Vitamin B12 Deficiency in Older Adults Living in Ontario Long-Term Care Homes: Protocols, Testing Procedures and Prevalence

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

BACKGROUND: Vitamin B12 status is a relevant topic to older adults and deficiency has avoidable clinical ramifications. While classical symptoms of B12 deficiency are either hematologic or neurologic, presentation of these symptoms are not reliable as diagnostic tools as they are not present in all cases. Furthermore, symptoms of subclinical deficiency are nonspecific; a blood test is required for B12 status determination and as a first step towards treatment. B12 screening and treatment protocols in LTC have not been documented and no studies have been designed to establish vitamin B12 deficiency prevalence at admission or to describe incidence within this population. Furthermore, studies on prevalence using a cross-section of the LTC population lack representativeness.

PURPOSE: The purpose of this thesis was to investigate vitamin B12 status in Ontario LTC homes. In particular, this thesis aimed to establish: what protocols and procedures are used in LTC for testing and treatment for B12 deficiency; the prevalence and what resident characteristics are associated with B12 deficiency at admission; and the incidence of B12 deficiency one-year post-admission.

METHODS & FINDINGS: Two studies were conducted as part of this thesis work.

Study 1: Forty five Ontario LTC homes were recruited using stratified random sampling from two sample frames, representing 5243 residents in this cross-sectional study. Phone interviews were conducted with directors of care using a standardized interview schedule for data collection. Interviewees were asked about policies and procedures for B12 testing, proportion of residents receiving B12 treatment and modality (oral, intramuscular, subcutaneous) used for B12 supplementation in their LTC home. Two-thirds (30/45) of participating sites routinely tested for B12 at admission, and 88% (34/40) of reporting homes conducted follow-up B12 testing. Furthermore, cut-off levels to define B12 deficiency varied between homes, presenting a challenge to determine prevalence. On average, 25% of current residents were receiving B12 supplements; the most common treatment modality was intramuscular injections (53%) followed by oral supplements (44%). Due to variability in practices relating to detection and treatment of B12 deficiency, LTC residents are potentially at risk for undetected deficiency.

Study 2: A retrospective prevalence study was conducted across eight sites in Southwestern Ontario within one LTC organization. Random proportionate sampling was used to identify resident charts for inclusion, with replacement as necessary. Eligibility criteria were: residents being 65 years or older at admission and presence of admission bloodwork, including serum B12 levels. Resident

characteristics considered for this study included: basic demographics, B12 supplementation prior to admission, medications, diagnoses, functional independence, cognitive performance, and nutritional status. 1005 charts were screened to obtain 412 total eligible residents. Average B12 deficiency prevalence at admission was 14% but only 48% of residents had normal B12 status at admission. Admission B12 status was significantly and positively associated with supplementation prior to admission (χ^2=60.8, $p<0.001$). There was a significantly ($p<0.01$) higher proportion of B12 supplementation in those with dementia than those without. Prevalence and incidence of B12 deficiency one year post-admission was 7% and 4% respectively. With numerous sites and large sample size, the present study provides better insight into B12 status than previous work, and is the first to report both admission prevalence as well as one-year incidence. In general, B12 status in this LTC sample was poor, yet supplementation was a protective factor.

OVERALL CONCLUSIONS: A relatively large proportion of LTC residents are impacted by B12 deficiency. While some protocols are in place for testing and treatment, some LTC residents are potentially at risk for undetected B12 deficiency. Treatment methods are generally effective, and should be used to deter progression to a deficient state. Prevalence was consistent with previous findings, including a high degree of variability depending on the cut-point used to define deficiency. This will remain a challenge, however screening, treating, and monitoring is important to prevent clinical manifestations, and deter symptoms associated with subclinical deficiency from presenting.

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Dedication

To my two best friends, my husband and my sister, who have always been there with me to: dream big, share in the wonderment of life, find courage, and who constantly inspire me to be a better person in every aspect of life. From you, I draw strength.

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List of Abbreviations

ADL: Activities of daily living

ATD: Alzheimer's-type dementia

AI: Adequate intake

BMI: Body mass index

CBLA: Competitive binding luminescence assay

Hcy: Homocysteine

holoTC: Holotranscobalamin

HPLC: High performance liquid chromatography

IF: Intrinsic factor

LTC: Long-term care

Mcg: micrograms

MMA: Methylmalonic acid

MTHFR: Methylenetetrahydrofolate reductase

OAHNSS: Ontario Association of Non-Profit Homes and Services for Seniors

OHTAC: Ontario Health Technology Advisory Committee

OLTCA: Ontario Long Term Care Association

RDA: recommended dietary allowance

SAM: S-adenosyl-methionine

SV: Schlegel Villages

TCI: Transcobalamin I (R-protein)

TCII: Transcobalamin II

TCIII: Transcobalamin III

Chapter 1
Introduction

This thesis has been a very personal journey for me. All of this work stemmed from my own B12 deficiency experience, rooted itself in my exposure to Professor Keller's work with micronutrients which then flourished into a quest to seek answers and advance our understanding of B12 deficiency in long-term care, with an underlying aim to enhance quality of life and care with these learnings. Vitamin B12 deficiency has tangible endpoints with known consequences such as anemia and neurological damage. When I was diagnosed with B12 deficiency, I had neither of those classic endpoints despite my deficient status by any of the prevailing cut-points. I went to the doctor with complaints of tiredness, inability to concentrate and, quite frankly, grumpiness. My experience and amelioration of symptoms with proper treatment opened my eyes to the magic of adequate B12 status and provided me with a healthy respect for how my nonspecific symptoms were related to a nutrient, which when deficient could impact my ability to live life to its fullest.

Working at the RIA opened my eyes further, this time, to the world of long-term care, a population where every ounce of independence counts. At one of Professor Keller's presentations at operational planning, she mentioned how older adults are more likely to be B12 deficient. With that, my interest was piqued. How big of a problem was this? What proportion of residents like me, are impacted by B12 deficiency and how could this impact their quality of life? How is B12 deficiency identified and treated in long-term care? These questions, based in personal experience, are the basis for this thesis.

This thesis consists of several chapters that summarize my learnings and answers many of the questions outlined above. Chapter 2 provides background on vitamin B12 including: what is B12; its biological roles; how it is metabolised and measured; how vitamin B12 status is defined; why vitamin B12 is important to residents in LTC and factors that can influence B12 status in this population and beyond. Chapter 3 provides rationale and poses research questions for the subsequent two research chapters. Two studies were conducted as part of this thesis work, each of which are outlined in an individual chapter in manuscript form (Chapters 4 and 5), followed by Chapter 6, the overarching discussion linking the manuscripts back to the research questions and providing main conclusions.

I hope you enjoy this journey through my curiosity and learnings.

Chapter 2
Background: The what, how, and why for studying B12 deficiency in LTC

2.1 What is vitamin B12?

Vitamin B12 is a water-soluble essential micronutrient required for: the production of blood cells, maintaining nerves, regulating B12-dependent processes (see *What are the biological roles of vitamin B12?* on page 3), and maintaining intact gastrointestinal mucosa [1]. Vitamin B12 is the only water soluble vitamin stored by the human body for any sufficient time; the liver is the primary storage site containing more than 1.5 mg of the vitamin [2]. Since turnover of vitamin B12 is approximately 0.1% per day, it would take between 2-10 years for B12 deficiency to develop even when a diet is completely lacking in the vitamin [2, 3].

Vitamin B12 refers to a family of complex molecules with a central cobalt atom surrounded by four reduced pyrrole rings, which is referred to as cobalamin. The form of cobalamin or B12 depends on the upper axial ligand bond linked to the cobalt atom [1, 4]. While there are five forms of B12 (methylcobalamin, cyanocobalamin, hydroxycobalamin, aquocobalamin, and adenosylcobalamin [1, 4]) methylcobalamin and adenosylcobalamin are the only forms of B12 used for metabolic processes in the human body.

2.1.1 Vitamin B12 transport proteins

There are four known proteins involved in the absorption and transport of vitamin B12: intrinsic factor, R-protein (transcobalamin I), transcobalamin II, and transcobalamin III. Intrinsic factor is made by parietal cells, which line the stomach. It plays a pivotal role in absorption of B12 from the small intestine into blood [4]. R-protein which is also known as haptocorrin or transcobalamin I is found in most body fluids [4]. While it has no known function, B12 bound to R-protein accounts for 80% of total plasma B12 [5, 6] and may contribute to falsely high B12 measurements [7]. Transcobalamin II, also known as holotranscobalamin (holoTC) when bound to B12, is found in blood plasma [4]. HoloTC is the only biologically active form of B12 and is responsible for the transport of B12 to cell membrane receptors (part of receptor mediated endocytosis) [4]. HoloTC accounts for 6-25% of total plasma B12 [1, 8, 9] and is believed to be the most sensitive marker to depletion or repletion of B12 [5, 9, 10]. Lastly, transcobalamin III is made by granulocytes [4, 11]

and has an unknown function [5]. However, it has clinical significance as elevated levels of transcobalamin III, as seen in chronic myelogenous leukemia, may cause falsely high measures of B12 [4].

2.2 What are the biological roles of vitamin B12?

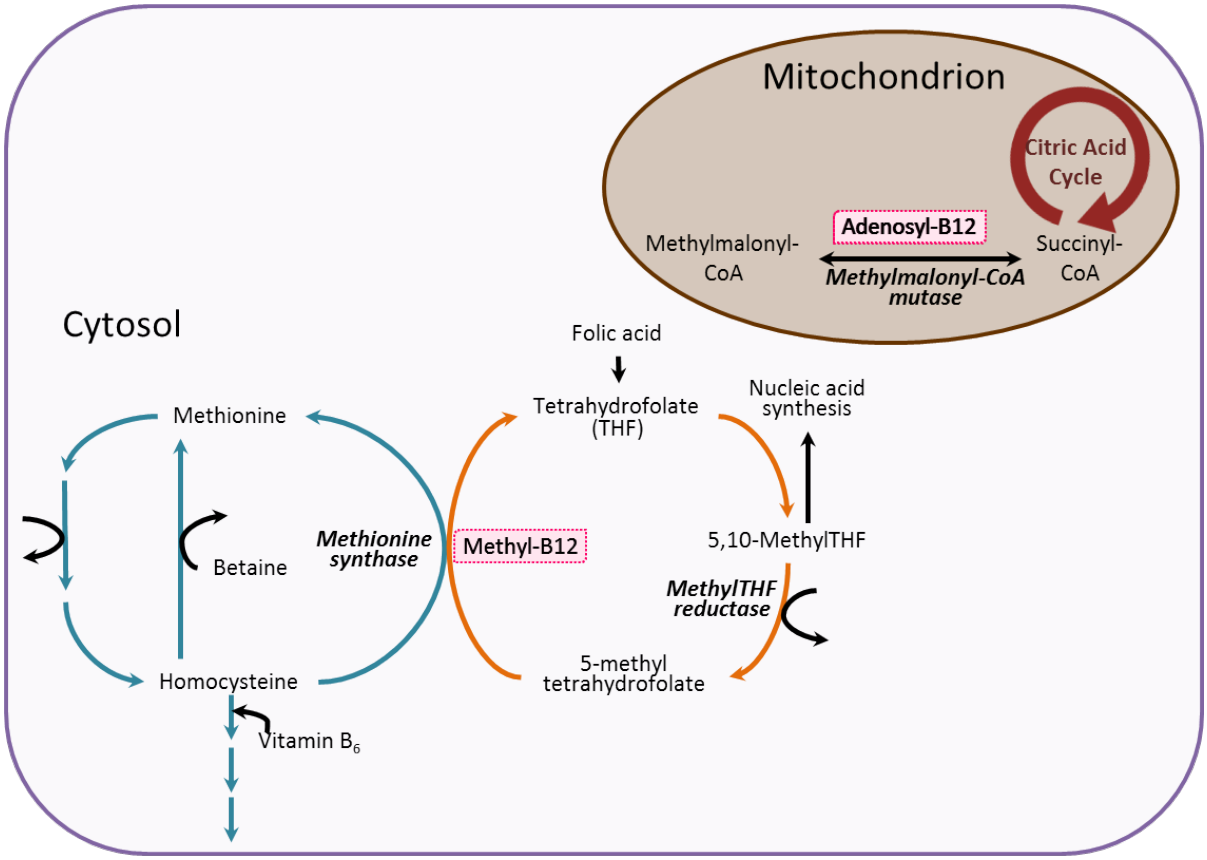


Figure 1: *A simplified model of vitamin B12-dependent processes*. Adapted from Andrès et al., 2004 [12].

While oversimplified, the following section provides an overview of the biological processes in which B12 is involved including its relationship to energy metabolism, folate and vitamin B6 and is summarized in **Figure 1**. Two forms of vitamin B12 are essential cofactors for biological processes: 1) adenosyl-B12 in the mitochondria for proper energy metabolism, and 2) methyl-B12 in the cytosol of cells for methylation reactions including nucleic acid synthesis.

In the mitochondria, vitamin B12 is involved in oxidation reactions of branch chain and odd-chain fatty acids (e.g., threonine, valine, methionine) [13-15]. Vitamin B12 (adenosylcobalamin) is required as a cofactor by the enzyme methylmalonyl-CoA mutase for the conversion of methylmalonyl-CoA to succinyl-CoA which feeds into the citric acid cycle for energy metabolism [13-15]. In vitamin B12 deficiency, methylmalonyl-CoA builds up and gets converted to methylmalonic acid (MMA) [14-16] which is a myelin destabiliser [17]. It also leads to less succinyl-CoA to feed into the citric acid cycle yielding less efficient energy metabolism [14-16].

In the cytosol, vitamin B12 is involved in the conversion of the amino acid, homocysteine into the amino acid methionine. Vitamin B12 (cobalamin) is required as a cofactor by the enzyme methionine synthase for the transfer of a methyl group from 5-methyltetrahydrofolate (derived from folic acid) onto B12 (methylcobalamin) to form tetrahydrofolate. The methylcobalamin is then used as a methyl donor in the conversion of homocysteine to methionine, through methyl group addition[18]. Generated methionine is required for the formation of s-adenosyl-methionine (SAM) which is a universal methyl donor for methylation reactions [18]. Vitamin B12 deficiency decreases the amount of methionine generated and downstream impacts every cell in the body from the formation of myelin [1, 18], neurotransmitters [1, 15], phospholipids [1, 15], to nucleic acid synthesis [18] and RNA and DNA methylation [10, 18].

Due to this metabolism, a by-product of vitamin B12 deficiency is elevated levels of homocysteine (Hcy), since it cannot be converted to methionine [17]. Elevated levels of Hcy have been shown to be associated with dementia [19, 20], Alzheimer's [20-22], carotid-artery stenosis [4, 23], stroke [4, 24], a three-fold increased risk for myocardial infarction [4, 23], and a four-fold increased odds ratio for venous thrombosis [4, 25, 26]. While association does not infer causation, a recent meta-analysis provides supporting evidence for an association between low vitamin B12 and folic acid with high homocysteine levels [27]. As such, elevated homocysteine levels may be a modifiable risk factor amenable to B12 and or folate therapy.

While vitamin B12 maintains homocysteine levels, so too does vitamin B6 and another compound, betaine. Vitamin B6 is responsible for transamination of homocysteine to cysteine, thus improved B6 status may also reduce elevated Hcy levels or may elevate Hcy levels in the case of B6 deficiency. In the cytosol of most cells of the body, betaine is present and is responsible for the conversion of homocysteine to methionine through an alternative pathway other than via vitamin B12. While betaine can help rescue the effect of B12 deficiency by preventing elevations of homocysteine [21,

22, 28, 29], it is not present in the brain, which may increase the importance for adequate B12 status with respect to healthy brain function.

2.3 What are food sources and how is vitamin B12 metabolised?

Meat and dairy products are the only naturally occurring sources of vitamin B12 as it is created by microorganisms. The amount of B12 per serving varies widely depending on the source. For example one 250 mL serving of skim milk contains 1.3 mcg; beef liver, tuna, ground beef and chicken contain between 52.9-64.4 mcg, 8.2-9.3 mcg, 2.4-2.7 mcg, 0.2-0.3 mcg of B12 per 2.5 oz (75 g) serving, respectively; two large eggs contain 1.5-1.6 mcg, and 175 g of fruit-bottom yogurt contains 0.8 mcg of B12 [30]. Plant-based foods require fortification [18]. Some examples of foods fortified with B12 are soy milk (1 mcg per 250 mL serving) [31], and dry cereals, such as All Bran (5.2 mcg per 31 g serving) and Multigrain Cheerios (6.2 mcg per 30 g serving) [32]. For foods naturally containing B12, vitamin B12 is bound to proteins or polypeptides. However, foods in which B12 has been added contain crystalline B12, [33] typically in the form of cyanocobalamin [34]. In the stomach, pepsin and hydrochloric acid are responsible for liberating the protein-bound B12 to its free form. The parietal cells which line the stomach are also responsible for producing the transport protein, intrinsic factor (IF) which plays a later role in absorption [18]. As naturally occurring B12 or non-physiologic doses of crystalline B12 empty from the stomach into the upper portion of the small intestine (duodenum), the transport protein, R-protein (transcobalamin I), binds to the B12 to form a complex. Once inside the duodenum, pancreatic proteases hydrolyze the R-protein to liberate free B12, which then binds to IF. The IF-B12 complex is carried from the duodenum through the middle portion of the small intestine (jejunum) and to the lower portion of the small intestine (ileum), which contains surface receptors for the IF-B12 complex. These surface receptors are called cubam and are comprised of a complex of two proteins, cubulin and amnionless. Cubam is responsible for the absorption of the IF-B12 complex into the intestinal absorptive cells (enterocytes) through receptor-mediated endocytosis and the B12 dissociated from IF [11, 13, 18, 35].

From here, vitamin B12 is absorbed into portal blood in one of two ways; through the major absorptive pathway or the minor absorptive pathway. In the major absorptive pathway, transcobalamin II (TCII) binds to the B12. This process accounts for at least 60% of oral B12 absorption [2]. However, the rate-limiting factor for absorption through this pathway is the capacity for the ileum mucosa to endocytose the IF-B12 complex; the maximum capacity is 1.5 μg [1]. The minor absorptive pathway is via passive diffusion, which, accounts for approximately 1-5% of

absorption [2, 11, 18]. However, physiologic doses of crystalline B12 primarily rely on this minor absorptive pathway [11]. Once in the portal blood, B12 is found bound to either TCI (R-protein) (~80%), TCII (~20%) or TCIII. Tissues have receptors for TCII and take up the TCII-B12 complex via endocytosis. Lysosomes then engulf the TCII-B12 complex and lyse it into free B12 for release into the cytosol of the cell [11, 13, 18, 35].

While malabsorption affects 10-30% (through atrophic gastritis) of older adults and impacts the ability to absorb B12 due to the major absorptive pathway, there is evidence to suggest that the minor absorptive pathway (i.e., through passive diffusion) remains intact with age [11, 36]. Thus food fortified with B12 is more bioavailable than food-bound B12 and older adults are advised to get B12 from B12 plant-based fortified foods instead of food-bound B12 [11].

2.4 How is vitamin B12 status measured?

Vitamin B12 status can be measured in one of two ways, either through blood (directly or indirectly measuring B12) or through urine biomarkers. The most commonly used method is through biomarkers directly measuring B12 in serum or by measuring holoTC. Indirect methods use serum metabolites of B12-dependent reactions such as methylmalonic acid (MMA) or homocysteine. Common methods for testing, a description of the method, reference ranges, sensitivity, specificity, and pros and cons are mentioned below; each measurement has its strengths and weaknesses.

Today, serum vitamin B12 is most commonly measured through a competitive binding luminescence assay (CBLA). First, vitamin B12 is liberated to the free form (i.e., not food-bound) of a patient's blood serum which competes with added labelled-B12 (LB12) for binding to a known quantity of purified intrinsic factor forming, either IF-B12 or IF-LB12. The IF bound to either B12 or LB12 is isolated by binding to paramagnetic beads. Then, a conjugate that specifically binds to the IF-LB12 is added followed by a substrate in which to bind the IF-LB12-conjugate. The result is chemiluminescence proportional to the amount of IF-LB12 with an inverse relationship of luminescence to the amount of B12 added from the patient [3]. The reference range for serum B12 varies but typically ranges between 78 pmol/L (105 pg/mL) to 258 pmol/L (350 pg/mL) [1, 37-41]. The sensitivity of serum B12 is high (between 90-95%) with clinical manifestations (i.e., hematologic or neurologic etiologies) [3]. While specificity has not been formally determined, it has been estimated to be between 40 and 80% but is worse in the absence of clinical symptoms [3]. One primary advantage of this method is that serum B12 tests are relatively inexpensive (i.e., €17 [5], $10-

15 CAD [42] per test) and is readily available. However, there is a risk of false-negative results from confounding factors due to the nature of CBLA. Anything that may interfere and cause decreased luminescence will yield an overestimation of B12 levels. In cases of pernicious anemia, there are IF antibodies naturally present in the blood sample which interact with the labelled IF [3]. This results in less luminescence falsely indicating higher levels of B12 and thus provides a false-negative for B12 deficiency [3]. Similarly, if luminescence is increased, indicating lower B12, as seen in haptocorrin deficiency, folate deficiency, multiple myeloma, HIV, pregnancy and oral contraceptive use, there is a risk of false-positive for B12 deficiency results [3]. These false positive and false negatives make it more difficult for prevalence estimates to be accurate. Especially in the case of pernicious anemia where 2-5% of older adults are affected [43], this may in part account for the wide margin of prevalence estimates.

