vintage of 63.5 months [range 5 – 241 months (20 – 964
weeks). As serum and RBC folate reach a steady-state over a median of thirteen and thirty-six weeks respectively (433,434), we can ascertain the serum folate values observed in our population reflect true folate status and are likely not affected by dietary variances.

4.3.2 Current Intake and Blood Vitamin B₆ and Vitamin B₁₂ Levels

This is the first study to describe dietary intakes of vitamin B₆ and vitamin B₁₂ in a Canadian ESKD population. In the general population, vitamin B₆ requirements for adults vary according to age and gender. Total mean vitamin B₆ intakes were near, at, or above the EAR for total subjects (1.3 mg/day) and no significant differences were observed among the three groups (p=0.85). Further stratification into age and gender found subjects aged 19-50 (male, female) and males 51+ years of age met recommendations for both the EAR (1.3 mg/day) and RDA (1.5 mg/day) general population targets. Prevalence of inadequate intakes (EAR) was similar at 42.4% and 42.8% for subjects 19-50 and males 51+ years of age, respectively. Females 51+ years of age were significantly below EAR recommendations, with a total mean intake of 0.97 mg/day and a high prevalence of inadequacy (66.7%) that mirrored the inadequacy seen with folate (67%). Rich sources of vitamin B₆ include root vegetables, tropical fruit, and certain protein-rich foods such as organ meats and fish; many of which are high in potassium and/or phosphorus. Similar to folate, restrictions of these food types are infrequently required in the home dialysis population. It is plausible that despite loosened dietary restrictions, a number of subjects continue to avoid certain food products; as the majority of them have been extensively educated early on in their diagnosis of chronic kidney disease. As well, a number of variables including age, diabetes, ethnicity and protein intake are known to be predictors of vitamin B₆ status (440). However, recent population data (NHANES 2015-2016) show females of similar age (50-59, 60-69, and 70+ years of age) with mean vitamin B₆ intakes well above our comparable cohort (1.73, 1.52, and 1.47 mg/day) (415). Prevalence of diabetes in this sub-group of patients was low (20%) and nearly two-thirds of the women were Caucasian (60%); however, mean protein intake was found to be appreciably suboptimal (58.1 g/day) compared to the mean observed for total subjects (76.6 g/day), providing a plausible reason for the discrepancy of vitamin B₆ status among the different age groups.
Contrary to our broader findings of adequacy with the total cohort and individual dialysis modality groups for this vitamin, low vitamin B₆ intakes have consistently been observed in other IHD and PD studies (266,341,343,344). For example; As’habi et al (2011) administered a 4-day food recall to 291 Iranian IHD subjects for assessment of micronutrient status, including vitamin B₆. Inadequacy of vitamin B₆ was recognized in 66% of patients, with a mean total intake of 0.8 (±1.5) mg/day (405). Another study investigated micronutrient intake stratified by nutritional (SGA) and inflammatory (CRP) status in 73 CAPD patients using a 24-hour recall. This study found reduced dietary vitamin B₆ intake to be associated with malnutrition and presence of inflammation (342); which was not observed in our study. To the best of our knowledge, there are no published studies examining vitamin B₆ intake in the HHD population.

Median (IQR) plasma vitamin B₆ (PLP) concentrations for all subjects (n=70) were high (121 [66,179] nmol/L), and significantly correlated with total (dietary and supplemental) vitamin B₆ intakes (p=0.001), a finding which has been observed in other supplemented ESKD populations (7,331). Ethnicity and dialysis vintage were also found to be predictors of plasma PLP status. Our study was comprised of 51.6% subjects who identified as being Caucasian, and the remaining 48.6% from diverse cultural backgrounds (p=0.022). Vitamin B₆-rich foods make up a strong component of certain cultural dietary patterns. Ethnicity has also been found to be a predictor of vitamin B₆ in the general population (441). Interestingly, parameter estimates for dialysis vintage show a 0.004% decline in vitamin B₆ for every additional month on dialysis, or approximately 5% year (p=0.006). This finding is of important clinical relevance, particularly in our HHD patients, who dialyze upwards of 40 hours per week and have a significantly longer vintage than IHD and PD (mean vintage; 101.3 [70.9] months). However, no significant differences were found among the three groups for plasma PLP, despite the HHD patients receiving double the amount of vitamin B₆ (20mg) compared to their IHD and PD counterparts (p=0.67). The opposite effect was observed in a study by Coveney et al (2011), where median plasma PLP of HHD subjects was significantly higher (93.9 [38.8-2436.9] nmol/L) than subjects on IHD (44.9 [10.9-1362.7] nmol/L) (p<0.001); however, a larger proportion of HHD subjects were on vitamin B₆ supplementation at the time of the study compared to the IHD group (331). Pyridoxal kinase, an enzyme required for the conversion of pyridoxine to PLP, is suppressed in
the uremic environment. It has been purported that even with incrementally higher dialysis doses, and greater clearance of urea, pyridoxal kinase will not respond as effectively in the presence of uremia, which may explain the lack of difference in PLP concentrations observed among our study groups. However, others have theorized dialysis would enhance the activity of pyridoxal kinase by reducing the uremic load (442,443). Total median (IQR) intake of vitamin B$_6$ from both dietary and supplemental use for all subjects was 11.7 (11.0,20.9) mg/day; nearly 8 times the RDA, if comparing against the 1.5 mg/day value for vitamin B$_6$ (19). Total median dietary and supplemental intake of vitamin B$_6$ was even higher in the HHD group at 21.2 (20.8,21.7) mg/day; 14 times the RDA (19), and statistically significant when compared to the other modalities (p<0.0001). A systematic review found vitamin B$_6$ deficiency, determined by plasma PLP levels, to be 24-56% in unsupplemented IHD patients (7,328,444,445); with similar results being observed in PD and HHD (353,456). These findings, in addition to the short half-life (15-20 days) and storage (3-4 months) of this vitamin suggest some form of supplementation may be required to sustain adequacy (7,323).

Unlike dietary folate (DFE), total mean daily intake of vitamin B$_{12}$ for all subjects exceeded both the EAR (2.0 μg/day) and RDA (2.4 μg/day) recommendations at 2.9 μg/day. Mean vitamin B$_{12}$ intakes for IHD, PD, and HHD patients were also above EAR and RDA recommendations at 2.6, 3.1, and 2.9 μg/day, correspondingly. Other dietary vitamin B$_{12}$ intake studies have yielded similar results in the IHD, PD, and HHD populations, where the majority of subjects have either met (341,342) or exceeded the EAR and RDA (266,343,344,405). As previously mentioned, serum vitamin B$_{12}$ concentrations have been reported as being comparable to or higher than the normal range in all three dialysis groups, with little to no presence of deficiency in other studies (6,7,328,331,346,353,362,363). No statistical significance was observed among the three modalities in our study (p=0.66); however nutritional status (SGA) (p=0.006) and education level (p=0.018) predicted serum vitamin B$_{12}$ levels after adjusting for confounders. Prevalence of inadequate intakes (intakes below the EAR) was 46%; much lower than what was observed for folate. This is not surprising, as all patients on dialysis are educated on the importance of consuming a high protein diet, with emphasis often placed on animal protein, a vitamin B$_{12}$-rich food group (447). Adequate protein consumption (characterized by ingestion of vitamin B$_{12}$-rich
animal protein) to circumvent dialysis protein losses is a key factor in maintaining an optimal nutritional status, and therefore provides a plausible rationale for the association between vitamin B$_{12}$ and SGA. For example, 95.2% (n=20) of our HHD group was classified as being well-nourished and only 4.8% (n=1) was classified as moderately malnourished. The HHD group also had the highest mean protein intake of the groups, although not statistically significant.

