FORTIFICATION OF PUREED FOODS FOR LONG-TERM CARE RESIDENTS

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By

Jennifer Lea Adolphe

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ABSTRACT

Elderly people living in long-term care (LTC) are at risk for malnutrition. Those who consume a pureed diet may be particularly at risk because of the food restrictions required on a pureed diet. Fortification of pureed foods with micronutrients may be an effective technique to treat malnutrition. The purpose of this study was to develop fortified pureed foods to incorporate into a menu at a LTC facility to assess if nutrient intakes and serum vitamin levels increased.

Fortification levels were determined using a combination of two techniques: the Dietary Reference Intakes report on planning formula, Estimated Average Requirement plus two standard deviations of intake; and Health Canada's method of using a defined nutrient contribution to the total daily intake. Fortification levels for 11 vitamins and 9 minerals were determined, which allowed for formulation of a vitamin/mineral mix and a vitamin-only mix. Seven pureed foods were fortified and triangle sensory tests were performed to determine whether fortification changed the flavour of the foods. Panelists were able to discriminate between the unfortified and vitamin/mineral fortified mix samples ($P > 0.05$). When the vitamin-only fortified foods were subjected to the triangle test, the panelists were unable to detect a difference ($P < 0.05$).

Four vitamin-fortified foods per day were incorporated into the pureed menu at a LTC facility. Nutrient intakes (n = 10) and serum vitamin B_{12}, folate, and 25-hydroxyvitamin D levels (n = 11) were analyzed at baseline and 8 weeks after the intervention. Nutrient intakes increased after the intervention for all vitamins assessed ($P < 0.01$). Serum 25-hydroxyvitamin D and folate levels increased from 41 ± 21 nmol/L and 10.7 ± 4.9 nmol/L at baseline to 66 ± 11 nmol/L and 25.2 ± 6.4 nmol/L after the intervention ($P < 0.01$). Serum vitamin B_{12} levels did not change ($P > 0.05$).

The development of acceptable vitamin-fortified pureed foods is feasible and fortified pureed foods are an effective way to increase the nutritional status of LTC residents.

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ABBREVIATIONS

AI	Adequate Intake
BD SST	Becton, Dickinson and Company Serum Separator Tube
BMI	Body Mass Index
DFE	Dietary Folate Equivalents
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
HPLC	High Performance Liquid Chromatography
IU	International Units
LC-MS	Liquid Chromatography-Mass Spectrometry
LTC	Long-Term Care
MMA	Methylmalonic Acid
ND	Not Determined
NE	Niacin Equivalents
RAE	Retinol Activity Equivalent
RBC	Red Blood Cell
RDA	Recommended Dietary Allowance
SD	Standard Deviation
SD_{in}	Standard Deviation of Intake
SPSS	Statistical Package for Social Sciences
TC	Transcobalamin
UL	Tolerable Upper Intake Level
25(OH)D	25-Hydroxyvitamin D

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The prevalence of malnutrition in the elderly is high, especially among those living in institutional settings (Keller, 1993). The consequences of malnutrition include frailty, poor immunity, and decreased functional ability and quality of life (Bartali et al., 2006; Keller, 2004; Lesourd, 2004). Long-term care (LTC) residents with dysphagia who are required to consume a pureed diet to facilitate swallowing may be at high risk for malnutrition because of the food restrictions required on a pureed diet.

Several studies have shown that energy, macronutrient, and micronutrient intakes are inadequate in LTC residents. Odlund Olin, Koochek, Ljunggvist, and Cederholm (2005) compared actual energy intakes to estimated energy requirements in LTC residents aged 79-90 years and found that intakes were significantly below requirements. In a study of LTC residents ≥ 65 years of age, Lengyel, Whiting, and Zello (In press) found low intakes of folate, magnesium, zinc, vitamin E, vitamin B_6, calcium, vitamin D, and dietary fibre. R. M. Johnson, Smiciklas-Wright, Soucy, and Rizzo (1995) found that women living in a nursing home and consuming either a regular or pureed diet had inadequate intakes of iron, zinc, calcium, and vitamin D.

Although intakes of many vitamins and minerals are low in this population, three vitamins of primary concern are vitamin D, vitamin B_{12}, and folate. The elderly are at increased risk for vitamin D deficiency due to lack of sun exposure, decreased ability to

synthesize vitamin D endogenously when the skin is exposed to sunlight, and the limited number of dietary sources of the vitamin (Armas, Hollis, & Heaney, 2004; Gloth, Smith, Hollis, & Tobin, 1995; MacLaughlin & Holick, 1985). Vitamin B_{12} is a concern for people over 50 years of age because of the increased prevalence of atrophic gastritis which results in decreased absorption of the vitamin (Andres et al., 2004). Folate is a concern because people consuming a pureed diet may have difficulty consuming enough folate-rich foods to meet their requirements since foods that are high in folate, such as leafy green vegetables and folic acid-fortified grain products, are difficult to make into a pureed consistency (Adolphe, Dahl, Whiting, & Tyler, 2006).

There is currently no consensus on the best way to treat micronutrient (vitamin and mineral) malnutrition among LTC residents consuming a pureed diet. Food fortification with vitamins and/or minerals is a possible solution but has not been studied in this population. Health Canada's fortification laws are currently under revision and may provide an opportunity for food manufacturers to develop fortified pureed foods under the category "special purpose foods" (Health Canada, 2005). However, there is currently no universally accepted method to determine fortification levels. Therefore, investigation into the appropriate methods to determine fortification levels needs to be completed. Subsequently, it needs to be determined whether the newly developed fortified foods are successful at improving the micronutrient status of those consuming them.

1.2 PURPOSE

A variety of methods are currently used to help prevent and correct malnutrition in LTC residents consuming a pureed diet. These methods include serving nutrient-

dense meals, increasing meal frequency, and providing oral nutritional supplements. However, there is evidence that these techniques may not be effective in achieving improved nutritional status (Milne, Avenell, & Potter, 2006; Taylor & Barr, 2006). Food fortification with micronutrients is a cost-effective way to provide nutrient-dense foods to a large number of people. Using fortified foods to improve the nutritional status of LTC residents consuming a pureed diet has the potential to improve their health status and quality of life. However, evidence is still needed as to how to determine appropriate fortification levels and whether or not fortification is feasible and effective in this population.

1.3 OBJECTIVES

The objectives of the study are as follows:

1. *To determine appropriate micronutrient fortification levels for pureed foods for consumption by LTC residents.*
2. *To assess if fortification results in detectable flavour changes.*
3. *To incorporate nutrient-dense fortified pureed foods into the menu at a LTC facility.*
4. *To determine if the fortified pureed foods increase the nutrient intakes of the residents consuming them.*
5. *To determine the effect of fortified pureed foods on serum levels of vitamin B_{12}, 25-hydroxyvitamin D, and folate of long-term care residents.*

1.4 HYPOTHESES

The hypotheses of the study are as follows:

1. *By using the Dietary Reference Intakes menu planning methods and Health Canada fortification guidelines, fortification levels can be determined that will result in adequate micronutrient intakes of LTC residents consuming a pureed diet, but prevent excessive intakes.*
2. *The addition of micronutrients to fortified pureed foods will increase the nutrient intakes of LTC residents prescribed a pureed diet.*
3. *The concentrations of serum vitamin B_{12}, folate, and 25-hydroxyvitamin D will increase in the residents consuming the fortified pureed diet.*

CHAPTER 2

LITERATURE REVIEW

2.1 NUTRITION AND AGING

2.1.1 Dietary Recommendations for Adults over 50

The Dietary Reference Intakes (DRI) provide nutrient reference values for healthy individuals and are categorized by age and gender. As shown in Table 2.1, the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA) and/or Adequate Intake (AI) for most nutrients do not change for younger adults (31-50 y) compared to older adults (≥ 51 y) (Otten, Pitzi Hellwig, & Meyers, 2006). However, there are a few nutrients for which the recommendations increase or decrease between the two age groups, which are highlighted in Table 2.1.

According to the DRI report on vitamin B_6, adults over 50 appear to have a slightly higher requirement for the vitamin compared to younger adults, although the report states that there has been only a limited number of studies that examined vitamin B_6 requirements in older adults (Institute of Medicine, 1998). The AI for calcium is higher in the older age group based on data that demonstrated a positive reduction in bone loss with calcium intakes above 1,000 mg/day and because calcium absorption declines with age (Institute of Medicine, 1997). Bone loss was used as an indicator of adequacy for vitamin D in the 51-70 age group and the recommendation was increased

Table 2.1 Comparison of EAR and RDA values for adults 31-50 y and ≥ 51 y.