Holotranscobalamin II (holoTC) is another way to directly measure B12 in serum; blood samples are analysed using a holoTC-specific monoclonal antibody assay [9]. Essentially there are two forms of antibodies, one to capture the holoTC as it passes by, which is mounted to a sensor chip, and another form of free-floating antibodies to detect the holoTC; the holoTC is captured on the chip followed by detection that the holoTC is present on the chip [44]. The reference range for the holoTC-specific monoclonal antibody assay is said to be between 32-35 pmol/L yielding a sensitivity of 75-80% and a specificity of 55-60% [1, 5]. However, poor renal function results in elevated holoTC levels [45]. This measurement is still undergoing testing and is not commonly used. Specifically, its diagnostic power may not be sufficient to distinguish between deficient and nondeficient status and there is evidence to suggest that while holoTC may have low measurement variability at levels of B12 ingested from food, it has a high degree of diurnal variation with physiologic doses of B12 [9]. The relative cost of holoTC may also be a barrier for its use at €50 per test and no readily available cost estimate for Canada [42]. Additionally, holoTC is prone to a higher rate of false positives, especially in impaired renal function and liver disease [45].

Homocysteine (Hcy) is a surrogate biomarker for B12 that can be measured using high-performance liquid chromatography (HPLC) with fluorometric detection [19]. HPLC consists of a column with specific beads with characteristics complementary to the substance of interest for measurement. Once a sample is run through the column, molecules are separated based on their affinity for the column, other liquids are passed through the column that interact with molecules bound to the column in different ways, eluting them off at different times; this is referred to as

retention time [46]. If different molecules have the same binding affinity for the column, they will elute off the column at the same time which is problematic; thus, the substance of interest is contaminated with another. One way around this is the additional use of fluorometric detection where a known wavelength of light interacts with a compound of interest (in this case it is homocysteine) in a specific way to produce fluorescence. The degree of fluorescence is proportional to the concentration of the compound of interest thus the relative "glow" of the sample indicates how much homocysteine is present. Homocysteine is considered to be elevated at concentrations above 10 µmol/L [1]. The sensitivity of Hcy for vitamin B12 deficiency is very high at over 95%, yet it has an unknown specificity [3]. Positives of this test are that it improves diagnostic sensitivity [3] when used in tandem with serum B12 and it more accurately portrays the metabolic function at the tissue level than does B12 [4]. However, homocysteine is not a specific biomarker for B12 [4]; Hcy is elevated in folate deficiency and with certain genetic variants, which decreases the diagnostic utility of Hcy when used alone. It is also relatively expensive ($65 CAD per test [42]) compared to serum B12.

Methylmalonic acid is a surrogate biomarker of B12 since it is a metabolite of a vitamin B12-dependent reaction [4]. As such, MMA is a measure of functional B12 deficiency and is an unofficial gold-standard [3]. MMA can be measured using gas chromatography-mass spectrometry [5]. Molecules are separated based on their affinity for a stationary phase of a capillary column within the gas chromatograph and the retention time to elution of the molecule followed by a pass through a mass spectrometer further separating molecules based on their charge and mass [47]. The reference range of MMA is 50-300 nmol/L with a high sensitivity (95%), but undetermined [1] or low specificity [3]. Several advantages exist for this method of measurement: it can be assessed in urine [4] (using an adjusted reference range) which is less invasive than a blood draw; it can improve diagnostic sensitivity [3] when used in tandem with another measurement; it accurately portrays metabolic function at the tissue level [4]; and there is some evidence to suggest that it may be a more sensitive biomarker for cognitive function [5]. While MMA testing has considerable benefits, it is also an imperfect test; its relative cost compared to serum B12 at $105 CAD [42] per test (or €60 [5]) is a barrier for systemic use, and false positives are common in those with decreased renal function [4]. Since impaired renal function is more common in older adults, the diagnostic utility of this test decreases in this population.

One additional measure is the Schilling test which is used to determine if an individual can absorb B12. This test is not used as frequently as it once was because it relies on radio-labelled B12. In phase

one of this test, radioactive B12 is given orally, followed by a dose of nonradioactive B12. Phase two occurs 24 hours later where radioactive B12 plus intrinsic factor is given followed by a dose of nonradioactive B12. Interpretation of the results are as follows: if the results from phase one are abnormal and phase two are normal, this implies the individual has absorption problems consistent with pernicious anemia (i.e., they do not make sufficient IF for absorption). If both phase one and two are abnormal, this implies an alternative cause for B12 deficiency [4].

2.4.1 Dietary reference intakes (DRIs) for B12

The dietary reference intakes are a group of reference values (i.e., recommended dietary allowance (RDA), adequate intake (AI), tolerable upper intake level (UL), and estimated average requirement (EAR)) that provide quantitative estimates of nutrient requirements used in a healthy population for planning and assessing dietary intakes [36]. While the RDA is most relevant to this work, it is worth noting that no tolerable upper limit has been set [36], despite the relatively long-term storage of this vitamin. The RDA represents the intake level that meets the estimated nutrient requirement for 98% of a gender and age group within a healthy population. For vitamin B12, in those 70 years or older, the RDA has been defined by countries as follows: 2.4 mcg/day in the US, Canada, Australia and New Zealand [36, 48, 49], and 3 mcg/day in Germany, Austria and Switzerland [1].

It is unclear as to whether these recommendations for B12 are appropriate for older adults. There is evidence to suggest that mild B12 deficiency needs a daily oral dose of more than 200 times the RDA to normalize B12 levels and reverse hematological and neurological symptoms [1]. Yet, the average daily intake in the US for community dwelling adults aged 60 years or older, was estimated in males and females, respectively, to be 5.4 mcg and 3.7 mcg from dietary intake alone and 17.4 mcg and 31.0 mcg from supplemental intake [36]. This implies that, in individuals with mild deficiency, even in those taking supplements, older adults are not consuming more than 200 times the RDA and therefore cannot normalize their B12 levels without pharmacologic doses of supplements. This may be especially problematic for older adults living in long-term care where poor food intake is common [50].

2.4.2 Common Reasons for Deficiency

While vitamin B12 deficiency can arise for many reasons, generally, all factors fit into one of the following categories: inadequate intake; dysfunction of food-cobalamin absorption; or dysfunction of transport.

Inadequate intake of B12 may be of concern in a strict vegan diet where normal sources of food-B12 are avoided. While generally adequate intake is not a concern in older adults, those living in LTC may additionally be at increased risk of inadequate intake of B12 due to the high prevalence of malnutrition ranging between 30-70% [2, 51, 52]. Despite one study investigating the cause of B12 deficiency in older adults, reporting only 2% of cases due to insufficient B12 intake [12], others report B12 intake to be one third [53] and two thirds [51] below recommendations. It could be hypothesized that since older adults living in LTC are more likely to have low B12 intake and that it is impossible for this population to normalize their B12 levels through food alone, they are at an increased risk for reduced B12 levels, especially if they do not take supplements.

A fairly recent review identified the leading cause of B12 deficiency in older adults to be food-cobalamin malabsorption, accounting for 60-70% of cases [2]. Food-cobalamin malabsorption refers to the inability to adequately break B12 apart from food or from transport proteins [2, 54] and can be caused by a number of factors such as: gastric atrophy either related or unrelated to *H. pylori* infections [8]; bacterial overgrowth [8, 35]; alcoholism [8]; or use of certain medications (H2 blockers [8], PPIs [8, 55]) or metformin [2, 56-58]. Pernicious anemia occurs in those who lack intrinsic factor; older adults with pernicious anemia accounted for 15-33% of B12 deficiency cases [2, 8, 12, 35]. Diseases that impact absorption more broadly such as lymphoma, Crohn's disease, celiac disease, may also cause B12 deficiency [2].

Heredity of rare disease and genetic factors also play a role in B12 deficiency albeit to a much lesser extent in older adults since most appear to be autosomal recessive [2]. Imerslund-Grasbeck syndrome, a rare disease often diagnosed in the first few years of life, impacts intestinal transport of B12 in which cell surface receptors responsible for receptor-mediated endocytosis fail to function properly [59]. Additionally numerous other genetic mutations may impact B12 status through ineffective transport proteins or the ability to create intrinsic factor [13, 59].

2.4.3 Symptoms of B12 deficiency:

If left untreated, vitamin B12 deficiency will likely result in the classical endpoints of hematologic or neurologic complications. Seventy-five to 90% of those with B12 deficiency have neurologic symptoms and approximately 25% of patients with neurologic symptoms do not present with any hematologic abnormalities [1, 2, 4, 11]. Neurologic symptoms may result in paralysis from either subacute combined degeneration of the spine or funicular spinal cord disease, unsteady gait or skin numbness [1]. Hematologic abnormalities include pernicious anemia [8] and macrocytic anemia [8].

If left untreated, damage becomes irreversible. However there is evidence to suggest there is a window of opportunity in which to reverse these symptoms [60] highlighting the importance of diligent testing (in the absence of symptoms) and treatment of deficient cases [11, 61].

Additionally, symptoms relating to subclinical B12 deficiency are diverse making it difficult to pinpoint B12 status as the potential cause. These symptoms include: lethargy [22, 62] or fatigue [2], depression [22, 62, 63], cognitive decline or dementia [22, 62, 63], increased confusion [62], forgetfulness [22, 62], peripheral neuropathy [2, 14, 63], osteoporosis [14]. With such diverse symptoms, many of which affecting the ability to live life normally, regular testing and treatment when appropriate is important.

2.5 Defining vitamin B12 deficiency

Part of the challenge in establishing prevalence is inconsistency in defining vitamin B12 deficiency. Several cut-points have been suggested: 125 pmol/L [64], 148 pmol/L [38, 65], 150 pmol/L [39], 184 pmol/L [66] and 258 pmol/L[40]. Traditionally, a cut-point of 148 pmol/L has been used [59]. Cut-points are also lab dependent and drives the diagnosis dependent on the value used [67]. Several other definitions have also been introduced using a combination of cut-points for B12 and either other biomarkers (e.g., MMA, Hcy, holoTC) or other blood related symptoms [2, 40]. While surrogate biomarkers for vitamin B12 status (e.g., methylmalonic acid) may presently be regarded as the gold standard and improve diagnostic sensitivity [3], practicality of determining vitamin B12 deficiency is dependent on the measures that are covered by medicare and/or those available at a given commercial lab.

2.5.1 Defining a serum B12 level for “normal” and “subclinical deficiency”

For this thesis, a distinction was made between deficient, subclinical and normal B12 status as deficiency exists on a continuum [10, 68]. Some literature provides evidence to support that “normal” serum B12 should be a minimum value of approximately 300 pmol/L [1, 14, 69-71]. Eussen and colleagues used a range of 100-300 pmol/L to define mild deficiency in a dose-finding trial for vitamin B12 intake that resulted in optimal reduction in plasma methylmalonic acid [69]. Elevated methylmalonic acid is a sensitive biomarker for vitamin B12 deficiency. Additionally, Clarke and colleagues reported that the range of serum vitamin B12 that corresponded with a low risk for elevated homocysteine was 350-400 pmol/L [70]; homocysteine is a known cardiovascular risk factor [10, 59, 72], potentially a neurotoxin [1, 10] and vasculotoxin [10], and elevated levels

(hyperhomocysteinemia) are an independent risk factor for dementia and Alzheimer's disease [1, 19]. In line with this, Dhonukshe-Rutten and colleagues conducted a review of dietary intake pertaining to folate, B12 and their association with homocysteine and cardiovascular disease in Europe and used >350 pmol/L, 300-350 pmol/L and <300 pmol/L as favourable, moderate and marginal B12 status respectively [71]. Further, a 2003 review summarized evidence to suggest that a functional deficit may be present at levels under 332 pmol/L (400 ng/mL) and that approximately 15% of older adults with apparent normal B12 levels have evidence of metabolic abnormality [1]. There is evidence to suggest that subtle neurophysiologic changes can occur in the absence of clinical manifestations of B12 deficiency [59] and there is also some evidence to suggest that optimized formation of two types of blood cells, lymphocytes [73] and leukocytes [74], require a plasma B12 level of at least 300 pmol/L [14]. While changing the reference range for deficiency has been controversial [59], subclinical deficiency has clinical relevance. Therefore subclinical deficiency (instead of dichotomising serum levels with normal and deficient) in addition to deficiency will be determined in this study.

2.5.2 Understanding variance in B12 deficiency prevalence estimates: cut-points and samples

Due to variance in the cut-point used to define deficiency and the type of sample included in the study (e.g., excluded at risk groups), prevalence estimates for deficiency vary widely. However, when context of cut-point and sample are considered together, a few trends emerge (**Table 1**). The studies that included at risk groups tended to have higher estimates compared to other studies that used similar cut-points. The best examples to make this point are the studies which used <148 pmol/L to determine deficiency. Generally, community sample estimates tended to be fairly consistent (between 3-6% [75-77]), which was also consistent with a LTC hospital study that had no exclusion criteria for participants (6.7% [65]). This was contrasted with 15.4% prevalence in a LTC-related study that included an at-risk group (hospitalized older adults with oral dysphagia) [38].

The LTC-related samples tended to have higher estimates than general community samples. Excepting the 258 pmol/L cut-point, all estimates from LTC-related groups were either: higher than the community estimates, or were comparable to community estimates which used a higher cut-point and thus the prevalence estimate was higher. For example, LTC-related estimates of 2.5% to 35% [64, 65, 78-80] using cut-points up to 148 pmol/L. In addition, the LTC-related samples that included at risk groups tended to have estimates comparable to LTC-related samples which excluded at risk

groups but used higher cut-points. For example, studies which used cut-points up to 148 pmol/L and included at risk groups yielded prevalence estimates comparable to the studies that used higher cut-points (150-185 pmol/L) which excluded at risk groups (e.g., [38, 64, 79-83]). In light of this, when comparing prevalence estimates, it is important to consider which cut-point was used to define deficiency or low B12 status in addition to the sample the estimate represents.

Limited research exists on B12 status in this LTC population. A recent scoping review was conducted to provide an overview of the literature on vitamin B12 in LTC [84]. The purpose of this review was to summarize the published research on the prevalence of vitamin B12 deficiency, methods of B12 treatment and the efficacy of these treatment methods in LTC. This scoping review identified only 19 articles with only one study being conducted in Canada [65]. Consistent with community prevalence estimates, these LTC relevant studies indicated a high variance in prevalence (between 2.6% [78] to 35% [79]). One reason for variance among LTC studies again was the use of different cut-points. However, when similar cut-points were used, again the context of which groups were included must be considered. For example, range in LTC prevalence described above is based on two studies with relatively similar cut-points (110 pmol/L [78] vs 116 pmol/L [79]; 150 pg/mL [78] vs 157 pg/mL [79]). For these two studies, the sample was the main difference; one consisted of LTC hospital residents [78] (i.e., no exclusion or inclusion of more vulnerable groups) while the other analysed data only for those with low folate [79]. A sample comprised of those with low folate, may be indicative of poor nutrient status overall which may, in part, explain the wide variance of these two studies. This highlights the importance for consideration of diverse groups when attempting to determine generalizable prevalence estimates.

2.6 How is vitamin B12 deficiency treated?

The most common ways of treating vitamin B12 deficiency are through regular intramuscular or subcutaneous injections or through daily oral pills [34] of B12. Both treatments have been shown to be effective [37, 60, 64, 66] or equally effective at improving B12 status [1, 85, 86]. However treatment recommendations may vary by etiology. For example, in those with pernicious anemia, typical treatment is 1000 mcg/d of oral B12 each day throughout the lifecourse [11, 34]. Alternatively between 100-1000 mcg may be administered intramuscularly for five sequential days followed by lifelong monthly injections [11]. For those with food-cobalamin malabsorption, of most significance to the LTC population, treatment is typically a daily oral dose of B12 [34, 41]; BC Guidelines recommend 250 mcg/day until the underlying condition or diet is corrected [34]. For those with

neurologic symptoms, one to five doses of 1000 mcg are recommended through either IM or subcutaneous injections followed by a daily oral dose of 1000 to 2000 mcg until serum values are within normal range [11].

Less traditional methods of B12 replacement have also been successful. In a recent scoping review [84], three additional treatment methods (intranasal spray [40], oral nutritional supplements [51], and multivitamin preparations [81]) were also found to be effective for improving B12 status, but no studies contrasted these treatment methods. Further, other methods may exist at improving bioavailability of the B12 already ingested, which is a very relevant issue in older adults with a high prevalence for malabsorption [36]. For older adults, this could be accomplished through greater consumption of B12 through foods fortified with this vitamin (e.g., breakfast cereals), as the crystalline form does not suffer the same level of malabsorption as the food-bound form when faced with factors like atrophic gastritis or medications which alter gastrointestinal pH. An alternative may also be through generally improving gut health. One study focussed on improving food-B12 bioavailability through increased fibre intake [53]; 30 hospital residents aged 57-68 with low total energy intake were included in the study. Half received a regular diet as the control group and the other half received oat bran containing added fibre blended into common daily meals. At baseline there was no significant difference between treatment groups ($p>0.05$); yet, after 12 weeks, the B12 status of the treatment group was maintained while the control group B12 status decreased significantly ($p<0.05$). The authors concluded that fibre may help stabilize B12 status in this population. While this study is not pertinent to this thesis work, since there is some evidence that bioavailability of B12 can be modified, this may provide insight into complementary treatment methods which are acceptable to older adults and less focussed on medications.

2.7 Why is vitamin B12 important to Long-Term Care?

There is increasing evidence that nutritional status impacts quality of life [87], morbidity and mortality [88]. Among older adults (65 years or older), several nutrients including calcium, vitamin D and vitamin B12 [89] are of special concern due to decreased absorptive ability with age [43, 89]. This may be especially true in LTC where up to 70% of older adults are affected by malnutrition [51]. Moreover, LTC residents have a high degree of polypharmacy; of all Ontario LTC residents 65 years or older, more than 15% are dispensed at least 9 medications [90]. Vitamin B12 is one of the micronutrients most commonly affected by drug interactions due to decreased absorption [88]. This

further highlights the importance of monitoring vitamin B12 status especially when deficiency or subclinical deficiency can take on a wide range of symptoms.

The general lack of literature on vitamin B12 prevalence in LTC indicates an opportunity for further work in the field. Work on vitamin B12 deficiency in LTC has the potential to impact over 70,000 thousand residents in Ontario [91] approximately 140,000 residents in Canada [91] and approximately 1.4 million LTC residents in the United States [92]. Using a moderate LTC B12 deficiency prevalence estimate of 11%, this equates to directly impacting up to 165,000 North Americans living in LTC who are potentially deficient. Again, monitoring B12 status is important as the proportion of older adults potentially affected by B12 deficiency in LTC is nontrivial.

2.7.1 Factors potentially associated with B12 status

Malnutrition in general, has been associated with poorer quality of life [93] and affects 20-60% of LTC residents [52]. For older adults living in LTC, micronutrient deficiency, specifically vitamin B12 deficiency, is relevant yet preventable form of malnutrition [50]. While the leading cause of B12 deficiency is due to decreased absorptive abilities, poor intake is still a concern [88] and intake as determined by the RDA may not be sufficient. Risk for B12 deficiency is further exacerbated by additional factors including certain medications or conditions which affect absorption as outlined below.

2.7.1.1 Medications

Research suggests that the use of some medications may decrease vitamin B12 status, specifically proton-pump inhibitors (PPIs), H2 blockers, and use of metformin and antibiotics. PPIs are used to treat *H. pylori* infections which impacts up to 60% of older adults and increases the risk of atrophic gastritis [1]. There is some evidence to suggest that the use of PPIs may cause decreased bioavailability of B12 [1] and is associated with decreased B12 levels [40, 94]. The rationale is that the use of PPIs reduces the amount of hydrochloric acid in the stomach, both decreasing the ability to disassociate food-bound vitamin B12 from associated protein, and reducing acidity of the small intestine thus increasing the likelihood for bacterial overgrowth (i.e., the pH of the small intestine increases and becomes a more favourable environment than at a lower pH) [1]. Similarly, there is some evidence to suggest that H2 blockers may impact B12 status due to decreased release of IF [3]. With this in mind, we anticipate that residents taking PPIs or H2 blockers may be more likely to have lower vitamin B12 levels than those who are not.