To our knowledge, the association found between vitamin B$_{12}$ and education in our study has not been investigated or observed in other ESKD studies. However, studies in the general population have linked a higher socioeconomic status, as determined by education, income and occupation, with healthier eating patterns and increased access to higher cost food items such as animal proteins. Our analysis demonstrated a statistically significant difference between subjects who did not complete high school and our reference group, those who attained a university degree or higher. It is highly plausible our subjects characterized as having a limited education may also have financial barriers; this would thereby minimize their ability to purchase higher cost foods such as animal protein.

4.3.3 Strengths and Limitations

To date, this is the only Canadian study to investigate the status of folate and other methyl donor nutrients through dietary, supplemental and biochemical measures in the post-fortification era. It is also the only study to provide a comparative analysis among the three different dialysis modalities currently utilized within Canadian nephrology programs. Our study group was also culturally diverse and reflected a wide variety of dietary intake patterns. Another key strength was the use of the 3-day weighed food record. The difficulties around assessment of dietary intake have been well documented and that the method employed may influence results. The weighed food record utilized in our study is considered to be one of the most accurate methods for capturing intake data. Our subjects received on-hands training with the scale and follow up through in-person visits, e-mail and telephone to ensure clear instructions were provided. Intakes were recorded in a manner that would reflect the dietary patterns of the three modalities. Other strengths included the limited variability in fasting and timing of blood draws. Subjects were
instructed to fast for at least eight hours prior to their blood draw and all blood samples were drawn prior to lunchtime to control for diurnal variations. In IHD and HHD, all blood samples were drawn after one full day without dialysis, similar to current protocols for blood collection. This ensured bloodwork results were comparable between groups as well as other studies.

There are a few limitations to this study. First of all, our subjects were generally well-nourished and >60% reported having completed post-secondary education. These two factors could play a role in the types and quantity of food products consumed, as well as overall nutritional status. The more vulnerable patients on dialysis, including those who are unable to speak English, have cognitive barriers or reside in long-term care facilities as a result of reduced capacity were visibly absent from our study due to the limitations around intake recording. As well, less than 25% of our patients had a diagnosis of diabetes; while this is similar to what is observed in the HHD population, it is much lower than what is typically observed in the IHD and PD patients. Despite this, our intake results were similar in many facets when compared to other studies. Although our cohort reflects the diversity observed in a multi-ethnic city such as Toronto, our findings and subject study group may not represent all Canadians or typical North American populations. Another limitation was our use of ESHA for nutrient analysis, as the database did not provide the same degree of cultural variation as what was recorded in our subjects’ food records. Several cultural foods were absent, requiring the creation of recipes to align as closely to the prescribed food items as possible. It may also underreport true folate (DFE) amounts, as Canadian food products fortified with folic acid have been found to over fortify by an average of 151%, likely owing to overages by food companies to ensure adequacy (210). We were also unable to assess folate status using RBC folate concentrations. While serum folate is a reasonable assay to use in this population due to their daily, static intake of supplemental folic acid, having RBC folate concentrations would have enabled us to assess the long-term folate status in our study subjects. As well, it is important to highlight that blood concentrations of folate and vitamins B₆ and B₁₂ may not necessarily reflect target tissue concentrations as well as biochemical or functional outcomes in the target tissues. However, the higher cost and evasive nature of tissue biopsies made the collection of blood biomarkers a more reasonable approach. Finally, our sample size was small due to time and budget constraints; however, being
exploratory in nature, its intention was to characterize the intakes and folate status of the ESKD cohort and establish a framework for future studies of a larger scale.
Chapter 5: Overall Conclusions and Future Directions

The primary objective of this thesis was to estimate total folate and folic acid intake consumed through food and dietary supplementation in the dialysis population, and to describe its association with blood levels of folate and other key methyl donor nutrients. Relationships among nutritional status, dialysis modality and the intakes and blood levels of folate and folic acid were also examined. We hypothesized folic acid intake derived from fortified foods and daily vitamin supplementation would result in high folate/folic acid intakes and blood levels in our study subjects, and that nutritional status and dialysis modality would influence blood levels of folate and other methyl donor nutrients.

We were able to determine total dietary and supplemental intakes of folate, vitamin B₆ and vitamin B₁₂ in a Canadian cohort of patients with ESKD receiving IHD, PD or HHD dialysis therapy in the post-fortification era. Mean dietary intakes of folate fell short of the EAR, while adequate intakes of vitamins B₆ and B₁₂ were achieved by most age groups. We also ascertained folate, vitamin B₆ and vitamin B₁₂ status, as assessed by serum folate, plasma PLP and serum vitamin B₁₂ concentrations. Serum folate concentrations were high and strongly correlated to total folate (DFE) intake, likely owing to the sizeable doses of daily folic acid supplementation. Inadequate dietary folate intakes suggest folic acid is warranted, but given that folic acid supplementation is at or above the UL and serum folate levels are appreciably high, implies current folic acid supplementation recommendations are inappropriate. Plasma PLP concentrations were high, while serum vitamin B₁₂ concentrations were within the normal range, and no patient was found to be vitamin B₁₂ deficient. Plasma PLP concentrations were highly correlated with total vitamin B₆ intake, dialysis vintage, and ethnicity, while nutritional status and education were predictors of serum vitamin B₁₂ concentrations. Significant associations between dialysis modality and folate and vitamin B₆ status were observed, but this relationship was lost after covariate adjustments. Nutrition status had no effect on folate or vitamin B₆ status, likely due to the large supplemental doses received by the entire dialysis cohort.
Findings from our exploratory analysis suggest current renal recommendations regarding the use of high dose B vitamin supplements in dialysis cohorts, particularly folic acid, are likely unnecessary to sustain adequate body stores. A reduction in frequency or dose of folic acid supplementation is recommended, particularly in the HHD group. In IHD and PD, it would be reasonable to reduce their renal multivitamin to three times per week, similar to the dosing regimen of many patients for vitamin D. For the HHD group, whose serum folate levels were significantly higher than the other two groups, decreasing their renal multivitamin from two to one tablet per day would be beneficial. These recommendations should be brought forward for further discussion at continuous quality initiative meetings, where protocols are reviewed, updated and/or developed. Additionally, total folate (natural folate in diets + folic acid in fortified food and nutrition supplements + supplemental folic acid) (µg DFE) intake should be investigated in the pediatric population. Recommendations suggest children on dialysis follow the Dietary Reference Intake for folate, according to age group (1-3yrs: 150 mcg; 4-8yrs: 200 mcg; 9-13yrs: 300 mcg; 14-18yrs: 400 mcg). However, the pediatric renal multivitamin contains 1mg folic acid and only children 1-5 yrs are instructed to take half a tablet daily, meaning a large proportion of children with ESKD are also receiving high daily supplemental doses of folic acid (448). Micronutrients are essential in maintaining homeostasis within the body and depend upon physiological regulators such as the kidney to help uphold that balance. In the absence of optimal kidney function, it is critical to have appropriate protocols in place to ensure metabolic processes are maintained as much as possible. As such, the goal of vitamin therapy for persons receiving dialysis should be normalization, and not deficiency or excess of vitamins in serum and tissues. We suggest routine monitoring be implemented to help prevent toxicity or deficiency states.