Nutrient	EAR(AI)[1]		RDA[1]	
	31-50 y	≥ 51 y	31-50 y	≥ 51 y
Vitamin A (µg RAE)				
Males	625	625	900	900
Females	500	500	700	700
Vitamin B_6 (mg)				
Males	1.1	1.4	1.3	1.7
Females	1.1	1.3	1.3	1.5
Vitamin B_{12} (µg)	2.0	2.0	2.4	2.4
Biotin (µg)	(30)	(30)	-	-
Vitamin C (mg)				
Males	75	75	90	90
Females	60	60	75	75
Vitamin D (µg)	(5)	(10) 51-70 y (15) >70 y	-	-
Vitamin E (mg)	12	12	15	15
Folate (µg)	320	320	400	400
Vitamin K (µg)				
Males	(120)	(120)	-	-
Females	(90)	(90)		
Niacin (mg)				
Males	12	12	16	16
Females	11	11	14	14
Pantothenic Acid (mg)	(5)	(5)		
Riboflavin (mg)				
Males	1.1	1.1	1.3	1.3
Females	0.9	0.9	1.1	1.1
Thiamin (mg)				
Males	1.0	1.0	1.2	1.2
Females	0.9	0.9	1.1	1.1
Calcium (mg)	(1000)	(1200)		
Chromium (µg)				
Males	(35)	(30)		
Females	(25)	(20)		
Copper (µg)	700	700	900	900
Iodine (µg)	95	95	150	150
Iron (mg)				
Males	6.0	6.0	8	8
Females	8.1	5.0	18	8
Magnesium (mg)	350	350	420	420
Manganese (mg)	(2.3)	(2.3)	-	-
Molybdenum (µg)	34	34	45	45
Phosphorus (mg)	580	580	700	700
Potassium (g)	(4.7)	(4.7)	-	-
Selenium (µg)	45	45	55	55
Zinc (mg)	9.4	9.4	11	11

[1]Value shown is that for males and females unless otherwise indicated.

from 5 µg/day to 10 µg/day for this age group in order to prevent bone loss (Institute of Medicine, 1997). This recommendation was further increased to 15 µg/day for the >70 age group because of the evidence that serum 25-hydroxyvitamin D levels decrease and skeletal fractures increase in people over 70 (Institute of Medicine, 1997). The recommendation for chromium is slightly lower for older adults based on extrapolation from data from the younger age group. The level was calculated based on a chromium intake per 1,000 kcal which results in a lower value for older adults who on average consume less food (Institute of Medicine, 2001). Iron recommendations decrease for women over 50 due to menopause when women no longer lose iron due to menstruation (Institute of Medicine, 2001).

2.1.2 Malnutrition in Long-Term Care

Malnutrition is a major concern in LTC and numerous studies have shown that energy, macronutrient, and micronutrient (i.e. vitamin and mineral) intakes are inadequate in this population (Akner & Floistrup, 2003; Odlund Olin, Koochek, Ljunggvist, & Cederholm, 2005). The prevalence of malnutrition in LTC residents has been estimated to be as high as 85% (Keller, 1993). However, it is difficult to accurately determine the prevalence of malnutrition due to differences in how malnutrition is defined and measured. There are numerous criteria used to define malnutrition, including inadequate nutritional status, undernourishment characterized by insufficient dietary intake, poor appetite, muscle wasting, biochemical measurements such as serum albumin, and weight loss (Chen, Schilling, & Lyder, 2001). Malnutrition can also be classified based on its severity. Keller (1993) found that severe undernutrition was

present in 18% of elderly LTC residents and mild to moderate undernutrition was present in 27%.

As shown in Table 2.2, many of the risk factors for malnutrition are common among elderly people, especially in those living in LTC facilities. These factors can generally be classified into two groups: those that cause inadequate intake and those that increase nutrient requirements (Abbasi & Rudman, 1994). The consequences of malnutrition can be detrimental and improving the nutritional status of the elderly has the potential to make a tremendous impact on their overall health.

Table 2.2 Risk factors for and consequences of malnutrition.

Risk Factors for Malnutrition[1]	Consequences of Malnutrition[2]
Age	Sarcopenia
Female gender	Impaired muscle function
Low activity level	Decreased bone mass
Poor communication	Immune dysfunction
Medication use	Anemia
Poor oral intake	Altered drug metabolism
Eating dependency	
Chewing and swallowing difficulty	
Decubiti	
History of hip fracture	
Illness/infection	
Impaired cognition	

[1](Abbasi & Rudman, 1994; Fiatarone Singh, Bernstein, Ryan, O'Neill, Clements, & Evans, 2000; Keller, 1993)
[2](Donini, Savina, & Cannella, 2003; Fiatarone Singh et al., 2000)

The relationships between malnutrition, frailty, immunity, functional ability, and quality of life have been topics of recent interest. Frailty can be defined as an increased vulnerability to stressors and increased risk of adverse outcomes including disability and death (Ble et al., 2006). It appears that diet quality plays a role in muscle efficiency and that a low intake of nutrients is associated with frailty even independent of energy intake

(Bartali et al., 2006). Specifically, low levels of vitamins E, C, and D have been shown to be risk factors for frailty (Bartali et al., 2006; Ble et al., 2006). Frailty and immunity are interrelated because the decrease in food consumption that often accompanies the frailty state leads to a decline in nutritional status and decreased immune responses. Poor immune system functioning, which has been thought to be a consequence of aging, may be due to low micronutrient status (Lesourd, 2004). Frailty and poor immune function also affect functional ability and quality of life because if the diet is deficient in nutrients, chronic diseases can develop or be aggravated (Keller, 2004). By improving nutritional status, functional ability can also be enhanced (Hickson & Frost, 2004)

Micronutrient malnutrition is an important concern among the elderly because over time insufficient status for one or more micronutrients may contribute to the development of disease (Brouwer, Welten, Reijngoud, van Doormaal, & Muskiet, 1998). Increasing age is a strong risk factor for poor nutritional status and elderly persons are at higher risk of vitamin deficiencies than younger adults (Forster & Gariballa, 2005; K. A. Johnson, Bernard, & Funderburg, 2002). A summary of the Canadian provincial nutrition surveys from the 1990's found that nutrient intakes decreased with age among free-living individuals (Dolega-Cieszkowski, Bobyn, & Whiting, 2006). This decrease may be even more apparent in LTC facilities where people often have concomitant factors that impact nutrient intake.

Recent data collected locally in LTC facilities in the Saskatoon Health Region suggests a high prevalence of inadequacy for folate, magnesium, zinc, vitamin E, and vitamin B_6 (Lengyel, Whiting, & Zello, In press). R. M. Johnson, Smiciklas-Wright, Soucy, and Rizzo (1995) found that women living in a nursing home and consuming either a regular or pureed diet had inadequate intakes of iron, zinc, calcium, and

vitamin D. A study by Rudman, Abbasi, Isaacson, and Karpiuk (1995) found that 88% of nursing home residents who were dependent on a caregiver for feeding had intakes for three or more nutrients that were below 50% of the RDA (this study was performed prior to the development of the Dietary Reference Intakes so prevalence of inadequacy was not determined). It can be difficult to recognize someone who is vitamin deficient because deficiencies are often disguised as skin, neurological, and gait abnormalities (K. A. Johnson, Bernard, & Funderburg, 2002). This is why it is so important to develop effective solutions to correct the high prevalence of micronutrient malnutrition in LTC.

2.1.3 Dysphagia

Dysphagia, or difficulty with swallowing, is a common disorder among elderly people living in LTC facilities (Kayser-Jones & Pengilly, 1999; Keller, 1993; Wright, Cotter, Hickson, & Frost, 2005). Dysphagia can lead to other medical conditions, including dehydration, aspiration pneumonia, suffocation, and malnutrition (Kawashima, Motohashi, & Fujishima, 2004). Dysphagic residents are at high risk of malnutrition due to the dietary changes used to treat the condition as well as the underlying cause of the disorder, which includes stroke, dementia, neuromuscular diseases, and cancer (Wright et al., 2005). Dysphagic residents are usually unable to feed themselves which is a major risk factor for undernutrition in LTC facilities (Abbasi & Rudman, 1994).

The diets most often used to treat dysphagia are referred to as texture-modified diets, which include consistencies from soft to minced to pureed depending on the severity of the dysphagia. The energy and protein requirements of individuals receiving a texture-modified diet have been shown to be the same as the requirements of people

consuming a normal diet. However, inadequate intakes of energy and protein in texture-modified diet groups can be large (Wright et al., 2005).