Literature also suggests that metformin use may be associated with decreased B12 levels [3, 56-58, 95]. It is thought that metformin interferes with the transport of vitamin B12 into cells through disruption of calcium-dependent receptor-mediated endocytosis of IF-bound-B12 [1]. There is some evidence to suggest that antibiotic use may be associated with decreased B12 through blocked absorption [3]. We anticipate that residents on metformin or antibiotics may be more likely to have lower B12 levels than those not taking these medications. Antibiotic and metformin use are anticipated to be high in LTC, with the high incidence of infections and diabetes in this segment of the population.

2.7.1.2 Conditions and diseases potentially associated with deficiency

B12 is involved in many high level processes. Literature suggests that symptoms associated with subclinical B12 deficiency include: cognitive decline [1, 63, 70, 96], depression [7, 15, 16, 21, 62, 63, 97-102], and lethargy [62], all of which impact ability to perform activities of daily living. Having numerous diagnoses may also be associated with B12 status. Specific conditions to consider are hypothyroidism, depression, and dementia, the latter being prevalent in a median of 58% of in LTC residents [103].

There is some evidence to suggest that hypothyroidism may be associated with the risk of pernicious anemia in part due to a high prevalence of concomitant parietal cell antibodies in 33% of hypothyroid patients [104].A fairly recent study reported that 40% of a sample of 116 hypothyroid patients were vitamin B12 deficient [104]. B12 is involved in production of neurotransmitters through the formation of s-adenosyl methionine, a universal methyl donor, which among other reactions is involved in the methylation of neurotransmitters. Sometimes subclinical B12 deficiency presents as depression [7, 15, 16, 21, 62, 63, 97-102]. The literature is unclear on whether B12 status is associated with cognitive decline. While there is evidence to suggest that low serum B12 imparts an increased risk for Alzheimer's disease [74], more rapid cognitive decline [1, 63, 70, 96], increased risk of brain atrophy [105] or more pronounced deep white matter lesions [106], others have failed to report any association between cognitive status and B12 levels [2, 19, 106, 107]. A potential confounder between the potential association of dementia and B12 status could be age. Literature suggests that B12 status decreases with age, in part due to reduced absorption [108]. In addition, up to 30% of older adults have atrophic gastritis which decreases both HCl secretion but also IF secretion, thus reducing the ability to absorb vitamin B12 [11] and increases the likelihood of

deficiency. Any research attempting to understand B12 status in LTC needs to consider these diagnoses and characteristics of residents.

2.7.2 To test or not to test: Existing guidelines and recommendations for Vitamin B12

Despite evidence of an association between B12 status and health risk factors, relatively few guidelines pertaining to screening or testing exist [34, 86, 109-112]; of those that do, most pertain to cognitive functioning or dementia [86, 109-112]. Three guidelines related to, but not specific to, older adults, were identified on www.guideline.gov . These guidelines from Clinical Research Centre for Dementia of South Korea, the European Federation of Neurological Societies, and the European Federation of Neurological Societies-European Neurological Society respectively pertain to diagnosis and evaluation of dementia [110] (2011), diagnosis and management of Alzheimer's disease [109] (2010), and diagnosis and management of disorders associated with dementia [111] (2012). All of these guidelines recommend B12 testing as part of laboratory testing to rule out this potential cause of dementia. Specific to Canadian older adults, Garcia conducted a review summarizing the evidence pertaining to B12, folate and homocysteine for investigation of cognitive function [112]. The review concluded with recommending inclusion of B12 in dementia assessment; however insufficient evidence of effectiveness of vitamin treatment for other forms of dementia (e.g., Alzheimer's type) was available to warrant other recommendations [112]. Only one set of recommendations were found specific to LTC. In 2001, Smith recommended that within LTC, routine testing for B12 be completed in a targeted manner for those with neurologic abnormalities, cognitive impairment, macrocytic anemia, nonspecific symptoms including weakness and fatigue as well as those with risk factors for vitamin B12 deficiency [68].

Somewhat in opposition to the above, in 2013, the Ontario Health Technology Advisory Committee (OHTAC) released recommendations for vitamin B12 testing and cognitive function [86]. These recommendations can be summarized as follows: 1) serum B12 testing should be restricted to those with potential macrocytic anemia or malabsorption; 2) routine testing for investigating dementia, cognitive impairment or based on vague presentations such as alopecia, dizziness, fatigue should be avoided; and 3) oral B12 is recommended for treatment instead of IM unless there is evidence of malabsorption [86]. Somewhere between these extremes of targeted screening in LTC and no screening unless macrocytic anemia or malabsorption are evident, reside the British Columbia guidelines (BC Guidelines) and protocols for investigating and managing vitamin B12 deficiency [34]. These BC Guidelines suggest that there is no indication for routine screening. However, they do

recommend consideration of testing for adults over 75 years of age, for those with medical or surgical history that may impact B12 status (e.g., inflammatory bowel disease, gastric resection), for vegans and for those taking H2 receptor agonists, PPIs or metformin [34]. Again a targeted approach based on selected risk factors, which may or may not apply to the LTC population is advocated.

Although older adults living in LTC may be more vulnerable than those living in the community, there may be advantages to living in a residential setting. Despite conflicting recommendations for testing, the most relevant to LTC are recommendations to test for B12, at least in a targeted manner [68]. These recommendations, specific to LTC, may have influenced protocols for testing of vitamin B12, leading to potentially timely treatment for those who are deficient, as compared to older adults living elsewhere. It can be hypothesized that: routine screening for B12 status in LTC is a common practice, treatment protocols support improvement of status, and that prevalence of persons living in care for a period of time with a B12 deficiency is potentially lower than frail older adults living in the community. The BC guidelines also suggest repeat testing once after 4-6 months for those receiving IM or subcutaneous B12 [112], none of the other guidelines discuss monitoring of vitamin B12 status after initial assessment [34, 86, 109-112]. Thus, incidence of deficiency has not been addressed in this population limiting insight into how B12 status may change, which factors affect this change and how effective LTC is at addressing B12 deficiency.

2.8 Summary

Vitamin B12 deficiency is a potential problem in LTC that could impact a significant number of residents. Due to the nonspecific nature of symptoms associated with subclinical deficiency, prevalence of poor food intake, the high degree of polypharmacy and health conditions associated with poor B12 status, LTC residents are at a potentially increased risk for B12 deficiency as compared to older adults living in the community. There are few existing guidelines for testing and treating B12 deficiency directly relevant to the LTC sector, none of which provide recommendations on follow-up testing. In general, vitamin B12 status is a relatively unexplored field; few studies have addressed prevalence and of those, a lack of generalizability impedes the understanding of true B12 deficiency prevalence; and subclinical deficiency is also unknown. In addition, incidence of B12 deficiency has yet to be reported. Due to conflicting guidelines for testing, it is unclear as to how vitamin B12 status is addressed through testing and treatment within LTC. This thesis aims to begin to address these gaps in knowledge and practice.

Table 1: *Summary of B12 deficiency prevalence estimates in community and LTC-related sample by cut-point and group*

Ref	Sample	Inclusion/Exclusion	Group Included	110 pmol/L	116 pmol/L	125 pmol/L	133 pmol/L	148 pmol/L	150 pmol/L	153 pmol/L	185 pmol/L	221 pmol/L	258 pmol/L
[113]	Community	1/3rd most disabled women (African American; Caucasian)	Included at risk	-	-	-	-	-	-	-	-	13.5%; 22.1%	-
[77]	Community	Veterans >65 with no diagnosis associated with B12 deficiency	Normal	-	-	-	-	6.0%	-	-	-	16.0%	-
[76]	Community	Free living older adults Framingham	Normal	-	-	-	-	5.3%	-	-	-	-	40.0%
[75]	Community	Free living older adults NHANES (1999-2000; NHANES III)	Normal	-	-	-	-	3%; 5%	-	-	7%; 13%	-	-
[82]	LTC-related	Excluded heart disease, taking B12, organ or endocrine disease	Excluded at risk	-	-	-	-	-	-	34.0%	-	-	-
[81]	LTC-related	Dementia excluded	Excluded at risk	-	-	-	-	-	31.0%	-		-	-
[83]	LTC-related	Cancer, CVD, antiepileptic drugs, thyroid hormones, B12 supplements excluded	Excluded at risk	-	-	-	-	-	-	-	15.7%	-	-
[80]	LTC-related	Excluded B12 deficiency, renal insufficiency and life threatening diseases	Excluded at risk	-	-	-	7.8%	-	-	-	-	-	-
[79]	LTC-related	Psychogeriatric patients with low folate (Community; LTC)	Included at risk	-	32%; 35%	-	-	-	-	-	-	-	-
[55]	LTC-related	use H2 blockers or PPIs; B12 users excluded	Included at risk	-	-	-	-	-	-	-	-	-	29.0%
[64]	LTC-related	Suspected B12 deficiency	Included at risk	-	-	17.0%	-	-	-	-	-	-	-
[38]	LTC-related	Hospitalized with oral dysphagia	Included at risk	-	-	-	-	15.4%	-	-	-	-	-

Ref	Sample	Inclusion/Exclusion	Group Included	110 pmol/L	116 pmol/L	125 pmol/L	133 pmol/L	148 pmol/L	150 pmol/L	153 pmol/L	185 pmol/L	221 pmol/L	258 pmol/L
[65]	LTC-related	LTC hospital	Normal	-	-	-	-	6.7%	-	-	-	-	-
[78]	LTC-related	LTC hospital	Normal	2.5%	-	-	-	-	-	-	-	-	-

Chapter 3
Rationale and Research Questions

3.1 Rationale

Vitamin B12 status is a relevant topic to older adults [114] and deficiency has avoidable clinical ramifications, especially for those living in long-term care [115]. While classical symptoms of B12 deficiency are either hematologic or neurologic, presentation of symptoms are not reliable as diagnostic tools [43] as these symptoms are not present in all cases of B12 deficiency [11]. Furthermore, symptoms of subclinical B12 deficiency are nonspecific; a blood test is required for B12 status determination and as a first step towards treatment. B12 screening and treatment protocols in LTC have not been documented and no studies have been designed to establish vitamin B12 clinical and subclinical deficiency prevalence at admission or to describe follow-up incidence within this population. Furthermore, studies on prevalence using a cross-section of the LTC population lack representativeness. This thesis aims to address these knowledge gaps.

3.2 Research questions

The overarching aim of this research was to gain insight about the proportion of residents affected, what screening procedures are in place and what treatment methods are being used to address vitamin B12 deficiency in LTC.

The following specific research questions were posed:

1. What protocols and procedures are in place for testing and treatment of B12 deficiency upon admission and during residents' stay in Ontario LTC residents?
    1.1. What residential covariates (e.g., ownership, geography, for-profit status) are associated with protocols and procedures for testing and treatment of vitamin B12 deficiency?
2. What is the prevalence of B12 deficiency at admission for current residents of the Schlegel Villages?
    2.1. What is the proportion of residents receiving vitamin B12 treatment prior to their admission to LTC?
        2.1.1. Of those on this treatment, what is the prevalence of B12 deficiency and what is their mean B12 level?
    2.2. Which covariates (if any) are associated with B12 levels at admission to LTC?

3. What is the incidence of B12 deficiency one year post-admission to the Schlegel Villages?
    3.1. Which covariates (if any) are associated with B12 levels at one year post-admission?

To address these research questions, two studies were completed: an environmental scan involving interviews with directors of care from Ontario LTC home and a multi-site retrospective prevalence study. Results from this thesis may inform future work aimed at identification of treatment protocol effectiveness and future policy and intervention protocols.

Chapter 4

Variability in Ontario long-term care practices for screening and treatment of vitamin B12 deficiency

4.1 Overview

BACKGROUND: Vitamin B12 deficiency impacts multiple domains (e.g., anemia, neuropathy, depression) and is often misdiagnosed; however it is an avoidable condition with screening and proactive treatment. Although prevalence in long-term care (LTC) is largely unknown, vitamin B12 deficiency impacts up to 40% of community-living older adults. Screening and treatment practice to address this avoidable deficiency is also poorly described. **OBJECTIVE**: The primary purpose of this study was to describe the state of vitamin B12 screening and treatment in Ontario LTC homes. The secondary purpose was to investigate the impact of covariates (e.g., geography and corporate structure) on: (a) current policies or procedures for vitamin B12 testing upon admission to LTC; (b) the reported proportion of treated residents; and (c) current treatment protocols. **METHODS**: A stratified random sample of LTC homes was selected, and 45 phone interviews with the directors of care were completed representing 5243 residents in this cross-sectional study. A standardized interview schedule was used to collect data. Descriptive analyses were completed. **RESULTS**: Cut-off values for determining B12 deficiency varied among homes; the most common were <156 pmol/L (31%), <198 pmol/L (21%) and <148 pmol/L (21%). Two thirds (30/45) of the participating sites routinely tested for B12 levels at admission to LTC and follow up B12 testing was completed in 88% (35/40) of homes, presenting a potential gap in determining the efficacy of treatment. On average 25 ± 16% of current residents were receiving vitamin B12 supplements (41/45 homes reporting). The most common form of treatment was regular intramuscular injections (53%) followed by daily oral supplements (44%). **CONCLUSIONS**: There is variability in practices with respect to detection and treatment of B12 deficiency in LTC, potentially placing residents at risk for undetected deficiency. Consensus is needed on cut-points for determination of deficiency in this population, as well as clear treatment and monitoring protocols which begins at admission to LTC.

(Prepared for submission to the Canadian Journal on Aging)

4.2 Introduction

Vitamin B12 has been identified as a micronutrient of interest for older adults as it is readily depleted by drugs [11, 43, 88], and deficiency is commonly caused by atrophic gastritis which is highly prevalent in this segment of the population [43, 88]. Nonspecific symptoms associated with subclinical B12 deficiency make diagnosis challenging and include: depression [7, 15, 62, 63, 97-101, 116, 117], other psychiatric symptoms [62, 76, 100, 118], impaired neurotransmitter and monoamine synthesis [62, 116], cognitive decline or dementia [8, 15, 63, 117], increased confusion [7, 98] or memory problems [7, 98], balance and mobility issues due to peripheral neuropathy [14, 63, 117], and osteoporosis [14]. These symptoms are in addition to classic symptoms of vitamin B12 deficiency including macrocytic megaloblastic anemia, pernicious anemia and degeneration of the spine [14, 15, 43, 62, 63]. Specifically, pernicious anemia affects up to 5% of older adults [43] and is caused by B12 deficiency which further demonstrates that this micronutrient is an issue in this segment of the population [18, 41, 88]. One particularly vulnerable group for B12 deficiency are older adults (65 years or older) living in LTC, where malnutrition affects up to 65% [119] of this population. Low micronutrient intake commonly coincides with protein-energy malnutrition and there is a significant potential for deficiency. Generally, older adults in LTC are more vulnerable to micronutrient malnutrition due to any combination of: inadequate micronutrient content of the meal resulting from menu planning challenges; low food intake resulting from functional impairment, slow eating, taste changes, poor appetite [120, 121]; and the high use of medications [90]. While decreased absorptive abilities is the main cause of B12 deficiency, low intake is still a concern as there may be increased B12 requirements for those with absorption problems [88].

Prevalence of B12 deficiency (or marginal status) in community-living older adults (age 65+) is estimated at up to 40% [16, 39, 41, 75, 77, 113, 122, 123]. Relatively few studies have reported on B12 deficiency prevalence in LTC; estimates outside of Canada range from 7% [65] to 34% [82] and only one Canadian study representing LTC exist. Prevalence of B12 deficiency in LTC is anticipated to be similar or potentially higher, due to challenges in eating and deficits in menu planning [124], since there are relatively few foods in which B12 naturally exists (i.e., only in animal products) [30]. Further, symptoms of vitamin B12 deficiency are subtle, nonspecific and are frequently unrecognized or misdiagnosed [39, 41]. This highlights the importance of regular testing; vitamin B12 deficiency can only be determined by blood work as the classic symptoms as such are unreliable for diagnosis [43].

This potentially avoidable deficiency provides a potential opportunity for improving health outcomes if B12-related care practices are effective at addressing vitamin B12 deficiency in LTC. However, a gap exists in our understanding of LTC practices; detection, treatment and monitoring patterns of vitamin B12 status in Canadian LTC remain unknown. The purpose of this study was to describe the practices of Ontario LTC homes for the detection, treatment and monitoring of vitamin B12 status. Specifically,

1. Does vitamin B12 screening occur at admission to LTC?
2. What cut-points are used to diagnose a vitamin B12 deficiency?
3. What types of vitamin B12 treatment are used in LTC?
4. How often/does monitoring of B12 status occur in LTC?

Secondly, do these practices vary based on characteristics of the LTC home (e.g., location, profit status and corporate structure)?

4.3 Methods

4.3.1 Study Design and Participants:

This was a cross-sectional study with data collected through phone interviews with the directors of nursing (also referred to as directors of care) or an alternative knowledgeable informant from the long-term care home. Data were collected between August and December, 2013.

4.3.2 Sampling and Recruitment:

The Ontario Long Term Care Association (OLTCA) and the Ontario Association of Non-profit Homes and Services for Seniors (OANHSS) member listings were chosen to provide the widest and largest coverage of Ontario LTC homes. These listings have representation across private, municipal, charitable and non-profit long term care homes. OLTCA and OANHSS were selected instead of the Ontario Ministry of Health and Long-Term Care (MOH-LTC) public reporting site as they provided the necessary detailed information for stratification purposes, as well as up-to-date contact information for the home administrator. These combined associations' members provide care to approximately 106,000 residents living in LTC in Ontario (70,000 and 36,000 from OLTCA and OANHSS respectively) [125, 126]. A list of all Ontario LTC homes in the OLTCA and OANHSS was compiled (N=651). After removal of long-term care hospitals, recently closed homes, or LTC

homes with 0 reported beds (n=31), the remaining 620 homes were then stratified by covariates based on corporate structure and geography.

A home was classified as either for-profit (n=359) or not-for-profit (n=261) as defined by the OLTCA reporting website and/or OANHSS membership. It was hypothesized that for-profit homes may have different protocols than not-for-profit, with respect to preventative practices such as screening for B12 deficiency. A home was classified as either an umbrella (n=445) home or a non-umbrella (n=175) home. Umbrella status was defined as any group of two or more homes owned or operated by the same company; it was hypothesized that umbrella homes may have a different structure that supported policy and practice implementation. A home was classified geographically based on the Local Health Integration Network (LHIN) in which it resides. Geographical region was then categorized as South-Western (n=327), Eastern (n=94), Northern (n=56) or Central Ontario (n=143); it was hypothesized that geographical region and density of LTC homes within a geographical region may impact how policy and practice are implemented particularly with respect to ordering of blood tests. As this was a descriptive study, a random stratified sample consisting of approximately 10% of these homes was desired as potential participating sites. With a goal of recruiting 45-60 sites, 194 homes were invited, assuming a participation rate of approximately 30%.

Three methods of contact were used for recruitment: 1) initial hard copy letter to the LTC home explaining the purpose and importance of the project, 2) email to the administrator, and 3) phone call issued to director of care. **Figure 2** provides the flowchart depicting sample recruitment and inclusion. Only one hard copy letter was sent to each LTC home. If, in speaking with the informant, they failed to receive the letter, an electronic copy was sent. If there was no response after approximately 2 weeks, one initial email was sent to the administrator. If after three days there was no response, a follow-up email was sent to the same contact. A total of 83 emails and follow-up emails were issued. At least two phone contact attempts were made and up to two voicemails were left for the director of care. If the LTC home advised on calling back later, this added to the number of phone-call attempts made. A total of 61 follow-up phone calls were issued in efforts to determine interest or schedule interviews. Once initial interest in participation was established, at least two more contact attempts were made to schedule and conduct the interview. Recruitment stopped when the 60 homes (plus an anticipated 10% loss) agreed to participate. Of the 68 LTC homes initially recruited, 45 followed through with data collection.

4.3.3 Data Collection:

Interviews were completed over the phone by the first author. Informants were provided with the questions before hand to support completion of the interview. For missing data identified during the interview, at least one follow-up email or phone call was issued (n=14). Questions were developed by the authors based on the aims of the study around current policies or procedures for B12 testing at admission to LTC, the reported proportion of treated residents and treatment protocols. Some questions were modified to be more clear after trialling the questions with the first few homes. In addition, each home was asked about whether their home was aware of recently released recommendations on B12 testing for cognitive function by the Ontario Health Technology Advisory Committee [86].

4.3.4 Statistical Analyses:

Due to lower participation rates across three of the four geographic regions, Eastern, Northern, and Central Ontario were collapsed for comparisons against South-Western Ontario. This made for a more equal comparison of geographic groups and also allowed for grouping of geographically high density versus low density LTC homes.