Some potential future studies include: 1) the determination of RBC folate concentrations to assess long-term body stores; 2) the determination of UMFA levels in the three dialysis groups; 3) a nation-wide study to better characterize the folate status of the Canadian ESKD population within the 3 dialysis modalities; particularly the HHD population, as this group has unique characteristics not observed in the IHD and PD groups and is also prescribed significantly higher doses of folic acid, vitamin B₆ and vitamin B₁₂. Consider calculating a “total vitamin score” for
all three aforementioned nutrients and then further stratify results using a high/low categorization to look at other relationships; 4) the effects of folate and other B vitamins on DNA methylation and its functional ramifications; 5) clinical studies using a dose-response approach to determine the amount of folate and other B-vitamins in ESKD patients (adult and pediatric) required to maintain optimal nutritional and metabolic balance; 6) clinical studies to establish optimal supplemental levels of folic acid and other B-vitamins; 7) the determination of potential adverse health effects such as cancer promotion, resulting from high folate/folic acid intake and blood levels in this ESKD population. This could be achieved by utilizing data from national (Canadian Organ Replacement Registry) or provincial (Ontario Renal Network) dialysis registries and linking it with data from similar cancer registries (Canadian Cancer Registry, Cancer Care Ontario).

In conclusion, this research provides a framework for future studies investigating optimal intakes of folic acid, vitamin B₆ and vitamin B₁₂ in patients with varying nutritional states and modes of dialysis in the post-fortification era. Randomized, dose-response clinical trials are needed to better delineate micronutrient requirements in this complex population. Doing so will help update clinical protocols and provide guidance on dietary recommendations so clinicians can tailor micronutrient supplementation, if warranted, to individual needs, as they do with other aspects of the renal therapeutic diet. This study can also serve as a basis for future studies aimed at elucidating the effects of supplementation of folic acid and other methyl donor nutrients on DNA methylation and its functional ramifications in patients with ESKD.
1. Canadian Organ Replacement Register Annual Report: 2018


112


73. Plumptre, L. Maternal one-carbon nutrient status and effects on DNA methylation and hydroxymethylation in newborn infants. (University of Toronto, 2016).


153. Selhub J, Morris MS, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proc Natl Acad Sci USA 2007;104(50):19995-20000.


182. Stevens VL, McCullough ML, Sun J, Jacobs EJ, Campbell PT, Gapstur SM. High levels of folate from supplements and fortification are not associated with increased risk of colorectal cancer. Gastroenterology 2011;141(1):98-105.


http://kidneyfoundation.cachefly.net/professionals/KDOQI/guidelines_nutrition/doqi_nut.html


Appendix A: Supplementary Data
Figure A.1: Flowchart of study recruitment, in-centre hemodialysis
Figure A.2: Flowchart of study recruitment, peritoneal dialysis

- Number of patients screened: N = 151
  - Ineligible: N = 36

- Patients eligible: N = 115
  - Patients approached: N = 66 (57.3%)
    - Declined to hear about study: N = 9

- Agreed to hear about study: N = 57
  - Declined: N = 18
    - Medical issues: N = 9
      - Did not give reason: N = 6
      - Too difficult: N = 3
  - No reply: N = 13

- Consented: N = 26
  - Did not complete: N = 3
    - Transplanted: N = 1
      - Changed to HD: N = 1
    - Extended vacation: N = 1

- Completed study: N = 23
Figure A.3: Flowchart of study recruitment, home hemodialysis
### Table A.1 Cancer diagnoses

<table>
<thead>
<tr>
<th>Types of cancer</th>
<th>All Subjects</th>
<th>IHD</th>
<th>PD</th>
<th>HHD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous cancer n (%)</td>
<td>11 (15.7)</td>
<td>2 (7.7)</td>
<td>4 (17.4)</td>
<td>5 (23.8)</td>
<td>0.31</td>
</tr>
<tr>
<td>Skin</td>
<td>5 (46.0)</td>
<td>2 (18.2)</td>
<td>1 (9.1)</td>
<td>2 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>3 (27.2)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td>2 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>2 (18.2)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>2 (18.2)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>2 (18.2)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>1 (9.1)</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Head &amp; Neck</td>
<td>1 (9.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td></td>
</tr>
</tbody>
</table>
### Table A.2 Total dietary intakes, individual days: 3-day food record

<table>
<thead>
<tr>
<th>Dietary Intakes</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1806 (755)</td>
<td>1753 (855)</td>
<td>1669 (504)</td>
<td>2020 (843)</td>
<td>0.28</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>75.4 (36.0)</td>
<td>68.9 (41.9)</td>
<td>72.1 (26.7)</td>
<td>86.8 (36.2)</td>
<td>0.21</td>
</tr>
<tr>
<td>Folate (μg DFE)</td>
<td>297.1 (233.4)</td>
<td>372.1 (278.7)</td>
<td>269.6 (239.8)</td>
<td>238.1 (131.5)</td>
<td>0.12</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.3 (0.8)</td>
<td>1.2 (1.0)</td>
<td>1.2 (0.6)</td>
<td>1.3 (0.8)</td>
<td>0.90</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>2.9 (3.1)</td>
<td>2.7 (2.2)</td>
<td>3.4 (3.9)</td>
<td>2.5 (3.2)</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1764 (667)</td>
<td>1695 (505)</td>
<td>1575 (736)</td>
<td>2055 (690)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>74.8 (29.8)</td>
<td>72.6 (25.4)</td>
<td>68.6 (31.5)</td>
<td>84.4 (31.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Folate (μg DFE)</td>
<td>280.5 (197)</td>
<td>239.8 (176.4)</td>
<td>292.8 (224.6)</td>
<td>315.4 (188.7)</td>
<td>0.41</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.2 (0.8)</td>
<td>1.1 (0.7)</td>
<td>1.3 (0.9)</td>
<td>1.4 (0.7)</td>
<td>0.60</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>2.9 (3.2)</td>
<td>2.7 (2.4)</td>
<td>3.1 (4.3)</td>
<td>3.0 (2.7)</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Day 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1813 (741)</td>
<td>1696 (777)</td>
<td>1653 (648)</td>
<td>2127 (731)</td>
<td>0.06</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>79.6 (35.2)</td>
<td>75.5 (36.2)</td>
<td>75.9 (35.9)</td>
<td>88.6 (33.3)</td>
<td>0.38</td>
</tr>
<tr>
<td>Folate (μg DFE)</td>
<td>280.8 (201.4)</td>
<td>292.6 (263.0)</td>
<td>309.4 (189.5)</td>
<td>235.6 (110.3)</td>
<td>0.45</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>1.4 (0.9)</td>
<td>1.4 (1.0)</td>
<td>1.5 (0.9)</td>
<td>1.2 (0.8)</td>
<td>0.74</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>2.8 (2.5)</td>
<td>2.4 (2.7)</td>
<td>2.9 (2.1)</td>
<td>3.2 (2.7)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