2.1.4 Strategies Used to Treat Malnutrition in Long-Term Care Residents

Dietitians and other healthcare professionals use a number of techniques to improve the nutrient and energy intakes of malnourished individuals. However, traditional dietary intervention methods intended to improve the nutritional status of LTC residents with or without dysphagia are not necessarily evidence-based. Some of the strategies used to prevent or correct malnutrition in this vulnerable population are discussed below.

Increased Meal Frequency: One technique used to improve an individual's nutritional status is to offer small, frequent meals in order to minimize fatigue while eating. However, until recently, the effectiveness of this strategy to increase food intake had not been studied quantitatively. Taylor and Barr (2006) recently reported on a small (n = 31) cross-over study comparing whether or not a five-meal pattern versus a traditional three-meal pattern improves energy intake among elderly LTC residents with dysphagia. Participants were randomly assigned to one of two meal pattern groups to receive either the three- or five- meal pattern menu for four days. Four weeks later, the groups were switched to the other meal pattern for four days. The three- and five-meal menus each provided approximately 1650 kcal/day and were of pureed or minced consistency. The investigators found that average energy intakes were similar between the three- and five-meal patterns (1,325 ± 207 kcal/day vs. 1,342 ± 177 kcal/day, respectively; $P = 0.565$) and that approximately 80% of the energy served for both meal patterns was consumed. The five-meal pattern also created some practical challenges for

the care staff, as many of the participants required feeding assistance. The researchers concluded that serving small, frequent meals did not improve energy intakes and that other nutrition intervention strategies need to be considered to appropriately nourish elderly people with dysphagia.

Increased Meal Energy Density: There is some evidence that the frequency of the meals may not matter as much as the meal energy density when the goal is to increase energy intakes. A study by Lorefalt, Wissing, and Unosson (2005) found that smaller energy- and protein-enriched meals improved energy intakes by 37% compared with the standard hospital menu in ten elderly patients consuming a regular texture diet at a geriatric rehabilitation ward. The first week after inclusion, the patients were offered a three-day standard hospital menu and in the second week, a three-day energy- and protein-enriched menu. Both menus consisted of three meals and two snacks per day, but the energy- and protein-enriched menu used portion sizes equal to half the standard size and the protein and energy content corresponding to a whole portion size. This study, while small and only short-term, suggests that the energy density of meals is an important factor to increase intakes.

The ways in which the menus where changed in each study may have contributed to the negative findings in the meal frequency study by Taylor and Barr (2006) and the positive findings in the energy density study by Lorefalt et al. (2005). The meal frequency study by Taylor & Barr simply redistributed the foods on the regular menu, while the energy density study by Lorefalt et al. used new menu items and enriched the foods with cream, butter, oils, and maize gruel. These changes would likely improve the palatability of the foods which can result in increased intakes. In addition, the energy density study by Lorefalt et al. did not use texture-modified foods, as did the food

frequency study by Taylor & Barr. It may be easier to make menu changes on a regular diet as there are fewer food restrictions than for a texture-modified diet.

However, making menu changes to increase intakes on a texture-modified diet has also been shown to be successful when an effort is made to prepare appealing texture-modified foods (Germain, Dufresne, & Gray-Donald, 2006). The study compared a newly developed dysphagia diet with a standard texture-modified diet. The dysphagia diet consisted of minced and pureed foods that were reshaped to look similar to regular texture foods. Compared to subjects consuming the standard texture-modified diet, the novel dysphagia diet was successful at increasing intakes of energy and several nutrients, including protein, magnesium, calcium, and vitamin D.

Oral Nutritional Supplements: Another dietary intervention that is often recommended to improve energy, protein, and nutrient intakes of LTC residents is the provision of oral nutritional supplements. There has been a lack of consensus about whether or not oral liquid supplements are effective at maintaining or improving nutritional status in LTC. Milne (2006) systematically reviewed oral nutritional supplementation on clinical and nutritional outcomes of older people in the hospital, institutions and the community. Fifty-five trials involving 9187 subjects were reviewed and the evidence suggested that there may be fewer complications and lower mortality with supplementation for short-term hospital stays in individuals that were undernourished at baseline. There was little evidence to suggest that longer-term supplementation was effective, although most studies were "small or had short follow-up times and used outcome assessors who knew which patients took supplements" (Milne, 2006). In addition, researchers have acknowledged that when a frail elderly

person is already malnourished, nutrition intervention alone may be too late and preventative strategies are needed to optimize nutritional status to minimize functional decline (Payette, Boutier, Coulombe, & Gray-Donald, 2002). Fiatarone Singh et al. (2000) were unable to increase energy and micronutrient status using oral liquid supplements after 10 weeks of supplementation despite high compliance of the frail, institutionalized elderly subjects. The researchers concluded that the subjects appeared to decrease their habitual food intake to compensate for the additional supplements. Furthermore, if usual foods are replaced with oral supplements, the overall acceptability of the diet and quality of life may decrease.

Low consumption rates of oral liquid or pudding supplements may be a problem, resulting in little or no improvement in nutrient intakes and considerable wastage. Wright et al. (2005) studied hospital patients who consumed either a texture modified diet (n = 30) or a normal hospital diet (n = 25). Sixteen patients from the texture modified group were prescribed liquid supplements but only 11 consumed some or the entire supplement. In a study by Remsburg, Sobel, Cohen, Koch, and Radu (2001), an energy-dense oral liquid supplement was dispensed with the routine medication pass at a LTC facility. They found that 35% of the supplements were wasted during a three day observation period. Thus, oral liquid or pudding supplementation does not appear to be a very effective method of nutrition delivery, especially if it is uncertain whether or not the supplements actually maintain or improve nutritional status.

Oral Micronutrient Supplements: Vitamin/mineral supplement pills are a possible solution to the high prevalence of micronutrient malnutrition in LTC. However, there are problems associated with the administration of these. It is difficult to have more than 50% of the target population consume vitamin supplements (Oakley, 2004). Due to large

administrative costs, supplement programs are more expensive than alternatives, such as fortification (Oakley, 2004). In general, geriatricians do not like their patients to take extra medication unless the benefits are very clear (F. Anderson, 2005). de Jong, Chin, de Graaf, Hiddink, de Groot, and van Staveren (2001) argue that medicalization of the elderly by prescribing vitamin pills is not a favourable direction and simultaneous intake of vitamin/mineral supplements with medications could induce a negative drug-nutrient interaction.

2.2 VITAMINS OF CONCERN IN THE ELDERLY POPULATION

2.2.1 Overview

Long-term care residents with dysphagia have inadequate intakes of many micronutrients (Adolphe et al., 2006; Germain et al., 2006; R. M. Johnson et al., 1995; Lengyel et al., In press; Rudman et al., 1995). Energy intakes in this population tend to be low (< 1600 kcal/day) which makes it difficult to obtain adequate amounts of vitamins and minerals (American Dietetic Association, 2005). Although it is important to ensure sufficient intake of all micronutrients, three vitamins that are of particular concern for this population are vitamin D, vitamin B_{12}, and folate. Elderly people living in LTC who have limited mobility are at increased risk of inadequate vitamin D status because they likely have limited sun exposure, and when their skin is exposed to sun, they may have a decreased ability to synthesize the vitamin in their skin (Lamberg-Allardt, 1984). Therefore, dietary sources of vitamin D become an important means for this population to obtain the vitamin, but there are only a limited number of foods that contain vitamin D. Vitamin B_{12} is a concern because of the increased prevalence of atrophic gastritis in the elderly which inhibits absorption of naturally-occurring

vitamin B_{12} from foods (Andres et al., 2004). Folate intake may be limited for people consuming a pureed diet since high folate foods, such as folic acid fortified grains and leafy green vegetables, are difficult to make into the appropriate pureed consistency.

2.2.2 Vitamin D

2.2.2.1 Metabolism

Vitamin D, or calciferol, is likely best known for its role in bone health by aiding in the absorption of calcium and phosphorus, thus maintaining normal serum levels of these minerals (Otten et al., 2006). Vitamin D is also involved in cellular metabolism through anti-proliferation and pro-differentiation actions (Otten et al., 2006). Serum 25-hydroxyvitamin D, or 25(OH)D, is the best indicator used to assess vitamin D status because it represents the summation of vitamin D from cutaneous synthesis and dietary intake of vitamin D_2 and D_3 (Institute of Medicine, 1997).