Data were analysed using IBM SPSS Statistics version 21. Descriptive statistics were as follows: dichotomous variables were presented as the proportion of responses for a given question; continuous variables were presented as the mean and standard deviations. A chi-square test was conducted to establish whether the characteristics of participating homes differed significantly from the Ontario LTC population identified in the sampling frames. Chi-square or Fisher's exact tests were conducted to determine whether site characteristics were associated with practices. Since LTC homes varied in size, the proportion of residents receiving vitamin B12 and which form of treatment were reported. A weighted average was calculated by summing these proportions and dividing by the total number of homes reporting residents receiving treatment.

4.4 Results

Forty-five participants/homes representing 5243 residents are included in this descriptive analysis; participation rate was 23%. The sample was not found to be statistically different from the sampling frame/population by geography, umbrella status or profit/not profit designation (χ^2=2.11 (df=1), p>0.1, see **Figure 3**). **Table 2** provides a summary of descriptive variables by LTC characteristic.

4.4.1 Detection:

There was diversity in cut-off values used to determine B12 deficiency among the participating homes. The range of values used was 100-300 pmol/L. The most common cut-points to determine B12 deficiency were <156 pmol/L (n=12; 31%), <198 pmol/L (n=8; 21%) and <148 pmol/L (n=8; 21%); (response to this question: 87%; 39/45). Generally, these cut-points were associated with the labs chosen for blood work. For example, <156 pmol/L was used to define a B12 deficiency at LifeLabs.

Fifty-nine percent (26/44) of homes commented that their home currently had a B12 testing protocol. Almost all (44/45) of LTC homes reported that they requested a vitamin B12 test at some point during selected residents' stay with the remaining 1/45 , for-profit, non-umbrella, medium sized home (between 75-100 beds) in Eastern Ontario reporting that they never did B12 testing, but in their home, 21% of residents were receiving B12 (85% on oral, 15% on IM). However, only 67% (30/45) of the participating sites routinely tested for B12 levels at admission of the resident to their LTC home. No significant differences were found on screening practices between LTC home characteristics for geography, for-profit status and umbrella status ($p>0.20$).

4.4.2 Treatment

Current treatment was used as a proxy for identified deficiency. One of the 41 homes reported that 100% of residents, regardless of admission blood work, were treated. This was a not-for-profit, not-umbrella, large home (between 150-175 beds) located in southwestern Ontario; all residents received monthly IM injections. Of the remaining 40 homes which reported treatment of B12 deficiency, a range of prevalence of residents currently receiving treatment was identified (**Figure 4**). Most homes reported having between 20-29% of their residents on vitamin B12 and 61% of homes reported that at least 20% of their residents were currently receiving vitamin B12 treatment(s). No significant differences were found between proportion of residents receiving vitamin B12 treatment and LTC characteristics for geography, for-profit status and umbrella status ($p>0.20$).

Homes were asked to comment on treatment modality. Most homes offered more than one modality (38/43; 88.4%); 87.8% (36/41) of homes used oral, 97.6% (40/41) of homes use IM and 7.3% (3/41) of homes used another form of B12 treatment (subcutaneous injections). Only 5 homes (11.4%) exclusively offered vitamin B12 treatment via IM injections; 36 (81.8%) offered oral and IM; one home (2.3%) offered oral and subcutaneous; and two homes (4.5%) offered either oral, IM or

subcutaneous B12 treatment. No significant differences were found between which forms of B12 treatment therapy was offered by an LTC home and characteristics for geography, for-profit status and umbrella status ($p>0.10$).

Homes were also asked to comment on the proportion of residents receiving each type of treatment modality. Of the residents receiving B12 treatment at a given LTC home on average, 53.1% (range (RG) 0-100%) received treatment in the form of IM B12, followed by 44.3% (RG 0-91.7%) through oral B12 and, 2.58% (RG 0-50%) through another form (e.g., subcutaneous B12). In terms of dosage, IM ranged from 500 to 1000 mcg administered either monthly, bimonthly or biweekly and oral doses ranged from 50 to 1200 mcg administered daily. The remaining 4.5% (2/44) received vitamin B12 through regular subcutaneous injections of 1000 mcg administered either monthly or quarterly. No significant differences were found between the proportion of residents receiving a form of B12 therapy at a given LTC home and LTC characteristics for geography, for-profit status and umbrella status ($p>0.10$). Reported estimates ($n=31$) of adherence to B12 treatment were high at 97.4% with no significant difference across LTC characteristics ($p>0.10$). Several interviewees commented that adherence was high because administration of medications is the responsibility of medical staff, not the residents themselves.

4.4.3 Monitoring

Almost all (44/45; 97.8%) of the participating sites reported that they conduct B12 follow-up testing. Yet, there was no consistent procedure for frequency of follow-up tests. One out of 44 homes (2.3%) was not sure if their home did follow-up testing in light of providing treatment to everyone, 7/44 (15.9%) homes did not complete follow-up B12 testing. The remaining 36/44 (81.8%) of homes conducted the following form of follow-up testing: annually 22/44 (50%), semi-annually 2/44 (4.5%), quarterly 8/44 (18.2%), monthly 1/44 (2.3%), and only at the discretion of the doctor 3/44 (6.8%). Among the homes that reported some form of follow-up, no significant differences were found between frequency of follow-up testing and LTC characteristics for geography, for-profit status and umbrella status ($p>0.10$). Only 5/43 (12%) homes were aware of OHTAC recommendations; 3/5 reported awareness as a result of having received the questions in advance of the interview and had researched the recommendations.

4.5 Discussion

This work demonstrates a variety of practices with respect to detection, treatment and monitoring of vitamin B12 status in Ontario LTC. For example, one third of LTC homes did not screen at admission. This could mean that residents who are highly vulnerable and potentially malnourished are moving into some LTC homes with an undiagnosed deficiency that can progressively impact their quality of life and health. Symptoms associated with an untreated B12 deficiency may also result in other investigations or treatments that lead to further comorbidity due to the non-specific nature of vitamin B12 related symptoms. Testing at admission should be a minimum standard due to: the increased risk of older adults, especially in the vulnerable; the low cost of a serum B12 measure; and, the simplicity and effectiveness of treatment. Additionally, it is possible that presentation of classic symptoms or symptoms associated with subclinical vitamin B12 deficiency may overlooked due to the general high degree of comorbidity in LTC; a blood test is the only way to confirm B12 status. However, it would be remiss to ignore the inherent challenges in defining screening protocols and the economical cost of screening. With approximately 71,000 older adults 65 years or older living in Ontario LTC homes [91], assuming 30% new admissions per year, and an average cost of $12.50 per serum B12 test [42], the cost for B12 screening using serum B12 for older adults in Ontario is approximately 1.2 million dollars annually (not accounting for treatment and monitoring costs). With this in mind, there is a need for a formal cost-benefit analysis for screening and subsequent treatment.

While residents on treatment were commonly monitored in some way, there were no consistent practices noted by respondents, such as timing of the monitoring. Two contrasting examples are the one home (NFP, not-umbrella, 150-175 beds, in SW Ontario) who provided treatment for all residents regardless of admission or follow-up bloodwork levels and the one home (FP, not-umbrella, 75-100 beds, in Eastern Ontario) who never conducted B12 testing yet reported that 21% of residents received B12, 85% of whom received IM. In the former case, it may be that all residents receive B12 because it is more efficient to treat everyone than to monitor select individuals. B12 monitoring may have been limited to those with low B12, thus treatment of all residents avoids potential for incident cases of deficiency. In the latter case, it may be that this home relied exclusively on home care transfer paperwork and bloodwork, such as admission to hospital. Such differential follow-up practices may leave a treatment gap for residents and lack of routine screening post admission could result in the missing of an emerging vitamin B12 deficiency while living in LTC. Furthermore, poor care transitions to LTC could also lead to care gaps. For example, a resident may have been receiving

B12 treatment in the community but after a poor care transition to LTC, the resident may have been identified as having normal B12 levels at admission to LTC and thus did not receive continued required treatment. Without a follow up test to indicate whether the resident's B12 level remained constant or decreased (or increased), a change in B12 level could go unnoticed, and thus deficiency could develop.

The proportion of residents receiving B12 treatment could be used to roughly estimate prevalence of identified deficiency; however, prevalence on admission as compared to developing during a resident's length of stay remains unknown. Based on the reported proportions receiving treatments across the sample homes, a rough estimate of B12 deficiency prevalence in Ontario LTC homes is approximately 20%. This estimate is consistent with prior LTC work outside of Canada which indicates vitamin B12 deficiency prevalence is between 7% and 34% [37, 39, 65, 82]. Future work needs to be aimed at an appropriately powered study with a generalizable population to accurately determine the prevalence of B12 deficiency on admission to LTC. Another opportunity for future work is to investigate the incidence of B12 deficiency after admission and during a resident's length of stay within an LTC home. These studies will lay a foundation for policies around vitamin B12 testing and treatment, as well as how to support positive care transitions. Prior work demonstrates that B12 deficiency in older adults does respond readily to treatment [37, 40, 51, 64, 81], potentially improving the quality of life, cognition and function of older adults living in long-term care.

4.6 Strengths and Limitations

While it is anticipated that there is some selection bias in these data as those homes that decided to participate are likely different in some way, one strength of this study was that the sample was found to not significantly differ from the population of the sampling frame in terms of geography, for-profit and umbrella status (χ^2=2.11, p=0.15). In addition the minimum sample desired of 45 was met. That said, since there was some missing data, and the sample size was less than 10% of the population, potentially limiting external validity. At the outset, a relatively low participation rate was anticipated, and borne out in the recruitment; this impacts the generalizability of results. It is anticipated that these selection and response biases have resulted in an underestimate of the potential problem with vitamin B12 in LTC. The sampling frame is a limitation of this work. Two organizations in Ontario which provide lists of members were available and used to stratify this sample based on key characteristics; yet coverage of the entire population of Ontario LTC homes was not 100%. However, random selection of homes to participate promotes some generalizability of findings.

This study contained minimal missing data due to extensive attempts for follow-up (range of proportion of missing data per question: 0 to 21%). Interviews were conducted by only one interviewer which implies good internal validity and consistency of how questions were presented. Finally, these results are based on the report of a knowledgeable informant in the LTC home and not all questions were answered by participants. To facilitate accurate responses, the questions were provided to the respondents before the interview. However, it is unknown how systematic these respondents researched their answers, such as the proportion of residents currently on B12 therapy.

Objective measurement that can be confirmed by audit needs to be used in future research to understand prevalence and care practices. In spite of these limitations, this is the first comprehensive study to examine vitamin B12 testing protocols in Ontario. These data provide a baseline for future studies on the vitamin B12 status of older adults living in LTC homes.

4.7 Conclusion

Although two-thirds of a stratified random sample of LTC homes in Ontario reported conducting admission bloodwork for screening B12 deficiency, there is no consistency in the detection, treatment and monitoring of this potential deficiency. Lack of standardization in cut-points to diagnose deficiency is a significant challenge for understanding prevalence and success with treatment. Once these concepts are better understood, evidence may further inform policy and practices that support adequate vitamin B12 status in LTC residents.

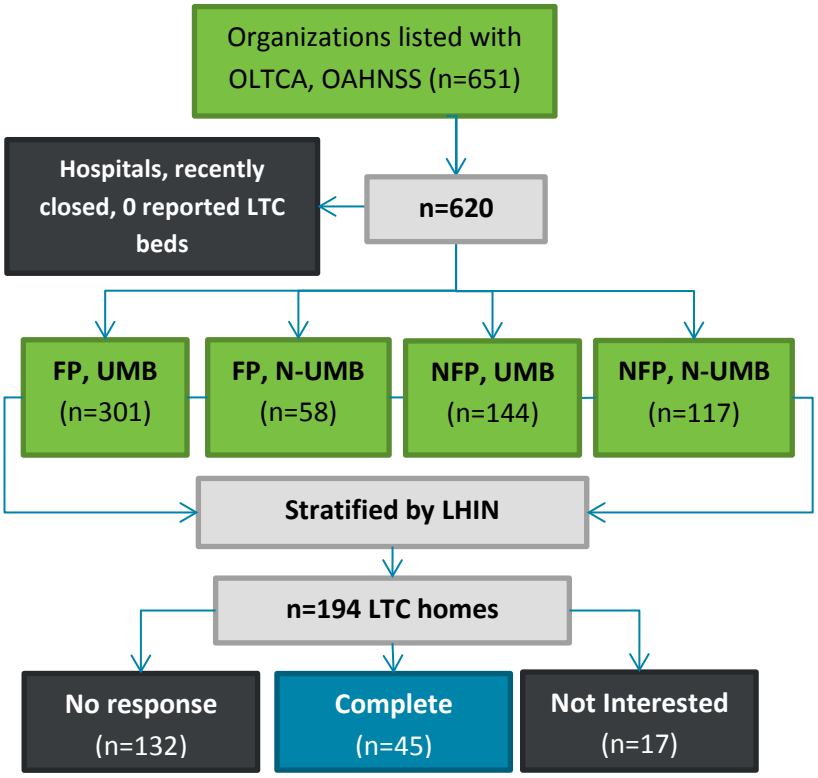


Figure 2: *Sampling framework for environmental scan. FP: for-profit, NFP: not-for-profit, UMB: umbrella home (i.e., at least 2 homes run by the same organization), N-UMB: not-umbrella home.*

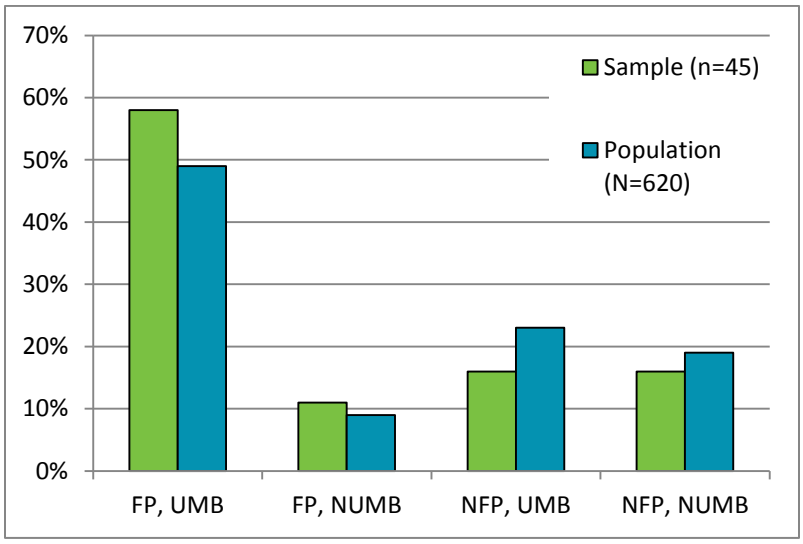


Figure 3: *Sample representativeness of environmental scan.*

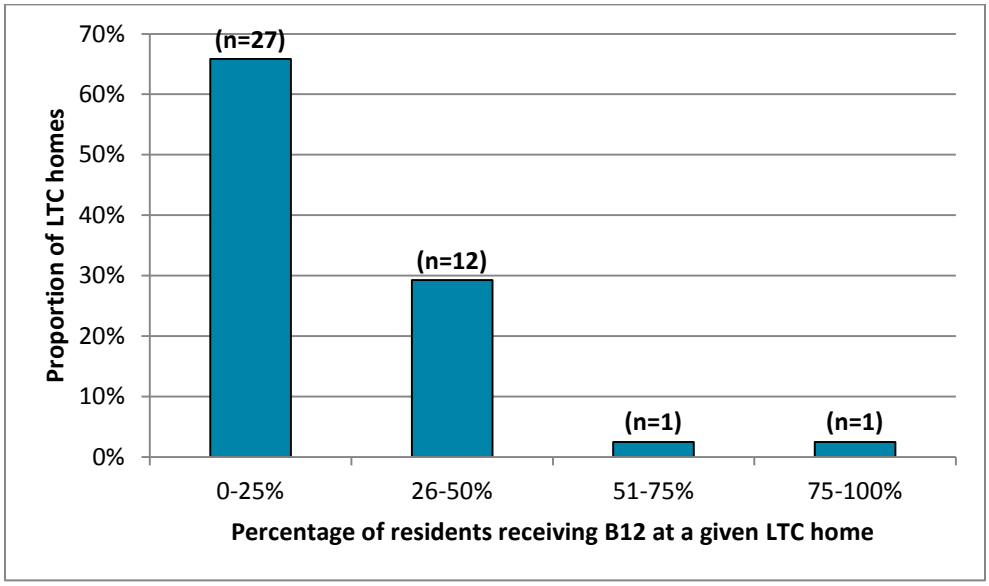


Figure 4: *Distribution of the proportion of current residents reported receiving B12 at a given LTC home.*

Table 2: *Summary of B12 practices by LTC home characteristics (n=45).**

Question	Total across all homes % positive responses	LTC Characteristics								
		Geography			For-Profit Status			Umbrella Status		
		C+N+E Ontario	**SW Ontario**	**P value**	**NFP**	**FP**	**P value**	**Not UMB**	**UMB**	**P value**
Does your LTC home currently have a B12 testing protocol? (N=44)	**59.1%** (26/44)	n=20 **55.0%** (11/20)	n=24 **62.5%** (15/24)	n.s.[d]	n=14 **57.1%** (8/14)	n=30 **60.0%** (18/30)	n.s.[e]	n=12 **58.3%** (7/12)	n=32 **59.4%** (19/32)	n.s.[e]
Does your LTC home test for B12 levels on admission? (N=45)	**66.7%** (30/45)	n=20 **65.0%** (13/20)	n=25 **68.0%** (17/25)	n.s.[e]	n=14 **64.3%** (9/14)	n=31 **67.7%** (21/31)	n.s.[e]	n=12 **66.7%** (8/12)	n=33 **66.7%** (22/33)	n.s.[e]
At any point during a resident's stay, does your LTC home test for vitamin B12? (N=45)	**97.8%** (44/45)	n=20 **95.0%** (19/20)	n=25 **100%** (25/25)	n.s.[c]	n=14 **100%** (14/14)	n=31 **96.8%** (30/31)	n.s.[e]	n=12 **91.7%** (11/12)	n=33 **100%** (33/33)	n.s.[c]
Does your LTC do follow-up B12 measurements for those identified to be deficient? (N=40)	**85.0%** (34/40)	n=18 **77.8%** (14/18)	n=22 **90.9%** (20/22)	n.s.[c]	n=12 **75.0%** (9/12)	n=28 **89.3%** (25/28)	n.s.[c]	n=10 **70.0%** (7/10)	n=30 **90.0%** (27/30)	n.s.[b]
Is your home aware of the recent OHTAC recommendations around vitamin B12 testing? (N=43)	**11.6%** (5/43)	n=18 **11.1%** (2/18)	n=25 **12.0%** (3/25)	n.s.[e]	n=13 **7.7%** (1/13)	n=30 **13.3%** (4/30)	n.s.[e]	n=11 **9.1%** (1/11)	n=32 **12.5%** (4/32)	n.s.[e]
How many homes have between 0-25% of residents currently receiving vitamin B12 treatment? (N=41)	**65.9%** (27/41)	n=17 **52.9%** (9/17)	n=24 **75.0%** (18/24)		n=13 **53.8%** (7/13)	n=28 **71.4%** (20/28)		n=11 **63.6%** (7/11)	n=30 **66.7%** (20/30)	
How many homes have between 26-50% of residents currently receiving vitamin B12 treatment? (N=41)	**29.3%** (12/41)	n=17 **41.2%** (7/17)	n=24 **20.8%** (5/24)	n.s.[c]	n=13 **38.5%** (5/13)	n=28 **25.0%** (7/28)	n.s.[c]	n=11 **27.3%** (3/11)	n=30 **30.0%** (9/30)	n.s.[c]
How many homes have between 51-75% of residents currently receiving vitamin B12 treatment? (N=41)	**2.4%** (1/41)	n=17 **5.9%** (1/17)	n=24 **0.0%** (0/24)		n=13 **0.0%** (0/13)	n=28 **3.6%** (1/28)		n=11 **0.0%** (0/11)	n=30 **3.3%** (1/30)	