DFE, dietary folate equivalent

### Table A.3 Total supplemental intakes: Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements

<table>
<thead>
<tr>
<th>Supplemental Intakes</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (μg DFE)*</td>
<td>2002.7 (946.9)</td>
<td>1556.9 (649.2)</td>
<td>1627.6 (343.0)</td>
<td>2965.7 (1021.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B₆ (mg)</td>
<td>14.9 (15.2)</td>
<td>12.7 (20.1)</td>
<td>10.8 (5.7)</td>
<td>22.2 (13.1)</td>
<td>&lt;0.027</td>
</tr>
<tr>
<td>Vitamin B₁₂ (μg)</td>
<td>24.1 (119.8)</td>
<td>10.7 (20.6)</td>
<td>51.1 (208.3)</td>
<td>11.2 (2.54)</td>
<td>0.425</td>
</tr>
</tbody>
</table>

*Supplemental folic acid converted to DFE (DFE: dietary folate equivalent)
### Table A.4 Percentage of subjects using Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements

<table>
<thead>
<tr>
<th>Supplementation</th>
<th>All Subjects [n (%)]</th>
<th>IHD [n (%)]</th>
<th>PD [n (%)]</th>
<th>HHD [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replavite</td>
<td>66 (94.3)</td>
<td>23 (88.0)</td>
<td>22 (96.0)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Vitamin supplement (folic acid, B₆, B₁₂)</td>
<td>9 (12.9)</td>
<td>2 (7.7)</td>
<td>4 (17.4)</td>
<td>3 (14.3)</td>
</tr>
<tr>
<td>Nutrition supplement</td>
<td>12 (17.1)</td>
<td>8 (31.0)</td>
<td>4 (17.4)</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table A.5 Total dietary and supplemental intakes, individual days: 3-day food records, Replavite, individual vitamin supplements (folic acid, B₆, B₁₂), oral nutrition supplements

<table>
<thead>
<tr>
<th>Dietary Intakes</th>
<th>All Subjects [Mean (SD)]</th>
<th>IHD [Mean (SD)]</th>
<th>PD [Mean (SD)]</th>
<th>HHD [Mean (SD)]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1848 (750)</td>
<td>1770 (907)</td>
<td>1702 (506)</td>
<td>2020 (843)</td>
<td>0.37</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>77.4 (35.8)</td>
<td>70.1 (43.7)</td>
<td>73.7 (26.5)</td>
<td>86.8 (36.2)</td>
<td>0.28</td>
</tr>
<tr>
<td>Folate (μg DFE)</td>
<td>2329 (945.4)</td>
<td>1915 (744.7)</td>
<td>1897 (434.4)</td>
<td>3204 (1080.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B₆(mg)</td>
<td>16.4 (15.2)</td>
<td>13.9 (20.0)</td>
<td>12.0 (5.8)</td>
<td>23.5 (13.4)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Vitamin B₁₂(μg)</td>
<td>26.8 (120.4)</td>
<td>12.0 (20.8)</td>
<td>54.5 (207.7)</td>
<td>13.7 (4.3)</td>
<td>0.40</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1808 (656)</td>
<td>1714 (588.1)</td>
<td>1608 (736)</td>
<td>2055 (689.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>74.3 (30.1)</td>
<td>68.3 (29.7)</td>
<td>68.6 (31.2)</td>
<td>84.4 (31.7)</td>
<td>0.15</td>
</tr>
<tr>
<td>Folate (μg DFE)</td>
<td>2264 (931.1)</td>
<td>1787 (646.8)</td>
<td>1920 (434.4)</td>
<td>3122 (1126.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B₆(mg)</td>
<td>16.4 (15.2)</td>
<td>13.8 (20.0)</td>
<td>12.1 (6.3)</td>
<td>23.5 (12.9)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Vitamin B₁₂(μg)</td>
<td>27.0 (120.6)</td>
<td>12.0 (20.7)</td>
<td>54.4 (208.0)</td>
<td>14.2 (2.1)</td>
<td>0.40</td>
</tr>
<tr>
<td>Day 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>1856 (722)</td>
<td>1715 (814.1)</td>
<td>1686 (637.1)</td>
<td>2127 (731.3)</td>
<td>0.09</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>81.6 (34.3)</td>
<td>76.5 (37.3)</td>
<td>77.5 (35.4)</td>
<td>88.6 (33.3)</td>
<td>0.46</td>
</tr>
<tr>
<td>Folate (μg DFE)</td>
<td>2313 (931.0)</td>
<td>1838.9 (706.3)</td>
<td>1938.7 (421.3)</td>
<td>3201.3 (1054.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B₆(mg)</td>
<td>16.8 (15.3)</td>
<td>14.1 (19.9)</td>
<td>12.3 (5.7)</td>
<td>24.4 (13.8)</td>
<td>&lt;0.02</td>
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<tr>
<td>Vitamin B₁₂(μg)</td>
<td>26.7 (120.6)</td>
<td>11.7 (19.8)</td>
<td>54.0 (208.2)</td>
<td>14.4 (3.5)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Supplemental folic acid converted to DFE (DFE: dietary folate equivalent)*
### Table A.6 Pill burden; stratified by dialysis group

<table>
<thead>
<tr>
<th>No. of Medications (Y) [n (%)]</th>
<th>All subjects</th>
<th>IHD</th>
<th>PD</th>
<th>HHD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 4</td>
<td>11 (15.7)</td>
<td>3 (11.5)</td>
<td>0 (0.0)</td>
<td>8 (0.3)</td>
<td>--</td>
</tr>
<tr>
<td>5 – 9</td>
<td>32 (45.7)</td>
<td>11 (42.3)</td>
<td>12 (52.2)</td>
<td>9 (42.9)</td>
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### Table A.7 Total dietary intakes: 3-day food record; stratified by nutritional status (SGA)

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<th>Moderately Malnourished [Mean (SD)]</th>
<th>Severely Malnourished [Mean (SD)]</th>
<th>p value</th>
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DFE: dietary folate equivalent
Figure A.4: Distributions of serum folate concentrations in IHD, PD and HHD groups in comparison to current recommended target range. *Deficiency cut-off based on Tiez, 1995; **Upper limit cut-off based on WHO 2015 guidelines for serum folate.
Figure A.5: Distributions of plasma B₆ concentrations in IHD, PD and HHD groups in comparison to current recommended target range.*Deficiency cut-off based on IOM,1998; **Upper limit cut-off based on UHN reference range for plasma B₆.
Figure A.6: Distributions of serum B<sub>12</sub> concentrations in IHD, PD and HHD groups in comparison to current recommended target range. *Deficiency cut-off based on IOM, 1998; **Upper cut-off based on UHN reference range for serum B<sub>12</sub>. 
Table A.8 BMI classifications according to renal guidelines

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Essential Guide for Renal Dietitians, 2018
### Table A.9: Correlations Among Independent Variables that have a Proposed Relationship with Serum Folate in ESKD (n=70)

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**Correlation is significant at the 0.01 level *Correlation is significant at the 0.05 level

*Serum folate = nmol/L
*DFE = dietary folate equivalents
*Kcal = calories
*BMI = body mass index
*SGA = subjective global assessment
Table 4.12 Correlations Between Total Dietary and Supplemental Intake and Associated Biomarker Parameters (n=70)