Vitamin D is fat-soluble and occurs in many forms. Vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) are the two dietary forms and are both similarly metabolized. However, vitamin D_3 has been shown to maintain serum 25(OH)D levels to a greater degree and is three to ten times more potent than vitamin D_2 (Armas et al., 2004). Vitamin D_2 and vitamin D_3 appear to create similar initial increases in serum 25(OH)D concentrations, but vitamin D_3 seems to maintain levels for substantially longer (Armas et al., 2004; J. L. Johnson, Mistry, Vukovich, Hogie-Lorenzen, Hollis, & Specker, 2005). Vitamin D_2 is derived from ergosterol in yeast and plants and vitamin D_3 originates from 7-dehydrocholesterol when it is synthesized in the skin (Otten et al., 2006). In the liver, vitamin D is hydroxylated to 25-hydroxyvitamin D which then enters circulation and can be used as an indicator of vitamin D status. The biologically active

hormone form of vitamin D is 1,25-dihydroxyvitamin D which is formed by hydroxylation of 25-hydroxyvitamin D in the kidney. This conversion is tightly regulated by parathyroid hormone in response to serum calcium and phosphorus levels (Otten et al., 2006). Serum calcium and phosphorus levels are regulated by 1,25-dihydroxyvitamin D which increases intestinal absorption of both minerals, increases calcium and phosphate resorption from bone, and reduces calcium loss in the urine (Mahan & Escott-Stump, 2004).

Vitamin D is obtained through synthesis in the skin from exposure to ultraviolet B rays from the sun or through dietary intake. Endogenous synthesis of vitamin D in the skin through sunlight exposure is limited in northern climates. During the winter months in Canada, the angle at which the sun's rays enter the earth's atmosphere does not allow for vitamin D synthesis (Holick, 2004). There are very few food sources of vitamin D. Naturally-occurring food sources of vitamin D are limited to fatty fish, some fish liver oils, and eggs from hens who were fed vitamin D (Institute of Medicine, 1997). Foods in Canada that are fortified with vitamin D include milk and margarine. Vitamin D can also be obtained with supplements and can be given in smaller daily doses of 20 – 25 μg (800-1000 IU), in larger weekly doses of 125 μg (5000 IU), or in even larger doses every four to six months in amounts as much as 2500 μg (100,000 IU) (Utiger, 1998).

2.2.2.2 Dosage

Defining adequate vitamin D status depends on the criterion used. The AI for vitamin D set by the DRI committee was based on the amount needed in the absence of sun exposure to maintain adequate concentrations of serum 25(OH)D (Institute of Medicine, 1997). Historically, the criterion used to determine adequate vitamin D status

was the level of vitamin D needed to prevent the onset of rickets or osteomalacia, which appears to be a level of serum 25(OH)D below approximately 20-25 nmol/L (F. Anderson, 2005). This was the criterion that was used to set the AI but there was insufficient evidence to know what the dietary intake should be to achieve this level of serum 25(OH)D. Thus, the vitamin D intake of a group of apparently healthy adults was used and multiplied by 2 for uncertainty (Whiting & Calvo, 2005).

Optimal serum 25(OH)D concentrations have been suggested to be ≥75 nmol/L (Bischoff-Ferrari, Giovannucci, Willett, Dietrich, & Dawson-Hughes, 2006; Heaney, 2006). In order to prevent increased bone resorption, 25(OH)D levels need to be increased to about 50 nmol/L and it has been proposed that even higher levels (75-125 nmol/L) are needed in order to prevent future disease onset (F. Anderson, 2005). Higher concentrations of 25(OH)D have been shown to have a positive benefit on calcium metabolism. Heaney, Dowell, Hale, and Bendich (2003) found that calcium absorption at a serum 25(OH)D concentration of 50 nmol/L was reduced compared to the amount of absorption at a concentration of 86 nmol/L. Dietary calcium intake may not need to be more than 800 mg/d if serum 25(OH)D concentrations are maintained above 45 nmol/L (Steingrimsdottir, Gunnarsson, Indridason, Franzson, & Sigurdsson, 2005).

An intake of 15 μg (600 IU) per day of vitamin D is needed to obtain a serum 25(OH)D level of 50 nmol/L and at least 20-25 μg/day (800-1000 IU) is needed to reach 75 nmol/L (Dawson-Hughes, Heaney, Holick, Lips, Meunier, & Vieth, 2005). A 12 week intervention trial that included women aged 65-85 years examined the response of serum 25(OH)D to three doses (5, 10, 20 μg/day) of vitamin D_3 (Viljakainen, Palssa, Karkkainen, Jakobsen, Lamberg-Allardt, 2006). An increase in serum 25(OH)D concentrations was observed after only two weeks in the supplemented groups compared

to placebo. Serum 25(OH)D levels reached a plateau at six weeks of supplementation and reached mean concentrations of 57.7 nmol/L, 59.9 nmol/L, and 70.9 nmol/L in the 5, 10, and 20 μg vitamin D supplemented groups, respectively.

A study that examined the dose response of serum 25(OH)D to vitamin D_3 suggests that if a 70 year old person receives 15 μg/day of oral vitamin D, the AI for this age group, and has no sunlight exposure, this amount is only sufficient to maintain a 25(OH)D concentration of about 12.5 nmol/L (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). Thus, the current AI for vitamin D is likely too low to maintain adequate levels of serum 25(OH)D. Heaney (2006) proposes that the UL of 50 μg/day (2000 IU) for adults is too low because the average daily requirement may be twice this amount. Healthy men may use as much as 75-125 μg (3000-5000 IU) of vitamin D_3 per day, with >80% of their needs met during the winter from endogenously synthesized vitamin D produced from sunlight exposure during the summer (Heaney, Davies et al., 2003). Intakes above 50 μg/day appear to be safe as demonstrated in a study by Vieth, Chan, and MacFarlane (2001) in which a 100 μg/day (4000 IU) dosage of vitamin D_3 increased 25(OH)D levels into the high-normal range (mean ± SD, 96.4 ± 14.6 nmol/L). Table 2.3 shows the classification of vitamin D status based on serum 25(OH)D levels and the amount of dietary vitamin D required to achieve these levels.

Table 2.3 Classification of vitamin D status by serum 25(OH)D and dietary vitamin D intake required to achieve serum 25(OH)D levels.

Serum 25(OH)D	Category	Dietary Intake to Achieve Serum Level	Notes
< 20 nmol/L	Vitamin D deficiency		
< 30 nmol/L	Not at risk for clinical rickets or osteomalacia		Cut-off used for DRI recommendation
30 – 75 nmol/L	Vitamin D insufficiency defined as at risk for chronic conditions except rickets and osteomalacia	5 – 20 μg/d	Upper end of range deemed lowest 25(OH)D for any health benefit
75 – 80 nmol/L	Threshold for vitamin D-dependent calcium absorption	25 μg/d	
90 – 125 nmol/L	Optimal status for some chronic conditions such as lower extremity function, periodontal disease, and fracture risk reduction	100 μg/d	
>220 nmol/L	Potential adverse effects are seen above this level	250 μg/d	No adverse effects were detected

Adapted from (Whiting, Calvo, & Stephensen, Submitted June 1, 2007) using references (Dawson-Hughes et al., 2005; Vieth, Chan, & MacFarlane, 2001; Viljakainen, 2006)

2.2.2.3 Analysis

Most analysis methods to measure serum 25(OH)D levels do not distinguish between $25(OH)D_2$ and $25(OH)D_3$, with the exception being a high performance liquid chromatography (HPLC) method (Gibson, 2005). In addition to HPLC, competitive protein-binding assays and radioimmunoassys are available to measure serum 25(OH)D (Gibson, 2005). Radioimmunoassays produce values that are about 30% lower than the competitive protein-binding assay which results in a cut-off value of 37.5 nmol/L for the radioimmunoassay method being comparable to a cut-off of 50 nmol/L for the

competitive protein-binding assay (Calvo & Whiting, 2003). Thus, the appropriate cut-off value must be used based on the analysis method. Serum samples used to measure 25(OH)D can be frozen, but repeated freezing and thawing should be avoided (Gibson, 2005).

2.2.2.4 Prevalence, Causes and Consequences of Deficiency

The reported prevalence of vitamin D deficiency in the elderly depends partly on the cut-off values used to define deficiency. Regardless of the definition used, elderly people living in LTC facilities are at high risk for vitamin D deficiency and the prevalence of deficiency among institutionalized elderly is typically greater than the prevalence in elderly people living in the community. A study by Hirani and Primatesta (2005) reported that mean serum vitamin D levels for older adults (≥ 65 years) living in institutions were 38.1 nmol/L for men and 36.7 nmol/L for women, which was significantly lower than the values found for age-matched people living in private households (56.2 nmol/L for men and 48.4 nmol/L for women).