Question	Total across all homes % positive responses	LTC Characteristics								
		Geography			For-Profit Status			Umbrella Status		
		C+N+E Ontario	SW Ontario	P value	NFP	FP	P value	Not UMB	UMB	P value
How many homes have between 76-100% of residents currently receiving vitamin B12 treatment? (N=41)	**2.4%** (1/41)	n=17 **0%** (0/17)	n=24 **4.2%** (1/24)		n=13 **7.7%** (1/13)	n=28 **0.0%** (0/28)		n=11 **9.1%** (1/11)	n=30 **0.0%** (0/30)	
Of the residents receiving B12 treatment what % of homes use more than 1 form of B12 treatment (e.g., IM and ORAL)? (N=43)	**88.4%** (38/43)	n=19 **94.7%** (18/19)	n=24 **83.3%** (20/24)	n.s.[c]	n=14 **85.7%** (12/14)	n=29 **89.7%** (26/29)	n.s.[e]	n=12 **75.0%** (9/12)	n=31 **93.5%** (29/31)	n.s.[b]
Of the residents receiving B12 treatment, what % of homes use only 1 form of B12 treatment (e.g., IM only)? (N=43)	**11.6%** (5/43)	n=19 **5.3%** (1/19)	n=24 **16.7%** (4/24)		n=14 **14.3%** (2/14)	n=29 **10.3%** (3/29)		n=12 **25.0%** (3/12)	n=31 **6.5%** (2/31)	
What proportion of LTC homes use ORAL B12 treatment therapy? (N=41)	**87.8%** (36/41)	n=17 **94.3%** (16/17)	n=24 **83.4%** (20/24)		n=13 **84.6%** (11/13)	n=28 **89.3%** (25/28)		n=11 **72.7%** (8/11)	n=30 **93.3%** (28/30)	
What proportion of LTC homes use IM B12 treatment therapy? (N=41)	**97.6%** (40/41)	n=17 **94.3%** (16/17)	n=24 **100%** (24/24)	n.s.[d]	n=13 **100%** (13/13)	n=28 **96.4%** (27/28)	n.s.[b]	n=11 **100%** (11/11)	n=30 **96.7%** (29/30)	n.s.[b]
What proportion of LTC homes use another form B12 treatment therapy? (N=41)	**7.3%** (3/41)	n=17 **11.8%** (2/17)	n=24 **4.2%** (1/24)		n=13 **15.4%** (2/13)	n=28 **3.6%** (1/28)		n=11 **0%** (0/11)	n=30 **10%** (3/30)	
Of the residents receiving B12 treatment, what percent of residents are receiving ORAL B12 at your LTC home? (N=40)	**44.3%** (RG 0-91.7%)	n=17 **40.3%** (SD±25.3)	n=23 **47.3%** (SD±30.3)	n.s.[c]	n=13 **51.1%** (SD±27.4)	n=27 **41.1%** (SD±28.4)	n.s.[c]	n=11 **43.0%** (SD±17.9)	n=29 **44.8%** (SD±25.3)	n.s.[e]

Question	Total across all homes % positive responses	LTC Characteristics								
		Geography			For-Profit Status			Umbrella Status		
		C+N+E Ontario	**SW Ontario**	**P value**	**NFP**	**FP**	**P value**	**Not UMB**	**UMB**	**P value**
Of the residents receiving B12 treatment, what percent of residents are receiving IM B12 at your LTC home? (N=40)	**53.1%** (RG 0-100%)	n=17 **56.5%** (SD ±28.8)	n=23 **50.5%** (SD±32.2)	n.s.[d]	n=13 **43.5%** (SD±30.2)	n=27 **57.7%** (SD±30.2)	n.s.[b]	n=11 **57.0%** (SD±31.4)	n=29 **51.6%** (SD±28.2)	n.s.[d]
Of the residents receiving B12 treatment, what percent residents are receiving another form of B12 supplementation at your LTC home? (N=40)	**2.58%** (RG 0-50%)	n=17 **3.1%** (SD±9.2)	n=23 **2.2%** (SD±10.4)	n.s.[d]	n=13 **5.4%** (SD±14.5)	n=27 **1.2%** (SD±6.4)	ǂn.s.[c]	n=11 **0%** (SD±0)	n=29 **3.6%** (SD±11.4)	ǂn.s.[b]
What would you estimate the % treatment adherence to be? (N=31)	**97.4%** (SD±4.5)	n=14 **98.7%** (SD± 1.82)	n=17 **96.4%** (SD± 5.74)	n.s.[b]	n=10 **98.3%** (SD± 3.3)	n=21 **97.0%** (SD± 5.0)	n.s.[c]	n=9 **95.9%** (SD± 5.3)	n=22 **98.0%** (SD± 4.1)	n.s.[c]

*Whenever expected values in cells were greater than five, the χ^2 test statistic was calculated with asymptotic 2-sided significance p-value. Whenever expected values in cells were less than five, the exact 2-sided significance p-value from the Fisher's Exact Test was calculated. (a=p>0.05, b=p>0.10, c=p>0.20, d=p>0.50, e=p>0.80). SD is the Standard Deviation of the mean. ** RG is the range. ǂequal variances not assumed.

Table 3: *Summary of additional phone interview responses by question.*

ADDITIONAL QUESTIONS	
What is the dosage ranges for Oral?	n = 29: 50 mcg/d - 1200 mcg/d
What is the dosage ranges for IM?	n = 38: 500 -1000mcg/ injection
What form of B12 is used for Oral? (e.g., cyanocobalamin, methylcobalamin)	n = 13: (cyanocobalamin > "vitamin B12")
What form of B12 is used for IM? (e.g., cyanocobalamin, methylcobalamin)	n = 15: (all cyanocobalamin)
At what level does your LTC home define B12 values to be "deficient"?	n = 39: avg =163.9 (SD=35 RG 100, 300)

Chapter 5
Vitamin B12 deficiency prevalence in older adults living in Ontario long-term care homes

5.1 Overview

BACKGROUND: Undernutrition is prevalent in long-term care (LTC) and can lead to mortality and morbidity, yet it may be amenable to intervention. Vitamin B12 is a micronutrient of which deficiency is relatively common in older adults and is treatable. As a first step towards improving B12 status, prevalence of deficiency and how it is currently being treated in the LTC setting needs to be understood. Small, ungeneralizable samples reported to date provide a limited perspective on this issue. **PURPOSE**: The primary aim of this study was to report prevalence of B12 deficiency at admission to Ontario LTC homes and incidence at one year post admission. The secondary aim was to explore associations between admission B12 status/level and resident admission characteristics in an attempt to understand if there is a subset of new admissions to LTC for whom vitamin B12 levels may be low. **METHODS**: This was a retrospective prevalence study representing 8 sites across south-western Ontario within one LTC organization. Eligibility criteria for inclusion of charts: resident was 65 years or older at admission and had admission bloodwork including B12 levels. Random proportionate sampling was used to identify resident charts for inclusion with replacements as necessary. 1005 chart reviews were conducted to obtain a total of 412 that were eligible. Only 142 of these charts had bloodwork at one year. Covariates of interest included: basic demographics, B12 supplementation, medications, diagnoses, functional independence, cognitive performance, and nutrition. **RESULTS**: Prevalence of B12 deficiency (<156 pmol/L) was 13.8% (range 4.1-27.1% across sites) at admission to LTC. Yet, only 47.6% of residents had normal B12 (>300 pmol/L) at admission, with the remaining residents categorized as subclinical. B12 supplementation use prior to admission was significantly associated with a better admission B12 status ($p<0.001$). There was a significantly higher proportion of B12 supplementation at admission in those with dementia compared to those without ($p<0.01$). Number of medications at admission was negatively associated with B12 level. Incidence of new B12 deficiency cases was 4%. **CONCLUSIONS**: The present multisite study, with a large sample, provides a better understanding than previous work on B12 status at admission to LTC. More than 50% of residents had B12 levels <300pmol/L indicating poor B12 status in this LTC sample. Supplementation use was significantly associated with better B12

status at admission to LTC. Future work should use prospective methods to identify treatment protocol effectiveness as well as incident B12 deficiency to inform future policy and intervention protocols.

(Prepared for submission to the Journal of Applied Physiology, Nutrition and Metabolism)

5.2 Introduction

Optimizing independence and quality of life is increasingly important with age, especially among the most vulnerable older adults living in long-term care (LTC) [127]. Research in LTC suggests risk undernutrition is prevalent at up to 65% [119], and can lead to mortality and morbidity [128]. Yet, some undernutrition in this setting may be amenable to intervention [129, 130]. Undernutrition is commonly thought of as protein-energy malnutrition, and although micronutrient malnutrition also exists in older adults, it is poorly studied in LTC [50, 121]. A specific micronutrient malnutrition is B12 deficiency; it is relatively common in community living older adults, but is treatable [11, 37, 41].

As a first step towards improving vitamin B12 status in LTC, understanding prevalence of B12 deficiency, characteristics of those who are at risk, and how B12 status changes over time is required. While, B12 deficiency prevalence estimates have been established in the community-living older adult ranging from 3-43% [11, 15, 16, 41, 75-77, 113], little work has been done in LTC. Variation in this prevalence is due to differences in cut-points used to define deficiency, as well as either inclusion or exclusion of population subgroups at greater risk for deficiency (e.g., frail older adults) [113]. Lack of prevalence data in LTC may be partially attributable to: difficulty in gaining access to LTC homes, or determination of prevalence impeded by variance in defining “deficiency”. The confidential nature of resident files and the LTC population considered to be a vulnerable group have also been barriers to gaining access for anyone outside the residents’ circle of care [131].

The potentially high prevalence in the community, especially of physically and cognitively dependent older adults [113] suggests that screening for B12 status at admission to LTC is a potentially worthwhile process. Additionally, persons receiving transitional care are generally more vulnerable due to: the stress and anxiety of relocation [132]; the potential for breakdown in communication on medication and other treatments that are not incorporated into the resident’s care plan post admission [133]; and change in primary physician caring for the resident. As a result, B12 treatment started in the community (especially intramuscular modality (IM) which is normally provided on a monthly basis, typically in a doctor’s office), may be overlooked and lost as a

component of the resident's care plan. To date in LTC, there have been no studies that report on status of B12 deficiency at admission or incidence of developing this deficiency post admission. While understanding prevalence at admission to LTC is a first step for determining if standardized screening and treatment protocols are required, understanding incidence provides insight into how B12 status changes with age, and other factors and over time while living in residence.

Only four studies of the LTC population conducted in the past decade have reported B12 deficiency prevalence [39, 65, 80, 82] with only one, small study (n=75) based in Canada [65]. None of these studies considered the question of prevalence of deficiency on admission or incidence within one year of admission to LTC. The Canadian study took place in one LTC/chronic care hospital in Guelph, Ontario [65]. The primary research objective of this study was to determine prevalence of vitamin deficiencies (including B12) in LTC. Secondary objectives were to determine if associations existed between B12 status and cognitive function, vitamin supplementation, and medications impacting gastric acid [65]. With a sample size of 75, which was potentially biased as 35% refused consent, this study was likely insufficiently powered to detect these associations, and is unlikely to be representative of LTC homes in Ontario. The prevalence of B12 deficiency in this sample was 7%, using a cut-point for deficiency of <148 pmol/L (<200 pg/mL) [65].

The three non-Canadian studies found a range in prevalence from 8- 34% [39, 80, 82]. A study in one LTC home of male veterans in Taiwan [80] was designed to explore the association between vitamin B12 supplement use and cognitive function and depressive symptoms [80]. Although this was a large study (n=419), it was not designed to answer the question of prevalence and exclusion criteria may have biased the sample beyond the single gender inclusion. The prevalence of B12 deficiency in this sample was 8% using a cut-point for deficiency of <133 pmo/L (<180 pg/mL) [80]. A study in Jordan compared young adults to older adults living at home or in one of five LTC homes [82]. The primary research objective was to determine the prevalence of hyperhomocysteinemia and folate and B12 deficiencies [82]. Multiple comparison groups and several covariates including medical history, smoking habits, and lifestyle habits were measured. However, it was unclear how participants were selected and the sample size of each group was relatively small (77 young adults, 169 community living older adults, 38 older adults living in LTC). In addition, those with "coronary heart disease, systemic illness, serious-organ disease, endocrine disease, and those currently taking vitamins" (pg. 410) were excluded [82] which limits the generalizability of these results. The prevalence of B12 deficiency in this sample was 34% in both LTC and community living older adults and 9% in young

adults using a cut-point for deficiency of <154 pmol/L (<208pg/mL). The most recent study was from 2012 and took place across 5 LTC homes in Australia [39]. The primary objective was to determine the prevalence of undiagnosed B12 deficiency in Australian LTC homes. Strengths of this study include random sampling across more than one site, with a high representation from the sample population (35% of residents were included). With a sample size of 130, the study was well powered to detect prevalence of B12 deficiency. However, as the study included all current residents, it is unclear if B12 deficiency developed during stay or prior to admission to the home. The prevalence of B12 deficiency in this sample was 14% using a cut-point for deficiency of <150 pmol/L (<203 pg/mL) [39]. One study reported on B12 deficiency incidence in a sample of Alzheimer's disease patients recruited from a health science centre [134]. Over three years, incidence was identified in 7.3% of Alzheimer's patients, however this study lacks generalizability as those with dementia other than Alzheimer's were excluded in addition to those who used B12 supplements; residence was not considered [134].

It is apparent that there is limited evidence on prevalence to support LTC testing and treatment protocols for vitamin B12 deficiency. Despite this lack of evidence, general recommendations for B12 testing and treatment have been released [86, 112] and current practices for testing and treatment in Ontario LTC homes have been reported for the first time [67]. Specifically 67% (30/45) of LTC homes in Ontario test for B12 status on admission to the home. With regard to treatment, in the literature, there has been a change in understanding of the effectiveness of typical treatment methods (i.e., oral therapy vs. intramuscular therapy) which is consistent with the recommendations, however it is unclear how this translates into practice [37, 41, 64, 135]. Additionally, recommendations for screening and treatment are not specific to LTC. Further work is required to help establish such recommendations. For example, what is the effectiveness of treatment methods for older adults leading up to admission to LTC and during residence, and who is at risk for B12 deficiency upon entry into an LTC home. This knowledge would help to determine treatment modalities as well as if screening should be in place and if it should be targeted to specific subgroups of the population or for all new admissions. Several medications and diagnoses have been associated with decreasing B12 levels including: metformin [3, 56-58, 95], antacids such as proton-pump inhibitors [40, 94] and H2 blockers [3], antibiotics [3], hypothyroidism [104], gastrointestinal (GI) conditions increasing the risk for food-B12 malabsorption [2, 54], mood and mood related disorders [7, 15, 16, 21, 62, 63, 97-102], dementia (including Alzheimer's-type dementia (ATD)) [74] and cognitive decline [1, 63, 70, 96], as well as cardiovascular disease [4, 23-26] and elevated levels of homocysteine [27]. B12 status may be

associated with the performance of daily activities as well as nutrition related covariates such as weight changes and could thus influence the quality of life of older adults living in LTC. All of these are potential means of identifying targeted subgroups that should be screened on a routine basis, yet data is lacking in a single study to support such a targeted approach.

5.3 Purpose

The primary aims of this retrospective study were to determine the prevalence of vitamin B12 deficiency at admission to LTC across several sites and to describe the sample characteristics of persons identified to be deficient. Secondary research aims were to explore: how admission B12 status and B12 levels are associated with covariates of interest (e.g., supplementation use at admission, BMI, number of diagnoses, number of medications, type of medications, presence of mood related disorders, PPI use, etc.); if B12 status changed one year post admission; and if this change in status was associated with these covariates of interest.

5.4 Methods

5.4.1 Sample and Design

This was a retrospective prevalence study which took place in originally 9 of 11 (8 completed) of the Schlegel Villages (SVs). The SVs are a family-run group of continuum-of-care homes across Ontario (Windsor to Barrie) offering independent living (in retirement) to long-term care. This organization has an organization-wide policy for admission bloodwork to be completed for each resident and B12 testing is included in the work-up, making this study feasible. As a result, policies and procedures are similar across the organization and due to geographical differences, residents across multiple homes are diverse and potentially more representative of all Ontario LTC residents. Prevalence of B12 deficiency was determined using admission bloodwork. Due to differences in the cut-points for B12 deficiency defined by labs contracted by a home, a common B12 level was needed to determine potential B12 deficiency. Recent work by the authors involved an environmental scan of Ontario LTC home practices pertaining to vitamin B12 testing and treatment in 45 LTC homes across Ontario. Results also indicated that there was no consensus to define vitamin B12 deficiency; cut-points used were directed by the lab at which the blood samples were analysed and ranged between 100 pmol/L to 300 pmol/L. The most common cut-point used for defining vitamin B12 deficiency was less than 156 pmol/L in 31% of homes (12/45) followed by less than 198 pmol/L in 21% and less than 148 pmol/L in 21% of LTC homes. Based on this work, the cut-point at which to define vitamin B12 deficiency in

this study was defined as less than 156 pmol/L [41]. In addition, the cut-point at which to define normal vitamin B12 was a serum value greater than 300 pmol/L. The rationale for this definition comes from the authors' prior work in which this cut-point was found to be a conceivable level; values in between "deficient" and "normal" were defined as subclinical (156 pmol/L – 300 pmol/L).The subset of 9 LTC homes included in this study were limited to those within two hours of the University of Waterloo to promote feasibility with data collection.

5.4.2 Sample Size and Sampling Frame

A complete listing of all current residents in each of the 9 LTCs included in the sample was provided (n=1355) and this value was used to estimate the required sample size to determine prevalence of B12 deficiency. A prevalence as low as 5% with 95% confidence could be determined with a sample of size of 341 [136]. After data collection began, it was evident that the practices with respect to detection of B12 status at admission at one site were different than that of the other 8. To reduce bias, this site was removed from the sampling frame. In addition, one neighbourhood (unit) was on an infectious outbreak precaution at the time of the chart review; the number of charts to be collected from this site remained constant but were only collected from the remaining neighbourhoods. The total population of older adults was thus recalculated, yielding n=1061. Thus the final required sample size to estimate a prevalence of 5% with 95% confidence with a finite population correction was identified to be 319.

Charts were only available for extraction of current residents. Eligible residents were those in the LTC portion of the home who were over the age of 65 years (on average 88% of residents; n=1183) and for whom there was admission bloodwork. Random proportionate sampling was used to identify which preliminarily eligible resident charts (i.e., > 65 years) were selected for data extraction. Bloodwork was only available on the physical chart in hard copy reports. Based on this random sampling, residents chart reviews were conducted for each neighbourhood and home until the predetermined proportionate number based on the home size and total sample size of the population was reached. Eligibility was confirmed at each site based on those for whom there was admission bloodwork; if a resident was selected randomly and did not have admission B12 bloodwork, this resident was replaced with the next one on the random number list. Of the 1061 residents 65 years of age and over at admission, 535 residents were excluded due to missing or inability to locate admission bloodwork. One resident's chart which was randomly selected was removed as the resident had previously opted out of research projects, and was thus excluded and replaced. The total number

of charts reviewed and included was 413. One resident was removed from analysis as their data from the MDS report corresponded to a different resident and thus the total number of residents data included in this prevalence estimate for admission bloodwork was 412 (see **Appendix A**).

5.4.3 Description of Extracted Variables

A data extraction from was created, reviewed by the research team and tested in 30 charts to ensure that all relevant information could be extracted. Except for serum vitamin B12 level at admission, missing values were permissible for any covariate as this did not affect determination of prevalence; therefore, proportions of missing data varied across covariates. Chart reviews were conducted by the first author and data were extracted as follows: basic demographics (e.g., sex, age, length of stay, marital status, etc.), serum vitamin B12 levels on admission to LTC and at first annual bloodwork if available, and the lab-based determination of deficiency; number of medications, prescribed medications at admission; number of diagnoses with note of cardiovascular disease, dementia, Alzheimer's-type dementia (ATD), hypothyroidism, gastro-intestinal (GI) conditions, mood conditions and selected variables from the Minimum Data Set (MDS), as described below.

Due to charting differences among sites and over time (as some residents had been residents for several years), the following process was used to extract a complete data collection and ensure consistency in values extracted. Admission lab results were reviewed and the value was recorded including the lab-specific cut-point used for defining B12 deficiency. In addition, first annual lab results were reviewed wherever possible to extract B12 level at 1 year post admission to LTC. Next, the new admission information form for basic demographics, medications and diagnoses was reviewed. When admission medications were unavailable on this form, nursing home admission documents were checked. If duplicates of medications were listed (e.g., 1 medication taken more than once per day or different doses at different times), these were counted as one medication. Medications for over the counter skin lotions (e.g., barrier, penaten) were not counted towards total number of admission medications. Those residents receiving *B12 treatment therapy* (resident receiving treatment: Yes/No and form: intramuscular injections or oral) prior to admission to the home were noted. If no admission form was available to determine diagnoses, the first post admission printout from the resident medical tracking software (GoldCare) was used. Diagnoses were counted only according to what was written in the admission diagnoses section. It was noted that there was inconsistency in this history with respect to prior surgeries; when surgeries were included as part of patient history, they were counted as diagnoses.