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<td><strong>p-value</strong></td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
<td>.13</td>
<td>.25</td>
</tr>
<tr>
<td><strong>Total B&lt;sub&gt;12&lt;/sub&gt;</strong></td>
<td>1.00</td>
<td>.76**</td>
<td>.25**</td>
<td>.25**</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>.000</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.51</td>
<td></td>
</tr>
<tr>
<td><strong>Total B&lt;sub&gt;6&lt;/sub&gt;</strong></td>
<td>1.00</td>
<td>.46**</td>
<td>.15</td>
<td>.26&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>.000</td>
<td>.21</td>
<td>.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serum Folate</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00</td>
<td></td>
<td>.12</td>
<td>.59**</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>.31</td>
<td></td>
<td>.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serum B&lt;sub&gt;12&lt;/sub&gt;</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00</td>
<td></td>
<td>.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Plasma B&lt;sub&gt;6&lt;/sub&gt;</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>**Correlation is significant at the 0.01 level</sup>  <sup>*Correlation is significant at the 0.05 level</sup>
<sup><sup>n</sup>=69</sup>
<sup>DFE = dietary folate equivalents</sup>
<sup>serum folate = nmol/L</sup>
<sup>serum B<sub>12</sub> = μmol/L</sup>
<sup>plasma B<sub>6</sub> = nmol/L</sup>
CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

Study Title:
Nutritional Status and Dialysis Modality: Their Effect on Folate Status and Methylation Capacity 15 Years after the Institution of a National Folic Acid Fortification Program.
"Folate, Nutritional Status and Dialysis"

Investigators/Study Doctors:
Christine Nash, MSc(C), RD
Phone: 416-340-4800 ext.6272
Dr. Young-in Kim
Dr. Joanne Bargman

Contact Information:
Christine Nash, MSc(C), RD
Phone: 416-340-4800 ext.6272

Funding:
Canadian Foundation for Dietetic Research

Introduction:
You are being asked to take part in a research study. Please read the information about the study presented in this form. The form includes details on study's risks and benefits that you should know before you decide if you would like to take part. You should take as much time as you need to make your decision. You should ask the study doctor or study staff to explain anything that you do not understand and make sure that all of your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish including your friends, family, and family doctor. Participation in this study is voluntary.

Background/Purpose:
In Canada, it is recommended that patients receiving dialysis treatment take 1mg of folic acid or more per day to replace what is being lost during dialysis. Mandatory folic acid fortification in grain products has greatly increased one’s exposure to folic acid and as such, current folic acid recommendations for patients receiving dialysis treatment may no longer be appropriate.

There have been very few studies that have looked at the intake and blood levels of folic acid and folic acid in Canadian dialysis patients after the addition of folic acid in grain products, especially in peritoneal and home hemodialysis. It is also unclear if a patient's nutritional status or type of dialysis can affect folate status.
The purpose of this study is to assess the total intake of folate, folic acid and other B vitamins in a Canadian dialysis population post-fortification. It will also look at important relationships among nutritional status, type of dialysis, intake and blood levels of folate, folic acid and other B vitamins.

You are being asked to participate because you have kidney disease and are receiving dialysis treatment in the form of peritoneal or hemodialysis.

The usual treatment for your kidney disease is dialysis. Taking part in this study will not alter your treatment in any way.

Up to 100 people receiving dialysis treatment at UHN will participate in this study and it will take 2 years to complete.

**Study Visits and Procedures:**

There will be 2 study visits during your participation in this study. Visit 1 will be approximately 30 minutes and visit 2 will be approximately 90 minutes. Both visits will take place at Toronto General Hospital where you receive your dialysis care.

The following procedures and assessments will be carried out for this study:

- **Blood draw**
  - In-centre hemodialysis study subjects
    - completed by the primary nurse assigned to you for dialysis treatment and will be done through your dialysis access
  - Peritoneal and home hemodialysis study subjects
    - completed by a lab technician at the Toronto General Hospital laboratory OR by your primary nurse within your unit
  - You will be asked to do not eat for at least 8 hours before your blood is drawn
- **Nutritional assessment**
  - Completed by the study team and includes the following:
    - height, weight, functional ability
    - medical conditions that may impact nutritional status
    - changes to weight, appetite, gastrointestinal symptoms
    - changes to body muscle and body fat
- **3 day weighed food records**
  - Completed by the study subjects using a food scale

The following information will also be collected from your medical chart and personal interview by the study team:

- age, gender, ethnicity, alcohol and tobacco use
- current education and employment status
- cause of kidney failure, date you started dialysis
- medications you are currently taking

Version Date: Feb 27, 2017
- other medical conditions and family history of medical conditions (if documented)
- vitamin, mineral and nutrition supplement use

**Summary of Tests and Procedures**

<table>
<thead>
<tr>
<th>Visit</th>
<th>Tests and Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td>Meet with the study team during your dialysis treatment OR prior to your dialysis clinic visit to review the study and ask any questions you may have about the study. You will be provided with a food scale and 3 day weighed food record collection sheets. Instructions will be given on scale use and how to record food and fluid intake. <strong>Subjects on in-centre hemodialysis:</strong> you will be instructed to collect food and fluid intake from 1 dialysis day and 2 non-dialysis days. The 2 non-dialysis days should consist of 1 weekday and 1 weekend day. <strong>Subjects on peritoneal and home hemodialysis:</strong> you will be instructed to collect food and fluid intake from 2 weekdays and 1 weekend day.</td>
</tr>
<tr>
<td>Visit 2</td>
<td>Meet with the study team before starting your dialysis treatment OR prior to your dialysis clinic visit to provide them with your 3 day weighed food records and return the scale. Your study team will review the food records and may ask some questions to ensure all of your food and fluid entries are understood. At this time your nutritional assessment will also be completed by the study team. <strong>Subjects on in-centre hemodialysis:</strong> blood samples for the study will be drawn once you are connected to your dialysis machine for your treatment. <strong>Please note:</strong> blood samples for all study subjects will be drawn in the morning for consistency. If you are on an afternoon or evening shift, but would like to be part of this study, your study team will work with the dialysis unit to coordinate a morning dialysis session for you. <strong>Subjects on peritoneal and home hemodialysis:</strong> blood samples for the study will be drawn down in the Toronto General laboratory where you typically get bloodwork drawn (a study team member will come with you to the laboratory) OR within your unit.</td>
</tr>
</tbody>
</table>
Risks:

Taking part in this study has risks. Some of these risks we know about. There is also a possibility of risks that we do not know about. Please call the study staff if you have any side effects even if you do not think it has anything to do with this study.

Risks of Blood Draw/Catheter Blood Draw - There is a possibility of pain, bruising, swelling, or infection related to giving blood.

Benefits:

You may not receive a direct benefit from being in this study. But information learned from this study may help your healthcare team in the future understand how much folate, folic acid and B vitamins patients need when they are on dialysis.

Reminders and Responsibilities:

It is important to remember the following things during this study:

- Weigh, measure and mark down all food and fluids that you have on the days you are recording your 3-day food record.
- Return food records and food scales to your study team.
- Do not eat for 8 hours before doing your study bloodwork.
- Ask your study team about anything that worries you.
- Tell study staff anything about your health that has changed.
- Tell your study team if you change your mind about being in this study.

Alternatives to Being in the Study:

If you choose not to participate in this study, your care remains unaffected. You will continue your dialysis treatments and receive your usual care from your healthcare team.

Confidentiality:

Personal Health Information

If you agree to join this study, the study doctor and his/her study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could identify you and includes your:

- name
- address
- full date of birth
- new or existing medical records that includes types, dates and results of medical tests or procedures.
The following people may come to the hospital to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study is following proper laws and guidelines:

- Representatives of the University Health Network (UHN) including the UHN Research Ethics Board

The study doctor will keep any personal health information about you in a secure and confidential location for 10 years. A list linking your study number with your name will be kept by the study doctor in a secure place, separate from your study file.