Certain segments of the population, such as the elderly, may be at particular risk of not meeting their vitamin D requirements (American Dietetic Association, 2005). Long-term care residents consuming a pureed diet are at increased risk of vitamin D deficiency for several reasons. Because of the limited endogenous vitamin D synthesis in northern climates and the limited number of dietary sources, Canadians are at risk of not receiving adequate amounts of vitamin D (Calvo & Whiting, 2003). The elderly LTC population may be at particular risk of limited sun exposure due to limited mobility. Lamberg-Allardt (1984) studied serum 25(OH)D levels in three groups of elderly people (65-80 years) over one year. Group 1 were long-stay geriatric patients, group 2 were residents in a seniors' home, and group 3 were elderly people living in their own homes.

The study found that the serum 25(OH)D concentration in group 1 was the lowest of the three groups, group 2 was the next lowest, and group 3 had the highest concentrations. The investigators concluded that the low concentrations were due to both low dietary vitamin D intake and limited sunlight exposure (Lamberg-Allardt, 1984).

Another factor that might contribute to low serum 25(OH)D levels is that when elderly people are exposed to sunlight they might have a reduced ability to synthesize vitamin D endogenously due to a decreased amount of 7-dehydrocholesterol in the skin (Armas et al., 2004; Gloth et al., 1995; MacLaughlin & Holick, 1985). It has also been suggested that intestinal absorption of vitamin D as well as metabolic activation of vitamin D in the liver and kidney may also diminish with age (L. T. Lee, Drake, & Kendler, 2002). However, this finding has recently been disputed (J. L. Johnson et al., 2005).

Vitamin D deficiency can lead to bone diseases such as rickets, osteomalacia, or osteoporosis (Otten et al., 2006). There is also increasing evidence of an association between vitamin D insufficiency and the risk of chronic diseases, including diabetes mellitus, cancer, autoimmune disorders, and osteoporosis (Calvo & Whiting, 2003; Natri et al., 2006). Vitamin D may improve lower extremity function, functional performance, reaction time and balance, which may have a positive effect on preventing falls (Bischoff-Ferrari et al., 2004; Bischoff et al., 2003; Dhesi et al., 2004; Jackson, Gaugris, Sen, & Hosking, 2007). Improving the vitamin D status of the elderly has the potential to decrease the risk of falls by 20% which may be mediated by the effect of vitamin D on postural or dynamic balance (Bischoff-Ferrari et al., 2006). Vitamin D has even been implicated in affecting mood and cognitive performance (Wilkins, Sheline, Roe, Birge, & Morris, 2006) and may be associated with seasonal epidemic influenza (Cannell et al.,

2006). Recently, a four year randomized trial provided evidence of a relationship between cancer risk and vitamin D status (Lappe, Travers-Gustafson, Davies, Recker, & Heaney, 2007). In addition, vitamin D deficiency causes deep bone and muscle pain (Calvo & Whiting, 2003). Thus, inadequate vitamin D status can impact quality of life and ensuring adequate status in individuals with limited ability to communicate, such as elderly people with cognitive impairment, is essential to preventing unnecessary discomfort.

2.2.2.5 Fortification

Studies suggest that current fortification practices in Canada and the United States are not effective in preventing low vitamin D status, especially among vulnerable populations such as the elderly (Calvo, Whiting, & Barton, 2004). Milk is fortified with vitamin D at a level of 9.6 μg (385 IU) per liter (Calvo & Whiting, 2003). Since milk is one of the major dietary sources of vitamin D, elderly persons need to consume approximately 1.5 L/day of milk to meet the current AI of 15 μg (600 IU). Most elderly people are unlikely to consume such a large volume of milk and the fortification of additional foods would help the elderly meet their vitamin D requirement.

A limited number of studies have investigated the effectiveness of fortifying various foods with vitamin D. Since vitamin D is fat-soluble, whether or not vitamin D is bioavailable from non-fat foods has been questioned. Tangpricha, Koutkia, Rieke, Chen, Perez, and Holick (2003) studied the bioavailability of vitamin D in two non-fat beverages, skim milk and orange juice. The study using milk compared the bioavailability of 625 μg (25,000 IU) vitamin D_2 in whole milk, skim milk, and corn oil on toast. Each subject visited the research clinic on three different occasions, at least two

weeks apart, and the sequence in which each subject received the three fortified foods was randomized. Serum was obtained at 0, 2, 4, 8, 12, 48 and 72 hours after ingestion of the fortified food to measure serum levels of vitamin D. The results did not find a significant difference in serum vitamin D concentrations between the three groups which suggests that fat is not necessary for vitamin D to be absorbed. Since milk is not consumed by all individuals due to intolerance or taste preferences, the investigators then studied the feasibility of vitamin D fortification of orange juice (Tangpricha et al., 2003). Two groups were included in the orange juice study: one group received 240 ml orange juice fortified with 350 mg calcium; and the other group received 240 ml orange juice fortified with 350 mg calcium plus 25 µg (1000 IU) vitamin D_3. Subjects were randomly assigned into one of the two groups and consumed one serving of the orange juice per day for 12 weeks. Blood was taken each week during the study to measure serum 25(OH)D. The group that received the orange juice containing vitamin D_3 had significantly higher serum 25(OH) D levels than at baseline as well as compared to the control group.

In a fortification study using bread as the delivery medium, Natri et al. (2006) provided 10 µg (400 IU) of vitamin D_3 from fortified wheat or rye breads daily for 3 weeks to 41 healthy, middle-aged women. The change in 25(OH)D in the two fortified bread groups were compared with a group receiving unfortified wheat bread, and a group receiving unfortified wheat bread plus a 10 µg vitamin D supplement. The changes in 25(OH)D in the fortified wheat bread, fortified rye bread, unfortified bread, and bread plus supplement groups were 16.3 ± 6.6, 14.9 ± 6.2, -0.3 ± 4.0, and 19.5 ± 10.1 nmol/L, respectively. The increase in the bread fortification groups was not significantly different from the group that received the same amount of vitamin D as a

supplement. The people with the lowest baseline vitamin D status seemed to respond the best to the vitamin D from the bread or supplement (Natri et al., 2006).

In another vitamin D fortification study, J. L. Johnson et al. (2005) showed that vitamin D in fortified processed cheese is bioavailable. However, the study did not show an increase in serum 25(OH)D concentrations after the elderly subjects consumed the cheese for two months, which provided a daily dose of 15 μg. The investigators speculated that this could have been due to higher baseline concentrations in the group receiving the fortified cheese compared to the groups not receiving the product.

2.2.3 Vitamin B_{12}

2.2.3.1 Metabolism

Vitamin B_{12}, or cobalamin, functions in two coenzyme forms: adenosylcobalamin and methylcobalamin. Adenosylcobalamin acts as a coenzyme for methylmalonyl-CoA mutase and leucine mutase which play a role in the metabolism of propionate and amino acids. Methylcobalamin acts as a coenzyme for methionine synthetase which functions in single carbon metabolism (Mahan & Escott-Stump, 2004). The role of vitamin B_{12} as a coenzyme is essential for normal functioning of all cells, and especially for cells of the gastrointestinal tract, bone marrow, and nervous tissue. Vitamin B_{12} is absorbed by active transport as well as about 1% by simple diffusion. Intrinsic factor, a binding protein found in gastric secretions, mediates the active transport of cobalamin. After absorption into the circulatory system and transportation by glycoproteins and transcobalamins I and II, vitamin B_{12} can be stored in the liver in amounts as much as 2000 μg, which can provide about 5-7 years worth of the vitamin (Mahan & Escott-Stump, 2004).

Vitamin B_{12} is found naturally in foods of animal origin as well as in some plant-based foods that have been fortified. Meat, fish, poultry, and milk are generally the greatest contributors to adult intake in Canada and the United States. Cyanocobalamin is the primary form of vitamin B_{12} supplement available in Canada (Institute of Medicine, 1998). The EAR and RDA for vitamin B_{12} for adults aged ≥51 years are 2.0 μg/day and 2.4 μg/day, respectively. The Tolerable Upper Intake Level (UL) for this vitamin is unknown (Institute of Medicine, 1998).

2.2.3.2 Biomarkers

The primary criterion used to set the EAR for vitamin B_{12} was the maintenance of hematological status and serum vitamin B_{12} values (Institute of Medicine, 1998). Table 2.4 summarizes the available markers for vitamin B_{12} status and the advantages and disadvantages of each.