Antibiotic use was recorded as "yes" if resident was prescribed oral antibiotics or an ointment containing antibiotics. *Metformin* use was recorded as "yes" if a resident was prescribed metformin. *PPI, H2 blocker* and *antacid* use was recorded as "yes" if a resident was prescribed a type of PPI, H2 blocker or antacid respectively. The presence of *cardiovascular disease* was recorded as "yes" if a resident had a history of stroke, CAD, peripheral vascular disease or atrial fibrillation. The presence of *dementia* was recorded as "yes" if a resident was diagnosed with dementia, mixed dementia, mild dementia, or diagnosed with a probable dementia; diagnosis of cognitive decline was not included in this definition of dementia. The presence of *GI conditions* was recorded as "yes" if the resident had a diagnosis of gastroesophageal reflux disease (GERD) or were prescribed a PPI, H2 blocker or antacid. The presence of *hypothyroidism* or thyroid dysfunction not including hyperthyroidism was recorded as "yes" if the resident had a diagnosis of hypothyroidism. The presence of *mood/psychiatric illness* or mood related disorders was recorded as "yes" if the resident had a diagnosis of depression, anxiety, or schizophrenia. If diagnoses were missing from the new admission information form or nursing home admission documents, admission diagnoses and number of diagnoses were regarded as "missing".

Several variables from the MDS at admission were extracted. For a description on relevant subsections, see **Appendix B**. A RAI-MDS 2.0 report was generated by the information specialist at the Schlegel Villages for all residents within an available timeframe (2010 – 2014). This timeframe captured admission data for over 95% of included residents; 19 residents moved in prior to 2010 and these data were thus not available. Cognitive performance scale (CPS) score corresponds closely with the Mini-Mental State Examination [137]; score values range from 0 (intact) to 6 (very severe impairment). The ADL-short (ADL-s) scores range from 0-16 and ADL-long (ADL-l) scores range from 0-28 where a higher score indicates greater difficulty in performing daily activities [138] were also extracted. The report also contained data on resident's weight, height, nutritional problems and weight change, although this data was missing from one site for the year 2010. Body Mass Index (BMI) was calculated by dividing weight (kg) by the square of the height (m) from the MDS data. Upon computation, three biologically implausible values were removed in accordance with prior work [139], assuming the data were improperly entered.

5.4.4 Statistical Methods

Data were entered into Excel and imported into SPSS version 23.0 for statistical analyses. Frequencies, proportions and descriptive analyses as well as graphing of variables were used to clean

the data. ANOVA were conducted to determine the association between B12 values and categorical variables. Where appropriate, the Tukey B test was used to establish between group differences [140]. Chi-square tests were used to determine associations between categorical variables. Paired t tests were conducted for all admission and first annual bloodwork comparisons. The level of significance used was $p \leq 0.01$ to account for multiple tests. A two-proportion z-test was used to establish whether the proportionate samples were significantly different than the planned sample proportions using a level of significance of $p < 0.05$ [141].

5.5 Results

5.5.1 Subject Characteristics

The proportion of charts included from each village did not differ significantly from the planned proportion of residents to be included to ensure a representative sample ($p > 0.05$). However the number of alternates needed to reach sufficient inclusion from each village ranged between 4 and 99 with a mean of 67 and a median of 72. See **Appendix A** for a flow diagram for this study.

Of the 412 reviewed charts, 286 (69%) of residents were female. The mean age of this sample at admission was 83 (±7yrs) with a range from 65 to 101. The average, standard deviation and mode for length of stay from admission to date of data collection was 22 ± 20 months and 9 months respectively. The most recent admission was 1 month prior to data collection and the longest length of stay prior to data collection was 11.2 years. Marital status in this sample was as follows: 48% widowed, 39% married, 6% divorced, 5% single or never married, and 2% were separated. The mean number of admitting diagnoses was 6.05 (SD 2.73) ranging from 1 to 18. The following key conditions had the following prevalence: diabetes (21.6%), cardiovascular disease (38.0%), GI conditions (28.1%), hypothyroidism (20.2%) mood or psychiatric disorders (57.7%), dementia (49.5%), and Alzheimer's type dementia (17.0%). When dementia and ADT were considered together, 66.1% of the sample was affected.

The mean number of medications at admission for this sample was 10 (SD 4.5) with a range from 0-28. In terms of medication use related to GI conditions, 26.3% of residents were taking proton pump inhibitors, 1.5% of residents were taking H2 blockers, and 2.7% of residents were taking antacids. Metformin was used by 10.1% of residents and antibiotics were used by 6.2% of residents. At admission, 26.0% of residents were receiving vitamin B12 treatment. Of these, 80.7% received oral B12, 15.9% IM injections and 3.4% received both oral and IM B12 treatment. Of the residents

receiving oral B12, 97.3% received oral B12 treatment daily with the remaining 2.7% receiving B12 twice daily. Dose of oral B12 treatment ranged from 100 to 1250 mcg; the most common dose was 1000 mcg (60.0%); the dose for four residents was missing. Of the residents receiving IM B12, 93.8% of residents received 1000 mcg while the remaining 6.3% of residents received 300 mcg; the dose for one resident was missing. Frequency for IM B12 was most commonly monthly (82.4%) followed by quarterly (11.8%), and every two months (5.9%).

At admission, 46.2% of the sample was cognitively impaired (i.e., CPS score of >3); the mean CPS score was 2.46 (SD=1.47) with a mode of 3 indicating that on average, residents were moderately cognitively impaired. The breakdown for residents' cognitive status is as follows: 12.5% cognitively intact (CPS=0), 10.1% borderline intact (CPS=1), 25.1% mild impairment (CPS=2), 37.0% moderate impairment (CPS=3), 5.5% moderate severe impairment (CPS=4), 6.1% severe impairment (CPS=5), and 3.7% very severe impairment (CPS=6). At admission the mean ADL-s and ADL-l scores were 6.60 (SD 4.24) and 12.69 (SD 7.64) respectively. Residents' scores ranged from 0 to 16 on the ADL-s and from 0 to 28 on the ADL-l indicating a high degree of variability in terms of physical functioning.

In terms of nutritional status, nutritional problems and weight change and BMI were extracted. Within the past 7 day period, 4.6% of residents complained about the taste of many foods, 1.0% of residents had regular or repetitive complaints about hunger, 27.7% of residents left 25% or more of food uneaten at most meals and 68.9% of residents had no evidence to suggest nutritional problems. Weight loss of 5% or more in the last 30 days or 10% or more in the last 180 days was observed in 5.8% of the sample, while weight change was recorded as unknown on MDS in 8.6% of the sample. Similarly, a weight gain of 5% or more in the last 30 days or 10% or more in the last 180 days was observed in 6.6% and was unknown in 8.6% of the sample. After the removal of three inconceivable values, the average BMI in this sample was 28.18 (SD 6.58) with values ranging from 13.22 to 60.59. When BMI was grouped according to low (BMI<20), normal (20≤BMI≤30) and high (BMI>30) ranges, 6.7% of residents had a low BMI, 58.1% had a normal BMI, and 35.2% had a high BMI.

5.5.2 Prevalence of B12 Deficiency, Covariates, Treatment and One-Year Status

The mean serum B12 level at admission was 358.3 ± 229.3 pmol/L and overall prevalence of B12 deficiency was 13.8% (57/412) using the definition of less than 156 pmol/L. Prevalence of subclinical deficiency as defined as serum B12 level less than 300 pmol/L at admission, was 38.3% (158/412) while 47.6% (197/412) of residents had a normal B12 status (>300 pmol/L) at admission to their LTC

home. While prevalence of B12 deficiency within a given home ranged from 4.1% to 27.1%; these differences were not significant ($p>0.01$). All villages had proportions of residents with normal admission B12 status less than 56%. Using cut-points defined by the various labs (deficient <148,110,107; subclinical: 148-220, 110-150, 107-133; normal: >220, 150, 133) yielded variable deficiency prevalence estimates of 11.4%, 2.7%, and 1.7% respectively. **Figure 5** provides the distribution of residents' B12 levels at admission with the cut-points for deficiency from the lab and as defined in this study, this illustrates how different cut-points drives variable prevalence estimates.

Table 4 and 5 provide a summary of results associating B12 status on admission with covariates of interest. Vitamin B12 supplementation use at admission to LTC was significantly associated with higher admission serum B12 ($p<0.001$; $\chi^2=60.784$ (df=2)). A significantly smaller proportion of residents receiving B12 at admission had deficient (2.3% vs 18.4%) or subclinical status (13.6% vs 45.6%) than those not receiving B12 at admission thus, a significantly larger proportion of residents receiving B12 at admission had normal admission B12 status compared to those not receiving B12 (84.1% vs 36.0%). Number of medications was significantly associated with admission B12 ($p<0.01$; $F=6.019$ (df=2,299)). Those with B12 deficient status (<156 pmol/L) at admission were, on average, receiving significantly fewer medications (mean=8.5 medications) than those with normal B12 status (mean=10.8 medications). There was no significant difference however, when subclinical B12 was compared to either B12 deficient or normal B12 status at admission. No other covariates were statistically significantly associated with B12 status.

The type of B12 supplementation method at admission approached significance with B12 status ($p=0.012$; $F=11.620$). **Table 6** provides a summary of results associating B12 supplementation use at admission with covariates of interest. In terms of oral-B12-prescribed residents, there was a higher proportion of normal (90% vs 57%) and a lower proportion of subclinical (8.5% vs 35.7%) and deficient (1.4% vs 7.1%) serum B12 than those who received IM B12.Significant associations were found between B12 supplementation at admission and presence of dementia ($p<0.01$; χ^2 (df=1)=12.028) and having either dementia or ATD ($p<0.01$; χ^2 (df=1)=9.547). However, ATD alone was not significantly associated with B12 supplementation at admission to LTC ($p>0.8$; χ^2 (df=1)=0.097).

Only 40% of included charts (163/412) had first annual bloodwork recorded, and as a result, efficacy of treatment post identification of deficiency at admission could not be determined and associations between resident characteristics and change in B12 status will not be presented.

Descriptive analyses on change in status in this subsample are as follows (see **Table 7**). Within this subsample, residents' B12 status was significantly improved at first annual bloodwork compared to admission ($p<0.001$; $F=25.6$). Specifically, the majority of residents initially with vitamin B12 deficiency with year one data (n=16) had improved B12 status after 1 year (75%); 25% of initially deficient residents improved to subclinical deficiency, while 50% improved to normal status after 1 year. Of the residents initially in the subclinical deficiency category for B12 status with year one data (n=64), the majority of residents maintained this status at one year (56.3%); 35.9% of residents improved their status to "normal" and 7.8% of residents initially subclinical moved to a deficient state. Of the residents initially with normal B12 status with year one data (n=62), the majority of residents maintained this normal status (74.2%), 24.2% worsened to subclinical deficiency and 1.6% worsened to deficient status. Based on this subsample, incidence for worsened B12 status within this LTC sample at one-year was 14.8% (21/142); 4.2% to deficiency (6/142), and 10.6% subclinical deficiency (15/142).

5.6 Discussion

The overall prevalence of B12 deficiency at admission to these eight LTC homes was found to be 14% ranging from 4.1% to 27%. This range of estimates is within that of both community estimates (3-43%) [11, 15, 16, 39, 41, 75-77, 113] and the few studies conducted in LTC (7-34%) [39, 65, 80, 82]. The variation in prevalence across the eight sites demonstrates the importance of assessing multiple sites to determine a more representative estimate of prevalence. Among these LTC-related studies, similar cut-points to define B12 deficiency were used (<148 pmol/L [65], <133 pmol/L [80, 142], <150 pmol/L [39], <154 pmol/L [82]) which simplifies comparison of prevalence estimates. Higher cut-points have also been used (e.g., <185 pmol/L [83], <258 pmol/L [40]) which translates to a greater proportion of the sample defined as deficient and fewer residents missed who may benefit from treatment. Conversely, a lower-cut-point (e.g., <74 pmol/L [142, 143], <116 pmol/L [79], <125 pmol/L [64]) results in a smaller proportion of the sample identified as having deficiency. Pertaining to this study, labs primarily used one of three cut-points (148 pmol/L, 110 pmol/L or 107 pmol/L) yielding lower prevalence estimates (11.4%, 2.7% and 1.7% respectively) than the 156 pmol/L used in this study. This likely affected treatment that was instituted, although this was not investigated, as it is anticipated that physicians would respond to a deficient result provided by the lab documentation.

Classically, presence of anemia has been used to diagnose B12 deficiency as it was thought progression of deficiency moved from hematologic to neurologic problems [11]. However these

symptoms are sometimes non-existent; more recent reports have described neurologic changes in the absence of hematologic changes [11]. Neurologic changes are present in most clinically deficient persons (75%-90%) and in up to 33% of these cases, is the only symptom present [11]. Furthermore, there is some evidence to suggest that nonspecific symptoms associated with subclinical B12 deficiency can be present even at normal serum levels [1, 11] as tissues are depleted of B12 to maintain serum levels. This latter point highlights the importance of considering the cut-point used to define deficiency in prevalence studies and makes a case for considering subclinical deficiency as a treatable state. In this present work, when subclinical deficiency (156 pmol/L < B12 < 300 pmol/L) was considered, an additional 38% of residents were affected. This means that more than half (52%) of residents have less than an adequate B12 status at admission to LTC. This is similar to previous estimates of inadequate, but not solely deficient, B12 status in LTC (45% [39], 41% [65] and 61% [82]). Vitamin B12 status is thus a relevant form of malnutrition which needs to be considered in LTC populations and addressed with appropriate interventions. These data provide evidence in support of using oral B12 supplements over IM for older adults living in the community as there was a higher proportion of those with normal status and lower proportion of those with subclinical and deficient status for those using oral; whether this remains true after transition to LTC is unclear. It could be hypothesised that this may speak to an access to care issue as community-dwelling older adults may have a difficult time getting to their physicians office for IM treatment and thus result in missed doses. These data on prevalence of deficiency and sub-clinical deficiency suggest that screening for B12 on admission is a relevant and appropriate practice for LTC in Canada.

Several intervention studies including a subgroup from LTC exist, many commenting on treatment effectiveness. While a handful of these additional studies also report on prevalence, inclusion or exclusion criteria were targeted at specific conditions [64, 66, 81] or medication use [40, 143] which would either overestimate or underestimate true prevalence. For example, there is evidence to suggest that PPI use increases the risk of reduced B12 levels [1, 40, 94] by decreasing the amount of hydrochloric acid of the stomach required to break B12 apart from food-bound sources [1]. In one study investigating associations between PPI use and B12 status [40] prevalence was 47%. Nevertheless for the purpose of describing effectiveness of treatment, these studies have merit. Treatment methods in these studies included: IM injections [66], oral pills [64], a comparison of oral and IM treatment [37], multivitamin supplementation use [81], liquid nutritional supplement fortified with B12 [51], and nasal spray [40]. All formats significantly improved B12 levels [37, 40, 51, 64, 81] or B12 associated biomarkers [66]. This suggests that the LTC resident‘s B12 status is amenable

to treatment. This is consistent with the present study as those receiving vitamin B12 therapy prior to admission had a significantly lower proportion of deficient B12 status' at admission to LTC (18% vs 2%) ($p<0.001$; $\chi^2=60.784$ (df=2)) and a significantly higher mean serum B12 level (538 (SD 259) vs. 291 (SD 172)) when compared to those not supplemented at admission ($p<0.001$; F=-8.298 (df=115)). In addition, when comparing treatment type prior to admission with admission B12 status, a significantly larger proportion of residents receiving oral B12 had normal B12 status (90% vs 57%) and a significantly smaller proportion had deficient (1% vs 7%) and subclinical (9% vs 36%) statuses compared to those receiving IM ($p=0.006$; F=0.045). This provides evidence to suggest that using oral B12 therapy and moving away from IM to treat B12 deficiency is feasible and effective and is consistent with other studies [37, 41, 86, 135]. Whether this remains true for older adults after admission to LTC remains unknown. One study comparing effectiveness of IM to oral supplementation in older adults indicated that while both were effective, IM was more effective [37].

A significant association was found between B12 status at admission and a few of the resident characteristics. Use of fewer medications was associated with lower serum B12 at admission and a deficient status. This was an unanticipated finding based on the prior literature which suggests that comorbidities and some drug classes are associated with B12 deficiency. It could be hypothesized that number of medications was confounded by dementia status. An exploratory association identified that having dementia or ATD was significantly associated with taking fewer of medications ($p<0.001$). In addition, a recent study reviewing the degree of polypharmacy in all residents in LTC homes of Ontario, revealed this same association between dementia and medication use [90]. Thus, the association between number of medications and B12 status is likely confounded by dementia status. This finding may also be spurious as an association was not seen with subclinical deficiency.

Similar to evidence suggesting that PPI use may lower B12, other antacids (e.g., H2 blockers) and antibiotics may also interfere with absorption or uptake of vitamin B12 [3]. In this present study, a very small proportion of residents were prescribed these medications; (antibiotics 6%; H2 blocker 2% and antacids 3%). Thus, the proportion of those taking these medications in addition to having poor B12 status at admission is even smaller. Similarly 49% and 66% of this sample was affected by mood or mood related disorders and dementia (including Alzheimer's-type dementia) therefore there was a low degree of variability in these covariates. This study was powered to detect a prevalence of B12 deficiency at admission of 5% with $\beta=0.2$; all other aims were secondary and more exploratory in nature. Thus, null findings regarding associations with rare covariates or covariates expressing low

variability may have been due to a type II error. Further work specifically sampling based on these covariates is required to determine if suspected associations between these covariates occur in LTC populations. Regardless, at this time, these data do not support targeted screening for B12 deficiency in users of these medications.

There was a significantly higher proportion of B12 supplementation in those with dementia compared to those without (35% vs 18%) and in those when dementia and ATD were considered together (32% vs 16%) ($p<0.01$). However the proportion of B12 supplementation use in those with ATD compared to those without (25% vs 27%) was not significant ($p>0.8$). This may be indicative of different care practices between those with ATD and other forms of dementia. Recommendations from 2007 for B12 testing in the work-up of dementia suggest that B12 testing should be conducted in those with suspected dementia or cognitive decline and that those with low B12 should be treated with B12 therapy [112]. However, it is noted that these recommendations may not be applicable to those with early-onset dementia including early-onset familial Alzheimer's disease [112]. This may explain in part why supplementation use was significantly associated with dementia status considered alone or with ATD, but not in ATD status alone. In 2013, the Ontario Health Technology Advisory Committee (OHTAC) released recommendations for B12 and cognitive function recommending against serum B12 testing when investigating potential dementia or cognitive impairment [86]. This study needs to be repeated at a later time to determine if this recommendation changes the current practice of diagnostic work-up and treatment of B12 deficiency in persons with dementia, as evidenced in this study.

Two thirds of the sample did not have readily accessible first annual bloodwork and thus the subset with available data was potentially biased. Therefore, the efficacy of treatment post identification of deficiency in this sample cannot be determined. Despite the bias in this subsample for which first annual bloodwork was found, changes in B12 status have clinical significance. Specifically, 25% (4/16) of those initially deficient continued to have deficiency at one-year, but the majority of those found to be deficient at admission had improved by this time point. Of concern, 15% of the sample had worsened B12 status after 1 year in LTC as compared to admission, which indicates, for the first time, that incidence of B12 deficiency is also relevant to this population. This further demonstrates the value and necessity of not only screening and treating, but also of monitoring B12 status on a routine basis in LTC. Due to the concern about bias, resident covariates were not explored to see if there were any associations with change in status post admission. Prospective

research needs to be conducted to determine these potential associations and what characteristics describe a subset of residents that do not respond to treatment.

5.7 Strengths and Limitations

A key strength is the eight sites included in this study resulting in a large sample with potentially greater diversity than previously reported LTC research. At this organization, the same blood testing protocol has been used for over 10 years which eliminates any potential for recency bias, which is a significant challenge with retrospective studies of this nature. This study also used random sampling and had excellent representation of eligible residents (i.e., residents aged 65 or older at admission to LTC). Specifically, the sample represented 37% of the total eligible population and between 31% and 43% of eligible residents at each of the eight sites. This was the first study to report on incidence of vitamin B12 deficiency providing a point for comparison for future studies designed to specifically address incidence and associated covariates.

This study collected data across one organization in one province of Canada which is a limitation as it may not be generalizable to other organizations or countries. It is anticipated that results are consistent with other LTC homes in south-western Ontario. The sample was drawn from this region and there is no bias in admission to homes based on demographic status of residents; all receive the same process for placement through the Community Care Access Centres in this province and have equal access with respect to key demographics and diagnoses that could influence B12 status. Another limitation to this study is that different sites handled resident information and charted data differently yielding some missing values; between four and 99 alternates were needed to meet the number of chart reviews containing complete admission B12 bloodwork at each of the sites. As a result, in some sites almost all charts were reviewed to determine an eligible sample, demonstrating the challenge of missing data with retrospective chart reviews. A final limitation was the high proportion of missing data on the first annual follow up. The subsample with this data was likely biased. This also resulted in relatively few completed data points to determine a change in vitamin B12 status and thus the exploratory research question concerning resident characteristics that could influence treatment efficacy could not be addressed. Yet, this is the first study to report on how B12 status changes over the first year of residence in LTC and as such does add to the field.