Your participation in this study will also be recorded in your medical record at this hospital. This is for clinical safety purposes.

Research Information in Shared Clinical Records
If you participate in this study, information about you from this research project may be stored in your hospital file and in the UHN computer system. The UHN shares the patient information stored on its computers with other hospitals and health care providers in Ontario so they can access the information if it is needed for your clinical care. The study team can tell you what information about you will be stored electronically and may be shared outside of the UHN. If you have any concerns about this, or have any questions, please contact the UHN Privacy Office at 416-340-4800, x6937 (or by email at privacy@uhn.ca).

Study Information that Does Not Identify You
Some study information will be sent outside of the hospital to study collaborators at Saint Michael's Hospital. Any information about you that is sent out of the hospital will have a code and will not show your name or address, or any information that directly identifies you.

Voluntary Participation:
Your participation in this study is voluntary. You may decide not to be in this study, or to be in the study now, and then change your mind later. You may leave the study at any time without it affecting your care.

You may refuse to answer any question you do not want to answer, or not answer an interview question by saying "pass".

Withdrawal from the Study:
The Researchers can take you off the Folate and Dialysis study early for reasons such as:
- Hospitalization
- New medical diagnosis that affects folate absorption
If you leave the study, the information that was collected before you left the study will still be used in order to help answer the research question. No new information will be collected without your permission.

If you decide to leave the study, you have the right to request withdrawal of your blood samples. Let your study team know. They will keep and use any research results that were obtained prior to your withdrawal of consent.

Your identifiable information will remain with the study site. If you would like to withdraw from this study, please let your study team know.

Costs and Reimbursement:

You will not have to pay for any of the procedures or items involved with this study. You will be reimbursed for any parking costs that may occur as a result of you having to come in to the hospital to provide bloodwork on a day that you are not typically scheduled.

You will receive a small financial compensation of $12.00 in the form of a meal voucher for your time spent completing 3 days of weighted food records. This will be provided to you once your food records and blood samples have been completed.

Rights as a Participant:

If you are harmed as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost.

By signing this form you do not give up any of your legal rights against the investigators, or involved institutions for compensation, nor does this form relieve the investigators, or involved institutions of their legal and professional responsibilities.

Conflict of Interest:

The Canadian Foundation of Dietetic Research will reimburse the hospital and researcher for the costs of doing this study. All of the researchers have an interest in completing this study. Their interests should not influence your decision to participate in this study.

Questions about the Study:

If you have any questions, concerns or would like to speak to the study team for any reason, please call: Christine Nash, MSc(C), RD at 416-340-4800 ext.6272.

If you have any questions about your rights as a research participant or have concerns about this study, call the Chair of the University Health Network Research Ethics Board (UHN REB) or the Research Ethics office number at 416-581-7849. The REB is a group
of people who oversee the ethical conduct of research studies. The UHN REB is not part of the study team. Everything that you discuss will be kept confidential.

You will be given a signed copy of this consent form.

Consent:

This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to the use of my information as described in this form. I agree to take part in this study.

Print Study Participant's Name ___________________________ Signature ___________________________ Date ___________________________

My signature means that I have explained the study to the participant named above. I have answered all questions.

Print Name of Person Obtaining Consent ___________________________ Signature ___________________________ Date ___________________________

Was the participant assisted during the consent process? □ YES □ NO

If YES, please check the relevant box and complete the signature space below:

□ The person signing below acted as an interpreter, and attests that the study as set out in the consent form was accurately sight translated and/or interpreted, and that interpretation was provided on questions, responses and additional discussion arising from this process.

Print Name of Interpreter ___________________________ Signature ___________________________ Date ___________________________

Relationship to Participant ___________________________ Language ___________________________

□ The consent form was read to the participant. The person signing below attests that the study as set out in this form was accurately explained to, and has had any questions answered.

Print Name of Witness ___________________________ Signature ___________________________ Date ___________________________

Relationship to Participant ___________________________
Appendix C: PD_HHD Food Record Forms

Home Dialysis
Instructions for Recording Food and Fluid Intake
*Please read carefully before beginning*

1. Keep a record of everything you eat and drink for 3 days. Include all snacks, beverages and alcohol.

2. Please do not change your regular eating habits. It important that we learn what food and drink you have on a regular basis. A complete and accurate record is important for the study.

3. Please record your food and fluid intake on the following days:
   - 2 weekdays that you are receiving dialysis treatment
   - 1 weekend day that you are receiving dialysis treatment

4. List the food item in the appropriate column. Include important descriptions such as brand name, where it was purchased (if eating out), and any condiments added.

5. Weigh all food and fluid items when possible.

6. It is also important to describe the amount eaten. For example:
   - 1 slice of cake or 4 cookies
   - 2 slices of roast beef
   - 1 cup of peas with 1 tsp butter

7. For mixed dishes such as casseroles, weigh the portion you are going to eat and then describe the main ingredients in the dish. For example:
   - 1 piece of lasagna (includes noodles, beef, tomato sauce, ricotta cheese and cheddar cheese)

Checklist
Before you return your food record, please check that you have included:
   - sugar and cream in coffee or tea
   - milk and sugar in cereal
   - salad dressings
   - fats and oils in cooking and frying
   - syrups, sauces, and gravies
   - chips, nuts, and popcorn
   - jams, jellies, honey, and candy
   - alcohol, and soft drinks

3 Day Weighed Food Record_PDHHD_Feb 27 2017
# Food Record – Day 1

<table>
<thead>
<tr>
<th>Name and Description</th>
<th>Amount</th>
<th>Weight of Item</th>
<th>Method of Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scrambled eggs with butter</td>
<td>2 tsp</td>
<td>75 grams</td>
<td>fried</td>
</tr>
<tr>
<td>Kraft cheddar cheese</td>
<td>2 tbsp</td>
<td>30 grams</td>
<td>Shredded</td>
</tr>
<tr>
<td>Red rose tea</td>
<td>1 cup</td>
<td>55 grams</td>
<td>None</td>
</tr>
</tbody>
</table>
## Food Record – Day 2

**Subject ID:** ____________________________  **Date:** ____________________________

<table>
<thead>
<tr>
<th>Name and Description</th>
<th>Amount</th>
<th>Weight of Item</th>
<th>Method of Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green beans with butter</td>
<td>½ cup, 1 tbsp</td>
<td>80 grams</td>
<td>Boiled</td>
</tr>
<tr>
<td>Chicken breast (no skin) with</td>
<td>1, 1 tbsp</td>
<td>140 grams</td>
<td>Baked</td>
</tr>
<tr>
<td>BBQ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

3 Day Weighed Food Record_PDHHD_Feb 27 2017  
Page 3 of 4
# Food Record – Day 3

Subject ID: ___________________________ Date: ___________________________

<table>
<thead>
<tr>
<th>Name and Description</th>
<th>Amount</th>
<th>Weight of Item</th>
<th>Method of Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Example:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red apple</td>
<td>1</td>
<td>105 grams</td>
<td>none</td>
</tr>
<tr>
<td>Breyers chocolate ice cream</td>
<td>1 ½ cups</td>
<td>90 grams</td>
<td>none</td>
</tr>
</tbody>
</table>

---

3 Day Weighed Food Record_PDHHD_Feb 27 2017

Page 4 of 4
In-Centre Hemodialysis
Instructions for Recording Food and Fluid Intake
*Please read carefully before beginning*

1. Keep a record of everything you eat and drink for 3 days. Include all snacks, beverages and alcohol.

2. Please do not change your regular eating habits. It important that we learn what food and drink you have on a regular basis. A complete and accurate record is important for the study.