The Merck Manual states that the normal range for serum vitamin B_{12} is 200-800 pg/mL (150-590 pmol/L) (Merck Research Laboratories, 2006) while others refer to the lower limit for normal serum cobalamin as 150 – 300 pmol/L (Dhonukshe-Rutten, van Zutphen, de Groot, Eussen, Blom, & van Staveren, 2005). It has been suggested that serum cut-off values for diagnosing vitamin B_{12} deficiency are too low and should be increased to 258 pmol/L (L. H. Allen & Casterline, 1994). Therefore, the normal reference range for serum vitamin B_{12} may be defined as 300-590 pmol/L, with < 150 pmol/L indicating deficiency, and 150-300 pmol/L indicating inadequate levels. Low or low-normal levels of serum vitamin B_{12} likely reflect a deficiency, even if it is subclinical, because other markers of vitamin B_{12} status (serum methylmalonic acid and

homocysteine) have been shown to respond to treatment with vitamin B_{12} in elderly populations (R. H. Allen, Lindenbaum, & Stabler, 1995).

Table 2.4 Vitamin B_{12} biomarkers and their advantages and disadvantages.

Indicator	Advantages	Disadvantages
Serum vitamin B_{12}	- Commonly used in research studies - Used in setting the EAR[1]	- No universally accepted cut-off value for defining cobalamin deficiency - Difficult to interpret in the elderly since many have low or low-normal levels without symptoms
Serum methylmalonic acid (MMA)	- Highly specific to vitamin B_{12} deficiency	- Could not be used as criterion for setting EAR because of a lack of direct data[1] - Levels affected by renal function which declines with age[2] - Difficult and costly to measure[3] - May not predict clinical manifestations of deficiency[4] - Using as the only indicator of B_{12} status may not predict clinical manifestations of deficiency[5]
Serum holo-transcobalamin	- Represents form available for use by tissue	- Test not readily available[6]
Serum total homocysteine	- Can indicate subclinical deficiency	- Levels also affected by folate and vitamin B_6 status[1]

[1](Institute of Medicine, 1998)
[2](Loikas et al., 2007)
[3](L. H. Allen & Casterline, 1994)
[4](Nilsson, 2002)
[5](Hvas, Lous, Ellegaard, & Nexo, 2002)
[6](Kapadia, 2000)

2.2.3.3 Prevalence, Causes and Consequences of Deficiency

Studies suggest that metabolically significant vitamin B_{12} deficiency in the elderly is more common than previously thought (Clarke, 2001). The prevalence of deficiency is about 20% in the general population of industrialized countries and increases to 30-40%

in elderly people living in institutions or who are sick (Andres et al., 2004). Even in geriatric specialty clinics where clinicians routinely screen their patients for cobalamin deficiency, the prevalence has been found to be high (13%) (Rajan, Wallace, Beresford, Brodkin, Allen, & Stabler, 2002). However, the exact prevalence is difficult to determine because of the several different definitions and indicators of vitamin B_{12} deficiency described above (Park & Johnson, 2006). Many people, with serum vitamin concentrations that are considered within the normal range, are metabolically deficient in cobalamin (Lindenbaum, Rosenberg, Wilson, Stabler, & Allen, 1994). Regardless of the definition used, it is evident that the prevalence of vitamin B_{12} deficiency increases with age (Clarke et al., 2004; Lindenbaum et al., 1994).

Vitamin B_{12} is of particular concern for the elderly population because 10-30% of older people may be unable to absorb naturally occurring vitamin B_{12}, most likely due to atrophic gastritis. More than 40% of patients over 80 years are estimated to suffer from this condition which inhibits the ability to release cobalamin bound to protein, resulting in decreased absorption (Andres et al., 2004). Food-cobalamin malabsorption may be associated with significant neurologic, psychologic, and hematologic abnormalities and is likely the main cause of vitamin B_{12} deficiency in the elderly (Andres et al., 2005). It is advisable that people over 50 years meet their vitamin B_{12} needs with the crystalline form of the vitamin, either by consuming foods fortified with vitamin B_{12} or by taking a supplement that contains it (Institue of Medicine, 1998). Crystalline vitamin B_{12} absorption is normal in most people with atrophic gastritis because intrinsic factor is usually still present in adequate amounts (Russell, Baik, & Kehayias, 2001). Oral crystalline cyanocobalamin is effective at treating vitamin B_{12} deficiency resulting from

food-cobalamin malabsorption and serum levels can be returned to normal in approximately one month (Andres et al., 2003).

Other factors that contribute to food-cobalamin malabsorption in the elderly include chronic *Helicobacter pylori* infection and intestinal microbial proliferation (which can be caused by antibiotic treatment), chronic alcoholism, gastric surgery, partial pancreatic exocrine failure, and Sjogren's syndrome (Andres et al., 2004). The long-term use of certain medications can also lead to low vitamin B_{12} status. Prolonged use of H_2-receptor antagonists and proton-pump inhibitors may impair the absorption of protein-bound vitamin B_{12} (Ruscin, Page, & Valuck, 2002). Metformin, a drug that is commonly used to treat people with type II diabetes, has been shown to decrease levels of folate and vitamin B_{12} (Wulffele, Kooy, Lehert, Bets, Ogterop, Borger van der Burg, 2003). Pernicious anemia, an autoimmune disease characterized by the destruction of the gastric mucosa, is another cause of cobalamin deficiency and results in a lack of intrinsic factor so vitamin B_{12} is not absorbed (Andres et al., 2004). Vitamin B_{12} is secreted in the bile, but most of it is reabsorbed in healthy individuals. In people without intrinsic factor, vitamin B_{12} is excreted in the stool rather than reabsorbed which results in a deficiency that can develop quite quickly compared to someone who does not ingest the vitamin (Institute of Medicine, 1998).

Vitamin B_{12} deficiency is often not recognized because the clinical signs are subtle (Andres et al., 2004). Hematological, neurological, and gastrointestinal effects can result from a deficiency in vitamin B_{12} (Institute of Medicine, 1998). The hematological effects include weakness, fatigue, shortness of breath, and palpitations. The anemia that results from a vitamin B_{12} deficiency produces oversized erythrocytes, or macrocytosis. There is also usually some degree of neutropenia and thrombocytopenia. These

hematological complications are completely resolved with vitamin B_{12} treatment. Neurological impairment due to vitamin B_{12} deficiency can be evident even in the absence of anemia (Lindenbaum et al., 1994) and is often the only clinical manifestation (Institute of Medicine, 1998). Neurological abnormalities are due to patchy demyelinization in the spinal cord, peripheral nerves, cerebral cortex, and cranial nerves (R. H. Allen et al., 1995). Symptoms of this nervous system damage include tingling and numbness in the extremities, gait disturbances, loss of concentration, memory loss, disorientation, dementia, mood changes, visual disturbances, insomnia, impotency, and incontinence of the bowel and bladder. Gastrointestinal effects of deficiency include sore tongue, loss of appetite, flatulence, and constipation (Institute of Medicine, 1998). Edema is another possible symptom of vitamin B_{12} deficiency (Henoun Loukili, Noel, Ben Abdelghani, Locatelli, Blickle, & Andres, 2005).

2.2.3.4 Dosage and Analysis

The intake of synthetic cobalamin appears to correspond to serum cobalamin levels and taking vitamin B_{12} supplements provides protection against low levels (Garcia, Paris-Pombo, Evans, Day, & Freedman, 2002). However, the exact dose needed to maintain adequate serum levels is unclear. Rajan, Wallace, Beresford, Brodkin, Allen, and Stabler (2002) found that only 4% of subjects with synthetic cobalamin intakes above 12 µg were deficient and that no subjects with intakes greater than 50 µg were deficient. Garcia et al. (2002) found a dose-effect relationship between oral cobalamin supplement intake and serum cobalamin, methylmalonic acid (MMA), homocysteine, and methylcitric acid levels. Bor, Lydeking-Olsen, Moller, and Nexo (2006) measured transcobalamin (TC), holo-TC, TC saturation, MMA, and total homocysteine levels and found that a daily dose of 6 µg of vitamin B_{12} will result in a steady concentration of

these indicators of vitamin B_{12} status and that vitamin B_{12} intake between 6 and 10 μg per day might help correct low vitamin B_{12} status. Oakley (2004) found that 6 μg/day of oral vitamin B_{12} is very effective in reducing the proportion of elderly people with low vitamin B_{12} status and that those who do not take supplements are 300% more likely to have a pre-clinical vitamin B_{12} deficiency. However, the researchers also stated that some people will need to consume more than 6 μg/day in order to prevent a pre-clinical vitamin B_{12} deficiency. Campbell, Miller, Green, Haan, and Allen (2003) found that subjects deficient in vitamin B_{12} consumed less of the vitamin from supplements and fortified beverages compared to subjects with marginal or normal vitamin B_{12} levels.