5.8 Conclusions

The prevalence of vitamin B12 deficiency at admission to eight LTC homes across one organization in Ontario was determined to be 14%. More than 50% of residents had vitamin B12 levels under 300 pmol/L indicating poor vitamin B12 status overall in this LTC sample. It could be argued that this prevalence of subclinical/deficient status warrants screening or treating at admission for all LTC residents. Furthermore, resident characteristics (excepting number of medications) were not associated with status, indicating that targeting a subset of residents at admission for screening is an inappropriate strategy at this point for detecting deficiency. Of the subsample with follow-up B12 measures at one year, B12 status was significantly improved for 75% of initially deficient residents, however 15% of those with an initial subclinical or normal B12 status worsened. Better admission B12 status and admission B12 levels were significantly associated with supplementation prior to admission. Future work to inform policy and intervention protocols should use prospective methods focusing on fidelity to treatment protocols, as well as to determine incident B12 deficiency in LTC and characteristics of residents that result in these outcomes.

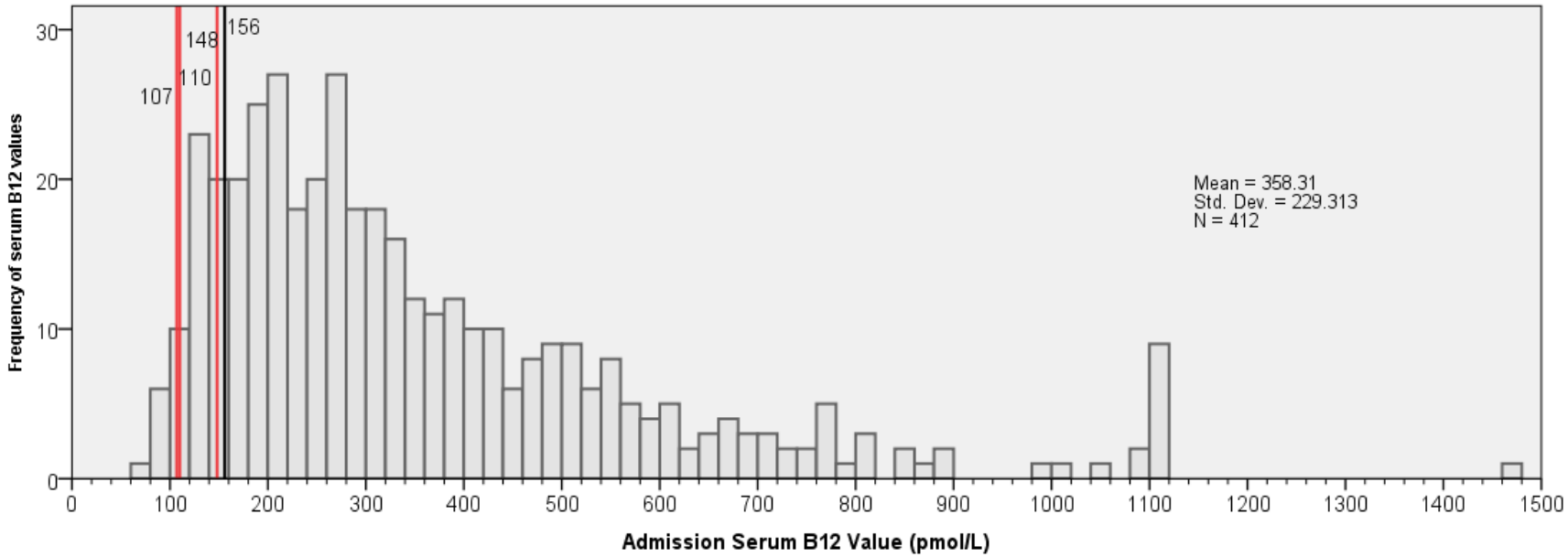


Figure 5: *Distribution of serum B12 levels at admission to LTC with lab cut-points (red) and author defined cut-point (black).*

Table 4: *Association between B12 status (deficient, subclinical, normal) and covariates.*

Characteristic	Total Sample % (n)	Category	Deficient	Subclinical	Normal
Sex	69.4% (286/412)	Female	12.2% (35)	38.8% (111)	49.0% (140)
		Male	17.5% (22)	37.3% (47)	45.2% (57)
Marital status (married)	38.5% (129/335)	Yes	14.7% (19)	40.3% (52)	45.0% (58)
		No	16.0% (33)	35.9% (74)	48.1% (99)
Taking B12 supplements at admission**	26.0% (88/338)	Yes	2.3% (2)	13.6% (12)	84.1% (74)
		No	18.4% (46)	45.6% (114)	36.0% (90)
Form of B12 supplements at admission **	26.0% (88/338)	Oral*	1.4% (1)	8.5% (6)	90.1% (64)
		IM*	7.1% (1)	35.7% (5)	57.1% (8)
		Oral + IM	0.0% (0)	33.3% (1)	66.7% (2)
		None	18.4% (46)	45.6% (114)	36.0% (90)
Hypothyroidism	20.2% (74/366)	Yes	12.2% (9)	31.1% (23)	56.8% (42)
		No	14.0% (41)	40.4% (118)	45.5% (133)
Metformin use	10.1% (34/338)	Yes	17.6% (6)	35.3% (12)	47.1% (16)
		No	13.8% (42)	37.5% (114)	48.7% (148)
Antibiotics	6.2% (21/338)	Yes	4.8% (1)	33.3% (7)	61.9% (19)
		No	14.8% (47)	37.5% (119)	47.6% (151)
CVD	38.0% (139/366)	Yes	12.2% (17)	43.9% (61)	43.9% (61)
		No	14.5% (33)	35.2% (80)	50.2% (114)
GI conditions	28.1% (103/366)	Yes	13.6% (14)	38.8% (40)	47.6% (49)
		No	13.7% (36)	38.4% (101)	47.9% (126)
Antacid use	2.7% (9/338)	Yes	22.2% (2)	22.2% (2)	55.6% (5)
		No	14.0% (46)	37.7% (124)	48.3% (159)
PPI use	26.3% (89/338)	Yes	11.2% (10)	40.4% (36)	48.3% (43)
		No	15.3% (38)	36.1% (90)	48.6% (121)
H2 blocker use	1.5% (5/338)	Yes	20.0% (1)	60.0% (3)	20.0% (1)
		No	14.1% (47)	36.9% (123)	48.9% (163)
Mood & mood related disorders	57.7% (211/366)	Yes	10.9% (23)	39.3% (83)	49.8% (105)
		No	17.4% (27)	37.4% (58)	45.2% (70)
Dementia	49.5% (181/366)	Yes	14.9% (27)	35.4% (64)	49.7% (90)
		No	12.4% (23)	41.6% (77)	45.9% (85)
Alzheimer's	19.1% (70/366)	Yes	11.4% (8)	40.0% (28)	48.6% (34)
		No	14.2% (42)	38.2% (113)	47.6% (141)
Dementia or Alzheimer's	66.1% (242/366)	Yes	14.0% (34)	36.8% (89)	49.2% (119)
		No	12.9% (16)	41.9% (52)	45.2% (56)
Categorical CPS [0,1,2 vs 3,4,5,6]	47.7% (156/327)	Yes	16.0% (25)	39.1% (61)	44.9% (70)
		No	10.5% (18)	35.1% (60)	54.4% (93)
Weight change – K3a Weight loss	5.8% (20/347)	Yes	20.0% (4)	40.0% (8)	40.0% (8)
		No	13.5% (40)	38.0% (113)	48.5% (144)
		Unknown	13.3% (4)	36.7% (11)	50.0% (15)
Weight change – K3b Weight gain	6.6% (23/347)	Yes	4.3% (1)	47.8% (11)	47.8% (11)
		No	14.6% (43)	37.4% (110)	48.0% (141)
		Unknown	13.3% (4)	36.7% (11)	50.0% (15)
Nutritional problems – K4a complains about taste of foods	4.6% (16/347)	Yes	18.8% (3)	37.5% (6)	43.8% (7)
		No	13.6% (45)	38.1% (126)	48.3% (160)

Characteristic	Total Sample % (n)	Category	Deficient	Subclinical	Normal
Nutritional problems – K4b regular or repetitive complaints of hunger	0.9% (3/347)	Yes	33.3% (1)	66.7% (2)	0.0% (0)
		No	13.7% (47)	37.8% (130)	48.5% (167)
Nutritional problems – K4c Leave 25% or more of food uneaten at most meals	27.7% (96/347)	Yes	14.6% (14)	39.6% (38)	45.8% (44)
		No	13.5% (34)	37.5% (94)	49.0% (123)
Nutritional problems – K4d None of the above	68.9% (239/347)	Yes	13.0% (31)	37.2% (89)	49.8% (119)
		No	15.7% (17)	39.8% (43)	44.4% (48)
B12 deficiency across Villages	13.8% (57/412)	Village1	25.8% (8)	25.8% (8)	48.4% (15)
		Village2	4.1% (3)	44.6% (33)	51.4% (38)
		Village3	12.9% (8)	38.7% (24)	48.4% (30)
		Village4	7.5% (3)	42.5% (17)	50.0% (20)
		Village5	27.1% (13)	31.3% (15)	41.7% (20)
		Village6	7.4% (5)	36.8% (25)	55.9% (38)
		Village7	21.1% (12)	40.4% (23)	38.6% (22)
		Village8	15.6% (5)	40.6% (13)	43.8% (14)
B12 status by lab cut-points (deficient; normal) (412)		<148; >220	11.4% (47)	20.6% (85)	68.0% (280)
		<110; >150	2.7% (11)	9.7% (40)	87.6% (361)
		<107; >133	1.7% (7)	5.3% (22)	93.0% (383)

** significantly different p<0.01; * indicates that significance is maintained, p<0.01 upon comparison between these two treatment methods.

Table 5: *Association between admission B12 status (deficient, subclinical, normal) and covariates.**

Characteristic (n)	Mean ± SD	Deficient (n)	Subclinical (n)	Normal (n)
Age (yrs) (n=412)	83.1 ± 7.1	84.4 ± 7.4 (57)	83.3 ± 6.4 (158)	82.5 ± 7.5 (197)
ADL-s (n=327)	6.6 ± 4.2	6.6 ± 3.3 (43)	6.3 ± 4.1 (121)	6.9 ± 4.8 (163)
ADL-l (n=327)	12.7 ± 7.6	12.6 ± 6.4 (43)	12.2 ± 7.7 (121)	13.1 ± 7.9 (163)
Number of diagnoses (n=366)	6.1 ± 2.7	5.7 ± 3.3 (50)	6.0 ± 2.6 (141)	6.2 ± 2.7 (175)
Number of medications** (n=338)	10.0 ± 4.5	8.5 ± 4.8** (48)	9.5 ± 4.2 (126)	10.8 ± 4.4** (164)
Length of stay (mo) (n=412)	21.8 ± 20.3	16.7 ± 21.7 (57)	24.3 ± 19.7 (158)	21.2 ± 20.2 (197)
Categorical BMI		BMI ≤ 19	Normal BMI	BMI ≥ 30
Admission B12 (pmol/L) (n=344)	359.5 ± 225.6	376.6 ± 310.1 (23)	339.3 ± 201.1 (200)	389.7 ± 243.0 (121)

**significantly different (p<0.01) * For all ANOVA analyses, Tukey B post-hoc analyses were conducted to report means and significance of difference between groups

Table 6: *Association between B12 supplement use at admission and covariates.*

Characteristic	Total Sample % (n)	Category	No Admission B12 Supplement	Admission B12 Supplement
Sex	67.5% (228/338)	Female Male	76.3% (174) 69.1% (76)	23.7% (54) 30.9% (34)
Marital status (married?)	40.9% (115/281)	Yes No	73.9% (85) 77.1% (128)	26.1% (30) 22.9% (38)
Hypothyroidism	21.8% (70/321)	Yes No	70.0% (49) 74.9% (188)	30.0% (21) 25.1% (63)
Metformin use	10.1% (34/338)	Yes No	64.7% (22) 75.0% (228)	35.3% (12) 25.0% (76)
Antibiotics	6.2% (21/338)	Yes No	66.7% (14) 74.4% (236)	33.3% (7) 25.6% (81)
CVD	38.0% (122/321)	Yes No	77.9% (95) 71.4% (142)	22.1% (27) 28.6% (57)
GI conditions	30.5% (98/321)	Yes No	75.5% (74) 73.1% (163)	24.5% (24) 26.9% (60)
Antacid use	2.7% (9/338)	Yes No	66.7% (6) 74.2% (244)	33.3% (3) 25.8% (85)
PPI use	26.3% (89/338)	Yes No	77.5% (69) 72.7% (181)	22.5% (20) 27.3% (68)
H2 blocker use	1.5% (5/338)	Yes No	80.0% (4) 73.9% (246)	20.0% (1) 26.1% (87)
Mood & mood related disorders	61.7% (198/321)	Yes No	70.7% (140) 78.9% (97)	29.3% (58) 21.1% (26)
Dementia**	49.2% (158/321)	Yes No	65.2% (103) 82.2% (134)	34.8% (55) 17.8% (29)
Alzheimer's	19.0% (61/321)	Yes No	75.4% (46) 73.5% (191)	24.6% (15) 26.5% (69)
Dementia or Alzheimer's**	66.0% (212/321)	Yes No	68.4% (145) 84.4% (92)	31.6% (67) 15.6% (17)
Categorical CPS [0,1,2 vs 3,4,5,6]	47.0% (133/283)	Yes No	73.7% (98) 72.7% (109)	26.3% (35) 27.3% (41)
Weight change – K3a Weight loss	6.3% (19/300)	Yes No Unknown	68.4% (13) 74.8% (193) 73.9% (17)	31.6% (6) 25.2% (65) 26.1% (6)
Weight change – K3b Weight gain	5.3% (16/300)	Yes No Unknown	50.0% (8) 75.9% (198) 73.9% (17)	50.0% (8) 24.1% (63) 26.1% (6)
Nutritional problems – K4a complains about taste of foods	4.7% (14/300)	Yes No	78.6% (11) 74.1% (212)	21.4% (3) 25.9% (74)
Nutritional problems – K4b regular or repetitive complaints of hunger	0.7% (2/300)	Yes No	100.0% (2) 74.2% (221)	0.0% (0) 25.8% (77)
Nutritional problems – K4c Leave	27.0% (81/300)	Yes	75.3% (61)	24.7% (20)

Characteristic	Total Sample % (n)	Category	No Admission B12 Supplement	Admission B12 Supplement
25% or more of food uneaten at most meals.		No	74.0% (162)	26.0% (57)
Nutritional problems – K4d None of the above	69.7% (209/300)	Yes No	73.7% (154) 75.8% (69)	26.3% (55) 24.2% (22)
B12 status at ***admission*****	Deficient 14.2% (48/338)	Deficient Subclinical Normal	95.8% (46) 90.5% (114) 54.9% (90)	4.2% (2) 9.5% (12) 45.1% (74)
B12 status at ***first annual bloodwork*****	Deficient 7.9% (9/114)	Deficient Subclinical Normal	100.0% (9) 89.1% (41) 62.7% (37)	0% (0) 10.9% (5) 37.3% (22)

**p<0.01

Table 7: *Proportion of residents with improved (green), maintained (yellow) of worsened (red) B12 status at first annual bloodwork (n=125). (p<0.001).*

		Year 1 Status (% from baseline status) *% of year 1 status*			
		Deficient	**Subclinical**	**Normal**	Total
Baseline Status	**Deficient**: B12 < 156 pmol/L	4 (25.0%) *40.0%*	4 (25.0%) *7.3%*	8 (50.0%) *10.4%*	16 (100% $deficient_{baseline}$)
	Subclinical: 156 ≤ B12 ≤ 300 pmol/L	5 (7.8%) *50.0%*	36 (56.3%) *65.5%*	23 (35.9%) *29.9%*	64 (100% $subclinical_{baseline}$)
	Normal: >300 pmol/L	1 (1.6%) *10.0%*	15 (24.2%) *27.3%*	46 (74.2%) *59.7%*	62 (100% $normal_{baseline}$)
	Total	10	55	77	142

Proportion improved	35/142 (24.6%)
Proportion maintained	86/142 (60.6%)
Proportion worsened	21/142 (14.8%)

Chapter 6
Discussion – Bringing it all together

The purpose of this thesis was threefold: to describe which protocols are in place for LTC testing and treatment of B12 deficiency and which institutional covariates are associated with these protocols; to establish the prevalence of B12 deficiency at admission to LTC and association with resident covariates of interest; and to describe, for the first time, the incidence of B12 deficiency at one year-post admission to LTC. Key findings from this work include: no significant association was found between LTC testing and treatment protocols and geography, for-profit status or organizational structure; 67% of LTC homes conduct B12 testing at admission to LTC and 85% of homes report follow-up testing in deficient residents. In addition, 14% of residents were identified as B12 deficient at admission to LTC and B12 supplementation was significantly associated with better admission B12 status; yet, 96% of deficient residents were not receiving B12 supplements at admission. Lastly, at one year post-admission, B12 status was improved as prevalence of deficiency was reduced to 8% however, the incidence of new deficiency within this timeframe was 4%. The following discussion will be focused on issues that should be considered for screening, treatment and monitoring of B12 status in LTC, as well as methodological issues that need to be considered when conducting similar work in the future. Finally, preliminary recommendations based on the findings of this thesis are offered.

6.1 The importance of screening

Using a cut-point of <156 pmol/L to define B12 deficiency within the Schlegel Villages, the prevalence was 14% ranging between 4 and 27% across eight sites. This prevalence falls within the reported estimated for both community dwelling older adults (3-43%) [11, 15, 16, 39, 41, 75-77, 113] as well as samples within LTC (7-34%) [39, 65, 80, 82]. This result provides evidence to suggest that a significant proportion of residents are affected by B12 deficiency, and that it can be as high as one in every four residents in selected sites/residents. Since classic manifestations may or may not be present in B12 deficiency cases and subclinical deficiency symptoms are multifaceted, vitamin B12 admission screening is necessary to establish B12 status. This coupled with the prevalence, suggests that protocols for screening at admission to LTC should be instituted. Despite a lack of current standardized protocols or recommendations specific to LTC, the environmental scan identified that two-thirds of sampled homes were routinely screening for B12 deficiency at admission. It is unclear

from this analysis why this screening has been instituted and so highly implemented, considering that guidelines specific to LTC are relatively absent, and do not specifically address the issue of admission screening. This does however demonstrate that a screening strategy at admission is an acceptable practice. To fully reach 100% coverage with screening, recommendations specific to LTC still need to be published and disseminated. These need to be based on evidence, such as this thesis, which describe the prevalence and incidence of B12 deficiency at and during admission, but also requires further evidence to demonstrate the cost-benefit of a screening protocol that is followed with treatment and monitoring practices.

The next question that arises is if targeted or blanket screening should be instituted in LTC. In the environmental scan (Chapter 4), screening was completed for all residents if screening was done at admission. Results from the prevalence study (Chapter 5) suggest that targeted screening is inappropriate at this point, given the null findings between B12 status and a variety of resident characteristics (e.g. PPI use) at admission. Covariates chosen for this analysis had some basis in the literature, but failed to be significantly associated in this substantial sample. Prior work often focused on selected samples and populations [37, 39, 65, 78, 80, 82, 83], which may have led to some of these characteristics being considered as potential risks for B12 deficiency. Thus, if a screening protocol is administered, the evidence from this thesis indicates that it should be for all LTC residents at admission and not targeted for certain conditions or medication use.

Both wide variance for prevalence estimates in addition to economical valuation likely influenced the OHTAC's general recommendation for restriction of screening to those with anemia or malabsorption and their recommendation against investigating B12 status in persons who exhibit dementia and/or cognitive impairment (or alopecia, dizziness, and fatigue) [86]. Although this guideline is counter to those from geriatric and LTC experts [109-111], OHTAC did not limit these recommendations to younger adults or only community living older adults. OHTAC assessed the burden of illness based on the reported prevalence of vitamin B12-related anemia in the general population, which was estimated at 2% [86]. This estimate is inappropriate for several reasons. First, B12 deficiency may be present in the absence of hematologic manifestations [11, 59]; up to 90% of B12 deficiency cases present in the form of neurologic abnormalities and in 25% of these cases, neurologic symptoms are the only symptom [1, 2, 4, 11]. Thus, the burden of illness estimated at 2% is not representative of the majority of B12 deficiency cases. Second, there is a general lack of evidence (i.e., mixed findings) on prevalence of B12 deficiency with relation to outcome measures

upon which to base broad recommendations. Third, there are even fewer studies specific to the LTC population to guide recommendations. Finally, the prevalence in older adults living in the community has been documented to be as high as 43% [11, 15, 16, 39, 41, 75-77, 113] which indicates that this segment of the population is an especially vulnerable group to B12 deficiency. Those being admitted to LTC are in an even more vulnerable nutritional, functional and cognitive state, necessitating consideration of these factors when setting recommendations for screening. Thus, the OHTAC guideline needs to be critiqued more fully and revisited to consider exclusion of LTC residents.