3. Please record your food and fluid intake on the following days:
   - 1 weekday when you are receiving dialysis treatment
   - 1 weekday that you are NOT receiving dialysis treatment
   - 1 weekend day that you are NOT receiving dialysis treatment

4. List the food item in the appropriate column. Include important descriptions such as brand name, where it was purchased (if eating out), and any condiments added.

5. Weigh all food and fluid items when possible.

6. It is also important to describe the amount eaten. For example:
   - 1 slice of cake or 4 cookies
   - 2 slices of roast beef
   - 1 cup of peas with 1 tsp butter

7. For mixed dishes such as casseroles, weigh the portion you are going to eat and then describe the main ingredients in the dish. For example:
   - 1 piece of lasagna (includes noodles, beef, tomato sauce, ricotta cheese and cheddar cheese)

Checklist
Before you return your food record, please check that you have included:
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- milk and sugar in cereal
- salad dressings
- fats and oils in cooking and frying
- syrups, sauces, and gravies
- chips, nuts, and popcorn
- jams, jellies, honey, and candy
- alcohol, and soft drinks

3 Day Weighed Food Record_IHD_Feb 27 2017
# Food Record – Day 1

Subject ID: ___________________________ Date: ___________________________

<table>
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<th>Name and Description</th>
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</tr>
<tr>
<td>Red rose tea</td>
<td>1 cup</td>
<td>55 grams</td>
<td>None</td>
</tr>
</tbody>
</table>

---

3 Day Weighed Food Record_PDHHHD_Feb 27 2017
# Food Record – Day 2

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<tbody>
<tr>
<td>Green beans with butter</td>
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<td>80 grams</td>
<td>Boiled</td>
</tr>
<tr>
<td>Chicken breast (no skin) with BBQ</td>
<td>1, 1 tbsp</td>
<td>140 grams</td>
<td>Baked</td>
</tr>
</tbody>
</table>

3 Day Weighed Food Record_PDHHD_Feb 27 2017
## Food Record – Day 3

<table>
<thead>
<tr>
<th>Name and Description</th>
<th>Amount</th>
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</tr>
<tr>
<td>Breyers chocolate ice cream</td>
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<td>90 grams</td>
<td>none</td>
</tr>
</tbody>
</table>

---

3 Day Weighed Food Record_PDHHD_Feb 27 2017
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: __________________

DEMOGRAPHICS

Age: _______ Gender: □ Male / □ Female Race: __________________

Highest Level of Education:
□ Less than Secondary School
□ Some Secondary School
□ Secondary School Graduation
□ Some Postsecondary School
□ Postsecondary School Graduation

Employment Status:
□ Working FT __________________
□ Working PT __________________
□ Disability __________________
□ Retired __________________
□ Student __________________
□ Never Worked

Alcohol Use:
□ Current Drinker □ Former Drinker □ Never Drank

Tobacco Use:
□ Current Smoker □ Former Smoker □ Never Smoked

Marijuana Use:
□ Current Smoker □ Former Smoker □ Never Smoked
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: ______________

MEDICAL HISTORY

Primary Cause of Renal Failure:

☐ Glomerulonephritis / Autoimmune Disease __________________________
☐ Nephropathy, Drug Induced __________________________
☐ Polycystic Kidney Disease __________________________
☐ Congenital / Hereditary Renal Disease __________________________
☐ Diabetes __________________________
☐ Renal Vascular Disease __________________________
☐ Other* __________________________
☐ Unknown __________________________

*separate list attached for “Other” causes

Dialysis Modality:

☐ HD - Home
☐ HD – In-center
☐ PD - CAPD
☐ PD - APD
☐ PD - NIPD

Dialysis Vintage: _______ months
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: ______________________

MEDICAL HISTORY

*Primary Cause of Renal Failure (Other):

___ Pyelonephritis/interstitial nephritis, cause not specified
___ Pyelonephritis/interstitial nephritis due to acquired obstructive uropathy—specify
___ Pyelonephritis/interstitial nephritis due to urolithiasis
___ Pyelonephritis, other causes
___ Sickle cell nephropathy
___ Wilms’ tumour
___ Multiple myeloma
___ Amyloid
___ Multi-system disease, other—specify
___ Cortical or acute tubular necrosis
___ Tuberculosis
___ Gout
___ Nephrocalcinosis and hypercalcemic nephropathy
___ Balkan nephropathy
___ Kidney tumour
___ Traumatic or surgical loss of kidney
___ HIV nephropathy
___ Other identified renal disorders—specify
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: ________________

MEDICAL HISTORY

Co-Morbidities:

☐ Diabetes - Type _____ ☐ Retinopathy ☐ Neuropathy
☐ Hyperlipidemia
☐ Hypertension
☐ Heart Disease
  ☐ Hypertensive ________________________
  ☐ Ischemic ________________________
  ☐ Cerebrovascular ________________________
  ☐ Inflammatory ________________________
  ☐ Other ________________________
☐ Lung Disease
☐ Malignancy
  ☐ Blood ________________________
  ☐ Breast ________________________
  ☐ Digestive/Gastrointestinal ________________________
  ☐ Eye ________________________
  ☐ Endocrine/Neuroendocrine ________________________
  ☐ Genitourinary ________________________
  ☐ Osteoporosis ________________________
  ☐ Transplant ________________________
  ☐ Other ________________________

☐ Gynecological ________________________
☐ Head & Neck ________________________
☐ Musculoskeletal ________________________
☐ Neurologic ________________________
☐ Respiratory/Thoracic ________________________
☐ Skin ________________________
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

MEDICAL HISTORY

*Expanded List of Malignancies:

Blood:
- Leukemia
- Lymphoma
- Myeloma

Digestive/Gastrointestinal
- Anal
- Appendix
- Bile Duct
- Carcinoid
- Colon
- Esophageal
- Gallbladder
- Gastric
- Liver
- Pancreatic
- Rectal
- Small Intestine
- Stomach

Gynecological:
- Cervical
- Ovarian
- Uterine
- Vaginal
- Vulvar

Endocrine/Neuroendocrine:
- Adrenocortical
- Merkel cell
- Neuroendocrine, Lung
- Parathyroid
- Pituitary
- Thyroid
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

MEDICAL HISTORY

*Expanded List of Malignancies:

**Genitourinary:**
- Bladder
- Kidney
- Penile
- Prostate
- Testicular
- Urethral

**Gynecological:**
- Cervical
- Ovarian
- Uterine
- Vaginal
- Vulvar

**Head and Neck:**
- Laryngeal
- Mouth
- Nasopharyngeal
- Oral Cavity & Lip
- Oropharyngeal
- Parathyroid
- Salivary Gland
- Throat
- Thyroid

**Muscoskeletal:**
- Bone
- Soft Tissue Sarcoma

**Neurologic:**
- Brain
- Neuroblastoma
- CNS Lymphoma
- Pituitary
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

MEDICAL HISTORY

*Expanded List of Malignancies:

Respiratory/Thoracic:
- Lung
- Malignant Mesothelioma
- Thymoma
- Thymic

Skin:
- Cutaneous T-Cell Lymphoma
- Kaposi Sarcoma
- Melanoma
- Merkel Cell
- Skin
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: 