A study by Seal, Metz, Flicker, and Melny (2002) was performed to "establish the minimum daily dose of vitamin B_{12} to restore to normal the low serum vitamin B_{12} concentrations in older people". The study lasted four weeks and compared two dose levels of vitamin B_{12}, 10 and 50 μg/day, with placebo. The study concluded that supplementation with 50 μg/day of cyanocobalamin produced a significant increase in serum vitamin B_{12}, whereas 10 μg/day was not significantly different than placebo. Blacher et al. (2007) found that a dose of 5.9 μg/day of vitamin B_{12} is needed to increase serum concentrations of the vitamin by 37 pmol/L in elderly subjects with low serum vitamin B_{12} status. In a review by Park and Johnson (2006) the authors concluded that doses of vitamin $B_{12} \leq 50$ μg/day for short periods of time do not increase serum or plasma levels of vitamin B_{12} to levels above ~260 pmol/L and that more than 100 μg is needed for at least eight weeks to lower MMA to less than 300 nmol/L. Stabler, Lindenbaum, and Allen (1997) concluded that an adequate daily oral dose of vitamin B_{12} would be greater than 6 μg but less than 300 μg. Wolters, Strohle, and Hahn (2004)

stated that general supplementation in the elderly with > 50 μg/day of vitamin B_{12} should be considered.

A daily intake of oral vitamin B_{12} at the amount recommended by the RDA is likely not adequate to recover from vitamin B_{12} deficiency and there does not appear to be risks involved with higher amounts of vitamin B_{12} intake (Food and Nutrition Program of the Pan American Health Organization, March of Dimes, Centers for Disease Control and Prevention, 2004). Toxicity is not apparent even at levels up to 10,000 times the requirements, although the safe upper intake level is unknown (Dharmarajan & Norkus, 2001). Andres et al. (2003) found that an oral dosage of 250-1000 μg/day for one month may be an effective treatment for vitamin B_{12} deficiencies that are not related to pernicious anemia. Eussen et al. (2005) stated that a dose more than 200 times greater than the RDA is needed to correct a mild vitamin B_{12} deficiency. Kuzminski, Del Giacco, Allen, Stabler, and Lindenbaum (1998) found that 2 mg/day of oral cyanocobalamin was as effective as a 1 mg intramuscular dosage administered monthly. A daily oral dose of 1000 μg vitamin B_{12} was found to maintain serum vitamin B_{12} levels in patients who were previously maintained on vitamin B_{12} injections (Nyholm et al., 2003).

Older radioassays overestimated vitamin B_{12} status which led to individuals, who actually were deficient, to be classified as having sufficient levels. This occurred because the assays would measure non-functional analogs of the vitamin but this has been resolved with the use of purified intrinsic factor in commercial radioassay kits (Gibson, 2005). An even newer method to measure serum cobalamin levels is the non-isotopic serum vitamin B_{12} assay which uses a higher cut-off value for deficiency. Cut-off values for serum cobalamin are inconsistent in the literature and because cobalamin

levels depend on the type of analytical test used, the laboratory conducting the analysis, and the sample size, Gibson (2005) recommends that each laboratory set its own reference values.

2.2.3.5 Fortification

The level of vitamin B_{12} fortification needed to prevent cobalamin deficiency is uncertain. Seal et al. (2002) recommend that food fortification should aim to supply at least an additional 50 µg/day. Another study found that 1000 µg cobalamin per day for 12 weeks delivered via fortified milk was as easily absorbed as the same amount delivered as crystalline cobalamin in capsule (Dhonukshe-Rutten et al., 2005). This study concluded that additional studies are needed to determine whether lower doses of the vitamin can be used in fortification to prevent cobalamin deficiency. A study that used vitamin B_{12} supplements at doses of either 10 µg or 50 µg per day for four weeks concluded that food fortification should provide an additional 50 µg of vitamin B_{12} daily, because they did not find an improvement with an additional 10 µg/day (Seal et al., 2002). However, another study found that lower levels of fortification are capable of improving vitamin B_{12} status. A breakfast cereal fortified to provide 4.8 µg vitamin B_{12}, 440 µg folate, and 1.8 mg vitamin B_6 per one cup serving significantly improved vitamin and homocysteine levels. The study included adults over 50 who consumed one serving of the cereal daily for three months (Tucker, Olson, Bakun, Dallal, Selhub, & Rosenburg, 2004). Oakley (2004) suggests that vitamin B_{12} fortification should be at a level of 10 µg per 100 g of grain which would result in a median vitamin B_{12} intake of approximately 15 µg/day.

A concern with fortification that has the potential to affect food manufacturers is that the natural colour of crystalline vitamin B_{12} may turn grain products pink. However,

no difference in colour was found between vitamin B_{12} fortified and unfortified bread with 25, 100, or 500 µg of vitamin B_{12} per 100g of bread. Only at a high level of fortification (1000 µg/100 g bread) was a difference detected with the appearance of the bread being off-white with a trace of pink colour (Herbert & Bigaouette, 1997).

2.2.4 Folate

2.2.4.1 Metabolism

Folate is a B-vitamin that functions as a co-enzyme in single-carbon transfer reactions and is essential for the metabolism of nucleic and amino acids (Institute of Medicine, 1998). Foods that are rich in folate include fortified grain products, dark green vegetables, and legumes. Folate refers to the naturally-occurring folate found in food while folic acid refers to the form used in vitamin supplements and fortified foods, although these terms are often used interchangeably. Folic acid is more easily absorbed by the body than folate. In order to compensate for this difference in bioavailability, a conversion unit is used which is termed dietary folate equivalents (DFE). Dietary folate equivalents are calculated as 1.0 µg of food folate equals 1.0 µg DFE. Folic acid from a fortified food or supplement is equal to 1 µg of folate in food multiplied by 2.0 if consumed on an empty stomach and multiplied by 1.7 if consumed with food (Otten et al., 2006). The EAR and RDA for adults ≥51 years is 320 and 400 µg per day, respectively, and the UL is 1000 µg/day for folic acid from fortified foods and supplements (Institute of Medicine, 1998).

2.2.4.2 Biomarkers

Serum, plasma, whole blood, and red blood cell (RBC) folate assays are available to determine folate status (Hultberg, Nilsson, Isaksson, & Gustafson, 2002). Inadequate

folate intake first leads to a decrease in serum folate concentration, then to a decrease in erythrocyte folate concentration. This will eventually lead to macrocytic anemia, characterized by a low erythrocyte count, as well as low hematocrit and hemoglobin (Institute of Medicine, 1998). The Dietary Reference Intakes used RBC and serum folate levels as well as plasma homocysteine as criteria for determining folate requirements for several of the life stage groups (Institute of Medicine, 1998). Serum folate is a sensitive indicator of dietary folate intake and serum folate has been shown to respond to an increase in dietary folate intake in as little as four weeks (Venn et al., 2002).

K. A. Johnson et al. (2002) recommend using serum folate to determine folate status, unless there has been a recent change in diet in which case they recommend using RBC folate as it is more indicative of folate stores. A problem with using RBC folate in repletion studies is that because red blood cells have a life span of 120 days and only accumulate folate during their formation, a longer repletion period is needed to observe a change in RBC folate levels (Kauwell et al., 2000). A study using a daily 400 µg supplement measured erythrocyte folate and found an increase in folate concentrations after 12 weeks (Adank, Green, Skeaff, & Briars, 2003).

According to the Merck Manual, the normal value for serum folate is >4.3 nmol/L (1.9 ng/mL) (Merck Research Laboratories, 2006). However, the cut-off value depends on the testing method and other sources quote different cut-off values for serum folate. Several authors report using a cut-off value of 6.8 nmol/L (Pfeiffer, Caudill, Gunter, Osterloh, & Sampson, 2005; Rampersaud, Kauwell, & Bailey, 2003; Ubbink, 1998). Other evidence suggests that the cut-off value should be higher. Hultberg et al. (2002) suggests that, based on studies using homocysteine to redefine the lower limit, the cut-off value should be 10-15 nmol/L.