As noted in this thesis, variation exists in prevalence among LTC sites (including treatment in the environmental scan, which was considered a proxy for prevalence of deficiency). This is not only due to the variability in cut-points used by labs to determine deficiency, as seen in both the environmental scan and prevalence study, but also the natural variation in residents being admitted to LTC homes. Thus, this thesis adds to the prior evidence that there is considerable variation in prevalence, corroborating some of the reasoning upon which the OHTAC recommendations were made. However, this prevalence study was one of the largest for LTC and the environmental scan at 45 homes represented 5243 Ontario LTC residents. Considering the greater representativeness of these two studies than prior work, the overall average prevalence results from both studies (14% prevalence study; 25% environmental scan) support the need for screening at admission to LTC.

While there is evidence that screening at admission is likely appropriate, it would be remiss to ignore the inherent challenges in defining screening protocols and the economical cost of screening. With approximately 71,000 older adults 65 years or older living in Ontario LTC homes [91], assuming 30% new admissions per year, and an average cost of $12.50 per serum B12 test [42], the cost for B12 screening using serum B12 for older adults in Ontario is approximately $1.2 million dollars annually (not accounting for treatment and monitoring costs). With this in mind, there is a need for a formal cost-benefit analysis for screening and subsequent treatment. In 2001, one such analysis was conducted in the US with regard to vitamin therapy to lower homocysteine levels for coronary heart disease prevention [144]. While this analysis was not specific to older adults as it included adults aged 35 through 84, it concluded that by screening and supplementing men aged 45 or older with 1 mg folic acid and 500 mcg vitamin B12 daily, that it would save more than $2 billion USD over 10 years through primary and secondary prevention of coronary heart disease incidents [144]. Thus, there are potential savings associated with screening and a formal cost-benefit analysis is needed for an estimated quantification of savings. Another consideration is blanket treatment of LTC

residents, as seen in one of the homes of the environmental scan. This potential will be discussed further below.

Determination of B12 status is more complicated than the decision to screen. Consistent with the literature, different cut-points have been used to define deficiency which greatly impacts prevalence estimates. This was evidenced through both studies included in this thesis, with estimates varying based on the cut-point used by the lab. However, standardization of cut-points at the lab level is impractical, as different labs use different equipment each yielding different specificity and sensitivities [3, 4]. Furthermore, serum B12 tests are imperfect as they may yield falsely high or falsely low values [3, 4]. To address this, an additional biomarker specific to tissue level B12 status (e.g., MMA) would be beneficial for determination of B12 deficiency. Yet this would impart additional costs presenting a barrier for wide-spread use. There may be merit to using a higher cut-point for potentially identifying subclinical cases. While using a higher cut-point to identify a larger proportion of individuals to be treated would likely imply prevention of development to deficiency, it would also impart added costs as treatment would be more wide-spread. Thus, the issue of inconsistency in cut-point use for defining B12 deficiency also impacts treatment and presents another ongoing challenge.

6.2 How to treat B12 deficiency

Based on the environmental scan as well as the prevalence study, the most common forms of vitamin B12 treatment are through intramuscular injections and oral pills. In the prevalence study, B12 treatment prior to admission was significantly associated with a better B12 status at admission to LTC. This provides evidence to suggest that efficacy of treatment looks promising as the prevalence of deficiency in those not receiving B12 supplements at admission was significantly higher than those who were ($p<0.01$). However, even a small proportion (2%) of those receiving supplements had deficient admission B12 status, which highlights the importance of establishing efficacy of treatment and potentially different doses for those resistant to improved status. While those receiving supplementation had better B12 status, the form of treatment may be an important factor.

OHTAC recommends that oral B12 be used unless there is evidence for malabsorption, as both treatments have been shown to be effective [37, 60, 64, 66] or equally effective at improving B12 status [1, 85, 86]. The environmental scan, conducted at the same time as the OHTAC recommendations were released, indicated that 44% of residents received B12 in the form of oral

supplements, while 53% received intramuscular injections and the remaining 3% received subcutaneous injections. This suggests that practitioners providing care within LTC homes in Ontario were not implementing this current recommendation as they were generally unaware of these recommendations as evidenced by the environmental scan. Further work should be conducted to identify why prescribing patterns for B12 are not consistent with the evidence on efficacy. However, with time these proportions may change in light of the recommendations released by OHTAC and others on the equivocal effectiveness and ease of administration of oral supplements. Based on the prevalence study, at the Schlegel Villages 81% of residents receiving B12 received oral supplements, 16% receive IM and the remaining 3% receive a combination of both. One interesting finding from the prevalence study was that on admission to LTC, 90% of those on oral supplements had normal admission B12 status compared to 57% of those on IM and only 1% of those on oral B12 had deficient B12 status compared to 7% of those on IM. Based on these findings, oral supplementation appears to be more effective than IM for vulnerable older adults who are eligible for LTC. It can be hypothesized that physically and/or cognitively impaired older adults living in the community may have challenges attaining IM injections, which are typically conducted in a physician's office, affecting its efficacy.

Economically, from a health care perspective, oral B12 is also advantageous as it requires fewer physician visits, but oral B12 is not covered by the Ontario Drug Benefit Program (ODBP) [86]. This translates to an out-of-pocket expense, which, for some older adults, may be too burdensome. As well, dosing when left in the hands of the older adult could be subject to error, either intentional or unintentional. As a result, monitoring of B12 status when oral treatment is used, is required to ensure compliance. Compliance, however, is regarded as a non-issue in LTC since medications are administered by health care professionals.

Dose of treatment varies between oral therapy and parenteral (IM or subcutaneous). For oral therapy, larger doses are required at an increased frequency (e.g., daily) as this method of treatment relies primarily on passive diffusion, the minor absorptive pathway in which approximately 1% of oral dose is uptaken [2, 11, 18]. Guidelines suggest oral doses of 125-500 mcg daily for prophylactic treatment and maintenance [145], 250-500 mcg daily for those with food-cobalamin malabsorption [34, 145] and 1000 mcg daily for those with pernicious anemia [11, 34]. The majority of these suggestions are consistent with what was observed in this study, where residents on oral B12 therapy received 50-1200 mcg daily (environmental scan) or 100-1250 mcg daily (prevalence study). In

contrast, injections of B12 bypass the absorption process altogether and the B12 is deposited directly into tissue. As a result, the frequency of IM B12 therapy is reduced. Guidelines suggest a monthly administration of 1000 mcg of B12 via intramuscular injection for those with pernicious anemia [11, 34] or with neurological symptoms in which subcutaneous injections may alternatively be administered [34]. The environmental scan described IM therapy of 500-1000 mcg administered monthly, every two months or biweekly; the prevalence study reported 300 or 1000 mcg administered either monthly, quarterly or every two months. Discrepancies between the limited existing guidelines and what is observed in practice may be indicative of increased sensitivity of physicians to the issue of B12 status in LTC and using their better judgement to manage treatment on a case-by-case basis. Training physicians on appropriate dosing and an update on the evidence of treatment modalities and effectiveness may improve treatment of B12 deficiency as a relevant issue to older adults and may be a strategy to increase consistency of B12 treatment regimens.

Alternatively, given time constraints implicit in LTC, blanket treatment may be suitable for both treatment and prevention of vitamin B12 deficiency. In the environmental scan, in one LTC home treatment was provided to all residents regardless of admission B12 status. As a result of this treatment, this home reported that all residents were within their lab-defined normal range (198-615 pmol/L). Safety is an important consideration; while no reported risks are associated with high B12 intake and there is no defined tolerable upper limit [36], this lack of evidence was based on healthy individuals thus feasibility is contingent on favourable results from further research specific to older adults and excess from supplementation. Modality may also dictate feasibility for blanket treatment as IM B12 is but oral B12 is not covered by the Ontario ODBP [86]. A cost-benefit analysis considering cost from the health care perspective if oral B12 were to be added to the ODBP and from the resident perspective would be useful to inform feasibility. Using an estimated cost $0.08 for 500 mcg B12 per resident per day, a cost estimate (not including reductions in costs associated with screening, monitoring and clinician visits) would equate to approximately $29 per resident per year or $2.1 million CAD annually for blanket treatment of all Ontario older adults living in LTC.

6.3 Is monitoring in LTC required?

Beyond the issue of compliance, monitoring B12 status is necessary as evidenced by the prevalence study in which deficiency was present, even among those receiving B12 supplements at admission. Since the Schlegel Village protocol requires B12 testing at admission in addition to every year thereafter, for the first time in LTC, incidence of B12 deficiency can be reported. Incidence one year

post-admission was estimated at 4% in a subsample of the prevalence study and worsening of status in 15 %, providing further support for the need for monitoring. The environmental scan indicated that in LTC more broadly, there was no consistency around monitoring of B12 status in Ontario homes; follow-up was reported primarily in those who were deficient and even then, only in 85% (34/40) of LTC homes. Based on null findings between B12 status at one year and assessed resident characteristics, at this point, monitoring must be completed for everyone as no specific subgroup was found to be associated with B12 status at one year post admission. That said, incidence, was reported on a biased subsample due to the low proportion of follow-up measurements (142/412). This greatly impacts the power of the study to detect characteristics that may be associated with a poorer B12 status. However, based on the evidence at this point in time, a protocol is needed not only for the assessment of B12 status but also for monitoring.

The addition of monitoring B12 status does increase cost. Using the estimate of 71,000 older adults 65 years or older living in Ontario LTC homes [91], now assuming a 30% attrition rate due to death per year, and an average cost of $12.50 per serum B12 test [42], the cost for B12 monitoring using serum B12 for older adults in Ontario is approximately $620,000 Canadian dollars annually. There is a need for a formal cost-benefit analysis for monitoring and subsequent treatment and prevention of B12-health-related consequences.

6.4 Methodological lessons learned

The environmental scan was conducted via phone interviews, which were cumbersome, requiring multiple follow-up attempts to collect complete data. If a replication of this study were conducted, based on this experience, an online survey would be recommended. Such a survey may result in wider reach and response in that responders would complete the questionnaire based on their availability. Anonymity would also be assured, which could also have influenced the response in the environmental scan conducted. To promote completion, a meaningful incentive would be suggested. Provided another opportunity, a question specifically designed to distinguish between characteristics of those receiving follow-up testing would be beneficial. This distinction was made for the majority, but not all interviews. In addition, to get a sense for the average B12 status at each of the LTC homes, a mean and standard deviation of serum B12 levels would have been helpful, however this calculation may be infeasible to ask of the staff given time constraints and access to these data within a home. While interviewees were asked this question broadly, at best, a guestimate was provided.

Due to the Schlegel Village protocol for B12 screening, treatment and monitoring, it was believed that a retrospective chart review would be feasible with good internal validity. However, the high degree of missing data within the prevalence study potentially influenced the validity. Exclusion criteria were based on age at admission (at least 65 years) and admission bloodwork including a serum B12 level. Regarding admission bloodwork, 59% of charts reviewed were either missing admission bloodwork or this could not be located; 412 resident charts were eligible of 1005 charts screened for admission bloodwork. This was exacerbated at one year post admission, where 66% of first annual bloodwork was missing; of the initial 412 residents, only 142 first-annual bloodwork values were easily located. This suggests that despite implementing clear protocols for resident data (i.e., keep admission bloodwork and annual bloodwork in the chart) with best efforts to ensure implementation of these protocols, that practice may not always be congruent. For example, residents who have lived in LTC for a longer period of time, or have had more health-related incidents tended to have thicker charts. These charts are pruned regularly and through discussion with neighbourhood coordinators and nurses, it was clear that depending on the neighbourhood, pruning procedures varied. At each pruning of the charts, there was an opportunity for admission medications, diagnoses and bloodwork to be misfiled. Within a given chart, there were multiple sources of information that needed to be accessed from residents' physical charts (e.g., admission paperwork, CCAC paperwork, medications lists, lab results) and each section of residents' charts were seemingly treated differently with respect to chart pruning even within across different neighbourhoods at the same Village. For example, if admission bloodwork was included with resident background forms, it tended to be present more frequently than if it were filed under lab-work. Frequently review of archived data was required to find this admission bloodwork which provided additional challenges; systems for archival varied from site to site with some keeping all archives, others moved archived data offsite while a few had no filing system for archived data.While admission bloodwork tended to be fairly well maintained, annual bloodwork tended to be pruned and excluded from residents' primary charts. Based on exclusion criteria requiring only admission B12 bloodwork, time constraints and the primary objective of reporting prevalence, it was infeasible to review all archived data for first annual bloodwork.

An additional challenge for establishing B12 status was the inconsistency of cut-points used by labs. Even within the same village a given lab on a different date used different reference ranges. In at least one Village, the lab used for blood testing was recently switched and with this change, came a different cut-point. In addition to residents' physical charts, other sources of information needed to be

accessed (e.g., MDS) which presented additional opportunities for inconsistencies between sources and potential for missing data. Thus, due to the high degree of missing data, it was inappropriate to conduct thorough analyses around covariates associated with B12 status at one year post admission. Due to these challenges, retrospective chart audits across LTC homes, should be based on readily available data, preferably located in an electronic system that has greater likelihood of continual updating and completion.

6.5 Next Steps

In addition to providing some insight into B12 status, this thesis work also generated further research questions. For example, were the null findings of association between B12 status and key medications spurious? What is the incidence of B12 deficiency in a larger, non-biased sample and how does this compare to this work? What is the efficacy of treatment and are there differences based on treatment modality? What is the fidelity to treatment protocols and how might this differ across organizations? Does treatment of B12 improve or maintain health-related outcomes? Are there subgroups of older adults living in LTC that may be more likely to respond to treatment? Future work would benefit from a prospective longitudinal study to minimize missing data and designed to investigate change in B12 status over time and with respect to covariates of interest. Future work in this field will provide additional evidence and context which will translate into improved recommendations.

6.6 Concluding Remarks

This thesis work provided evidence that vitamin B12 deficiency is an issue within LTC as indicated through prevalence of 14% at admission, 8% one year post admission and the high proportion (25%) of residents in Ontario LTC receiving B12. This work also provides evidence that screening is required and that targeted screening would be inappropriate as no resident characteristics excepting supplementation status were associated with admission B12 status. While B12 status was generally improved, there was sufficient incidence of B12 deficiency and worsening of status that monitoring of B12 status is also needed. Prospective studies specifically designed to investigate the association between B12 status and risk factors (medications, conditions) are needed to confirm null findings or provide counter evidence. In addition, due to a lack of consistency for testing for B12 and monitoring B12 status as indicated through the environmental scan, guidelines specific to LTC outlining protocols for screening and treatment and monitoring may be warranted.

6.7 Recommendations

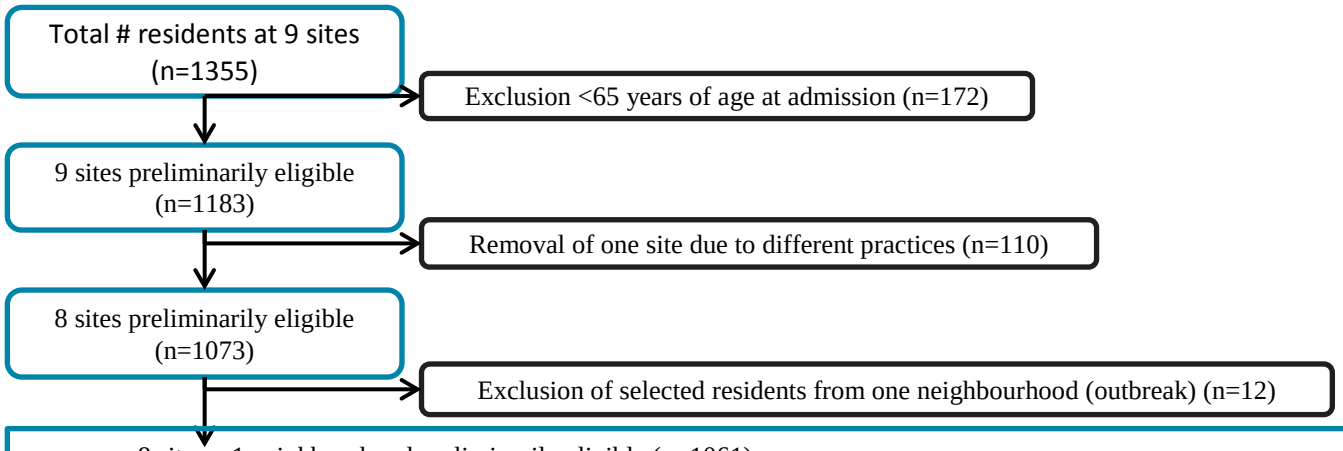
Based on the evidence provided by this thesis work, the following preliminary recommendations are proposed:

1. Admission screening is required for all LTC residents.
2. OHTAC guidelines need further critique and should consider exclusion of LTC residents in these recommendations.
3. Recommendations including a protocol for B12 assessment as well as monitoring is needed.
4. Physician training may be warranted for appropriate dosing and treatment effectiveness.
5. Blanket treatment with oral B12 for all LTC residents may be a suitable alternative to screening.

A formal cost-benefit analysis is needed for screening, subsequent treatment, monitoring and potential blanket treatment. This analysis should consider increased costs due to increased screening, monitoring and associated clinician visits, and addition of oral B12 to the ODBP as well as cost reductions due to fewer clinician visits with blanket treatment.

Appendix A

Flow diagram of included charts



8 sites – 1 neighbourhood preliminarily eligible (n=1061)

Village Code	Total # Resi-dents	# Resi-dents Eligible	Proportion Eligible	Planned # to Sample	Actual # Sampled	# Alternates required to get sample	Proportion planned to sample	Proportion Actually Sampled	Pooled Sample Proportion	SE	Test Statistic (z)	P
1	93	81	0.871	24.35	31	4	0.076	0.075	0.076	0.020	0.065	p >0.4
2	190	173	0.911	52.01	74	57	0.163	0.179	0.172	0.028	-0.573	p >0.2
3	187	160	0.856	48.11	62	99	0.151	0.150	0.150	0.027	0.026	p >0.4
4	119	105	0.882	31.57	41	66	0.099	0.099	0.099	0.022	-0.014	p >0.4
5	140	125	0.893	37.58	48	78	0.118	0.116	0.117	0.024	0.066	p >0.4
6	191	166	0.869	49.91	68	80	0.156	0.165	0.161	0.027	-0.299	p >0.3
7	155	148	0.955	44.50	57	87	0.139	0.138	0.139	0.026	0.057	p >0.4
8	119	103	0.866	30.97	32	64	0.097	0.077	0.086	0.021	0.938	p >0.1
		1061		319	413	535						

Appendix B

Sources for data collection for the prevalence study

Resident Assessment Instrument-Minimum Data Set 2.0 for LTC homes (RAI-MDS or MDS)

The RAI-MDS 2.0 is a standardized assessment tool used by all LTC homes across Ontario at regular intervals (admission, quarterly, after significant change in health status). LTC homes are required to conduct a full admission assessment within 14 days of a resident's admission to LTC. The MDS is used to assess a residents care and service needs [146]. For this study, the following subsections were of interest since information pertained to factors that may influence B12 status in LTC: weight at admission (kgs), height at admission (m), nutritional problem, and weight change. Where: nutritional problems is defined as within the past 7 days, the presence of "complaints about the taste of many foods", "regular or repetitive complaints about hunger", "leaves 25% or more of food uneaten at most meals", none of the above; and weight change is defined as a weight loss or weight gain of 5% or more in the last 30 days or 10% or more in the last 180 days.

Resident Assessment Protocols (RAPs) for cognitive performance and activities of daily living

Based on how questions are answered on the RAI-MDS 2.0, RAPs are used by clinicians in the development of a resident's comprehensive plan of care. For this study, the following RAPs were of interest since information pertained to factors that may influence B12 status in LTC: Cognitive Performance Scale (CPS) and Activities of Daily Living (ADL-s, ADL-l*).

A resident's CPS score is determined using five MDS items. Score values may take on values from 0 (intact) to 6 (very severe impairment) where a higher score indicates more cognitive impairment. CPS scores were used because this data was easily accessible and has been shown to correspond closely with the Mini-Mental State Examination [137].

*Activities of daily living are assessed on the MDS across 10 domains: bed mobility, transfer, walking in room, walking in corridor, locomotion on unit, locomotion off unit, dressing, eating, toilet use, and personal hygiene. From this overall assessment, an ADL short form scale (ADL-s) is calculated based on four ADL items: personal hygiene, toilet use, mobility and eating. ADL-s values range from 0-16 where a higher score indicates greater difficulty in performing daily activities. Similarly, an ADL long form scale (ADL-l) is calculated based on 7 ADL items including the same

four as ADL-s with the addition of bed mobility, transfers, and dressing. ADL-l values range from 0-28 where a higher score again indicates greater difficulty in performing tasks. The ADL-l has been shown to be more sensitive to clinical change than the ADL-s and has very good internal consistency.

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