MEDICATIONS

☐ Anemia
  ☐ EPO ☐ Aranesp ☐ Fe

☐ Anti-Anxiety
  ☐ Diazepam ☐ Lorazepam ☐ Sertraline ☐ Other

☐ Blood Pressure
  ☐ ☐

☐ Blood Pressure - Diuretics
  ☐ Furosemide ☐ HCL ☐ Metolazone ☐ Spironolactone ☐ Other

☐ Bone/Mineral Disorders
  ☐ PO4 Binder ☐ Vitamin D ☐ Cinacalcet

☐ Bowel Regulation
  ☐ Docusate Na+ ☐ Senna ☐ Lactulose

☐ Diabetes
  ☐ OHA
  ☐ Insulin

☐ Gastroparesis/Reflux
  ☐ ☐

☐ Heart
  ☐ ☐
Subject ID: ________________

MEDICATIONS

☐ Immunosuppressive
   ☐ ________________ ☐ ________________ ☐ ________________

☐ Lipid Lowering
   ☐ Atorvastatin ☐ Pravastatin ☐ Rosuvastatin ☐ Simvastatin ☐ Other _____

☐ Other
   ☐ ________________ ☐ ________________ ☐ ________________

VITAMIN AND MINERAL SUPPLEMENTS

☐ Multi-vitamin
   ☐ Replavite ☐ Other ________________ ☐ Other ________________

☐ Vitamins
   ☐ ________________ ___ times per day/week
   ☐ ________________ ___ times per day/week
   ☐ ________________ ___ times per day/week
   ☐ ________________ ___ times per day/week

☐ Minerals
   ☐ ________________ ___ times per day/week
   ☐ ________________ ___ times per day/week
   ☐ ________________ ___ times per day/week
   ☐ ________________ ___ times per day/week
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: ____________

MEDICATIONS

☐ Interacts with Folate metabolism:

____________________

____________________

____________________

____________________

____________________

____________________

____________________

____________________

____________________

____________________
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: ______________

NUTRITIONAL SUPPLEMENTS

☐ Beverage
  ☐ ______________  ___ bottle or tetra box / ___ times per day/week
  ☐ ______________  ___ bottle or tetra box / ___ times per day/week

☐ Pudding
  ☐ Boost __________  ___ pudding cup / ___ times per day/week
  ☐ Ensure __________  ___ pudding cup / ___ times per day/week
  ☐ Other: __________  ___ pudding cup / ___ times per day/week

☐ Powder
  ☐ Beneprotein  ___ scoop / ___ times per day/week
  ☐ Other: __________  ___ scoop / ___ times per day/week

☐ Gel
  ☐ Prosource  ___ ml / ___ times per day/week
  ☐ LiquaCel  ___ ml / ___ times per day/week
  ☐ Other: __________  ___ ml / ___ times per day/week

HERBAL REMEDIES

☐ ______________  taken ___ times/day
☐ ______________  taken ___ times/day
☐ ______________  taken ___ times/day
☐ ______________  taken ___ times/day
☐ ______________  taken ___ times/day
DATA COLLECTION SHEET FOR NUTRITION RESEARCH STUDY

Subject ID: _____________

NUTRITIONAL ANALYSES (FSHA)

☐ Kcal:
☐ Protein:
☐ Dietary Folate:
☐ Dietary B6:
☐ Dietary B12:

BIOCHEMICAL ANALYSES

☐ Hematocrit:
☐ Serum folate:
☐ RBC folate:
☐ UMFA:
☐ B6:
☐ B12:
☐ Hcy:
☐ CRP:
☐ Alb:
☐ Creat:
☐ Urea:

NUTRITIONAL INDICES

☐ Ht / M²:
☐ Wt:
☐ BMI:
### Appendix F: Subjective Global Assessment Form

<table>
<thead>
<tr>
<th>STUDY PARTICIPANT ID#:</th>
<th>Date:</th>
</tr>
</thead>
</table>

**WEIGHT/WEIGHT CHANGE:** *(Included in K/D01 SGA)*

1. Baseline Wt: ____________ *Dx weight from 6 months ago*
   
   Current Wt: ____________ *Dx weight today*
   
   Actual Wt loss/past 6 mo: ____________ % loss: ____________ *(actual loss from baseline or last SGA)*

2. Weight change over past two weeks: ____________ *No Change*  
   
   ____________ *Increase*  
   
   ____________ *Decrease*  

**DIETARY INTAKE**  

No Change ___  *Adequate*  

No Change ___  *Inadequate*  

1. Change:  

   - Sub optimal Intake: ____________  
     
   - Protein: ____________  
     
   - Kcal: ____________  
     
   - Full Liquid: ____________  
     
   - Hypocaloric Liquid: ____________  
     
   - Starvation: ____________

**GASTROINTESTINAL SYMPTOMS** *(Included in K/D01 SGA-anorexia or causes of anorexia)*

<table>
<thead>
<tr>
<th>Symptom:</th>
<th>Frequency:</th>
<th>Duration:</th>
</tr>
</thead>
<tbody>
<tr>
<td>None:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Never, daily, 2-3 times/wk, 1-2 times/wk  
  
- > 2 weeks, < 2 weeks

**2. FUNCTIONAL CAPACITY**

<table>
<thead>
<tr>
<th>Description</th>
<th>Duration:</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Dysfunction</td>
<td></td>
</tr>
<tr>
<td>Change in function</td>
<td></td>
</tr>
<tr>
<td>Difficulty with ambulation</td>
<td></td>
</tr>
<tr>
<td>Difficulty with activity (Patient specific “normal”)</td>
<td></td>
</tr>
<tr>
<td>Light activity</td>
<td></td>
</tr>
<tr>
<td>Bed/chair ridden with little or no activity</td>
<td></td>
</tr>
<tr>
<td>Improvement in function</td>
<td></td>
</tr>
</tbody>
</table>

**3. DISEASE STATE/COMORBIDITIES AS RELATED TO NUTRITIONAL NEEDS**

<table>
<thead>
<tr>
<th>Primary Diagnosis</th>
<th>Comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normal requirements</th>
<th>Increased requirements</th>
<th>Decreased requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acute Metabolic Stress:</th>
<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PHYSICAL EXAM**

<table>
<thead>
<tr>
<th>Loss of subcutaneous fat (Below eye, triceps, biceps, chest) <em>(Included in K/D01 SGA)</em></th>
<th>Some areas</th>
<th>All areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle wasting (Temple, clavicle, scapula, ribs) <em>(Included in K/D01 SGA)</em></td>
<td>Some areas</td>
<td>All areas</td>
</tr>
<tr>
<td>Edema (Related to undernutrition/use to evaluate weight change)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**OVERALL SGA RATING:**

Very mild risk to well-nourished=6 or 7 most categories or significant, continued improvement.

Mild-moderate = 3, 4, or 5 ratings. No clear sign of normal status or severe malnutrition.

Severely Malnourished = 1 or 2 ratings in most categories/significant physical signs of malnutrition.
Appendix G: Medications That Affect Folate Metabolism (Bailey, 2010 [24])

Anticonvulsants
Carbamazepine
Phenytoin
Valproic acid
Lamotrigine
Primidone
Phenobarbital

Antifolates
Methotrexate
Pemetrexed
Trimethoprim
Pyrimethamine
Proguanil
Triamterene
Sulfasalazine

Disturbance of Methyl Balance
Levodopa
Niacin
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