2.2.4.3 Prevalence and Causes of Deficiency

Elderly people have been shown to have an increased risk for low folate status (Clarke et al., 2004; Lokk, 2003) and those who are institutionalized have been shown to have lower levels than people in the community (Matthews, 1995). The prevalence of folate malnutrition in the elderly has been estimated at 11 - 28% (Keane, O'Broin, Kelleher, Coakley, & Walsh, 1998). There are several possible causes for folate deficiency. Insufficient food intake, which is often seen in the elderly, is a common cause of poor folate status (Lokk, 2003). People consuming a pureed diet may also be at increased risk for folate deficiency because it may be more difficult to provide high folate foods, such as leafy green vegetables and folate-fortified grain products, in a pureed consistency (Adolphe et al., 2006).

Folate absorption is optimized within a certain pH range and can be impaired in the elderly due to age-related achlorhydria (Lokk, 2003). Certain drugs that are commonly prescribed to elderly patients can affect folate status through their effects on absorption or metabolism. Methotrexate, a drug used to treat neoplastic disease, asthma, inflammatory bowel disease, psoriasis, and rheumatoid arthritis, acts as a folate antagonist. Phenytoin, an anticonvulsant drug, impairs folate metabolism, and sulfasalazine, which is used to treat inflammatory bowel disorders, can inhibit folate absorption and metabolism (Rampersaud et al., 2003).

2.2.4.4 Dosage and Analysis

To determine how much folate is required to restore normal folate levels in elderly women after a moderate folate depletion period, Kauwell et al. (2000) compared the effects of diets containing 200 or 415 μg/day of folate (provided as a mixture of folic acid and folate) on serum folate, RBC folate, and homocysteine concentration. The study

consisted of two phases. Phase I was a 49 day folate depletion phase during which time all subjects received a folate-restricted diet containing 118 ± 25 μg/d folate. Serum folate concentrations dropped from 45.7 ± 27.0 nmol/L at baseline to 13.7 ± 7.0 nmol/L after the depletion phase. Phase II was a 49 day repletion stage during which time subjects were divided into four groups, all of which continued to receive the folate-restricted diet. In addition, the groups received a folic acid fortified apple juice supplement and/or orange juice which naturally contains folate in the following combinations: *Group A* 10 μg folic acid, 70 μg folate; *Group B* 137 μg folic acid, 155 μg folate; *Group C* 82 μg folic acid, 0 μg folate; *Group D* 301 μg folic acid, 0 μg folate. The study found that the two groups that received 415 μg/d (groups B and D) had improved serum folate and homocysteine levels compared to the two groups that received 200 μg/d folate (groups A and C). Mean serum folate concentrations increased from 12.1 ± 7.2 nmol/L to 14.0 ± 7.6 nmol/L in the groups receiving 200 μg/d and from 15.6 ± 6.5 to 31.4 ± 9.9 nmol/L in the groups receiving 415 μg/d. Red blood cell folate did not improve which was likely due to the short study period (49 days) and long life span of red blood cells (120 days) during which time folate is acquired. Another study examined the effect of breakfast cereals fortified with either 100, 200 or 300 μg folic acid on serum folate concentrations (Venn et al., 2002). At baseline, serum folate concentrations for the control, 100, 200 or 300 μg/d groups were 18, 18, 17 and 18 nmol/L, respectively. Four weeks later, serum folate concentrations were 18, 23, 28 and 33 nmol/L in the control, 100, 200 or 300 μg/d groups, respectively (Venn et al., 2002).

Serum folate can be measured using a number of different techniques, including microbiological or competitive binding assays. The microbiological method is the best technique for assessing total serum folate because it responds to the greatest number of

folate derivatives (Gibson, 2005). The advantage of competitive binding assays is that they are rapid. A number of detective systems can be used with the competitive binding assays, including radioisotope dilution, enzyme-linked assays, and chemiluminescent tags (Gibson, 2005). Newer techniques to analyze folate include chemiluminescence, ion capture, HPLC with fluorescence detection, gas chromatography and liquid chromatography. Because of the variety of methods available, method-specific reference ranges need to be developed for which classification for deficiency is confirmed using other hematological symptoms (Gibson, 2005).

2.2.4.5 Fortification

In Canada, folic acid fortification of cereal grain products became mandatory in November 1998 at a level of 150 – 200 μg folic acid per 100 g of product (Public Health Agency of Canada, 2004). The United States Food and Drug Administration also mandated folic acid fortification at a level of 140 μg per 100 g of cereal grain product which was projected to increase the average folic acid intake by 100 μg/day (Choumenkovitch, Jacques, Nadeau, Wilson, Rosenburg, & Selhub, 2001). The introduction of mandatory folic acid fortification of grain products has been shown to improve folate nutritional status (Choumenkovitch et al., 2001; Ray, 2004). However, it is unlikely that the fortification has contributed to an increase in folic acid intake for elderly LTC residents consuming a pureed diet as there may be few grain products included on pureed menus due to the difficulty in producing pureed grain products (Adolphe et al., 2006). For example, bread, which is a staple food in regular-texture diets, is not appropriate for people on a pureed diet unless it is in a specially formulated pureed product.

Two previous studies have examined folic acid fortification of foods other than grain products. A cross-sectional study by Keane et al. (1998) examined the effect of milk fortified with folic acid on the folate status of an elderly institutionalized population. The subjects consisted of LTC residents from three facilities. One facility had been serving folic acid fortified milk (38 μg/100 ml) for at least six months prior to the study and two facilities that served as the control served milk without additional folic acid (4 μg/100 ml). Serum and RBC folate levels were significantly higher in the group that had been receiving the folic acid fortified milk. Mean serum folate concentrations were 5.81 μg/L for the fortified group and 2.16 μg/L for the control group ($P < 0.001$; reference range 2.7 – 20 μg/L). Mean RBC folate concentrations were 316.5 μg/L for the fortified group and 196.1 μg/L for the control group ($P < 0.001$; reference range 150 - 1000 μg/L). However, milk may not be the ideal medium in which to deliver folic acid as it may affect the bioavailability of folate. A study found that folate is better absorbed from fermented milk than from pasteurized milk due to the denaturation of folate-binding proteins in the fermented milk (Witthoft et al., 2006).

2.2.5 Folate and Vitamin B_{12} Interaction

2.2.5.1 Overview

Folic acid and vitamin B_{12} interact metabolically. Both folic acid and vitamin B_{12} deficiencies cause hematological abnormalities that are indistinguishable from each other. However, folic acid deficiency does not result in neurological disorders as does vitamin B_{12} deficiency (R. H. Allen et al., 1995). Supplementation with folic acid can correct the anemia associated with vitamin B_{12} deficiency but will not prevent or correct damage to the nervous system which is caused by vitamin B_{12} deficiency (Czernichow

et al., 2005). The masking of a vitamin B_{12} deficiency is particularly a concern in elderly populations since malabsorption and the prevalence of deficiency increase with age (Clarke, 2001; Koehler, Pareo-Tubbeh, Romero, Baumgartner, & Garry, 1997; Food and Nutrition Program of the Pan American Health Organization, March of Dimes, Centers for Disease Control and Prevention, 2004). To help avoid this problem, the UL for synthetic folic acid from fortification and supplementation was set at 1000 μg/day (Institute of Medicine, 1998).

As a result of the possible masking of vitamin B_{12} deficiency by folic acid, concern has been raised that vitamin B_{12} fortification should occur alongside folic acid fortification of grain products. Although this idea has a considerable amount of support, the amount of vitamin B_{12} that should be added is uncertain (Ray, Vermeulen, Langman, Boss, & Cole, 2003; Food and Nutrition Program of the Pan American Health Organization, March of Dimes, Centers for Disease Control and Prevention, 2004). Herbert & Bigaouette (1997) suggest that 25 μg of vitamin B_{12} per 100g of product or folic acid supplement should be added. At this level an individual consuming 227 g (8 oz.) of grain products per day would ingest more than 56 μg of vitamin B_{12} (Lavine, 1997), which is well above the recommendations put forth in the DRI report (Institute of Medicine, 1998). Such a high level of vitamin B_{12} fortification has been disputed when there is not sufficient evidence to support intakes this high for the general population (Lavine, 1997). Thus, vitamin B_{12} fortified foods developed specifically for populations at risk for deficiency, such as the elderly, may be more appropriate.

Vitamin B_{12} and folate are essential components in the metabolism of homocysteine. Since mandatory folic acid fortification has been implemented in the U.S. there has been a modest decrease in homocysteine levels (J. L. Anderson, Jensen,

Carlquist, Bair, Horne, & Muhlestein, 2004). Homocysteine is a thiol-containing amino acid which, in the presence of catalysts, produces reactive oxygen species resulting in cell damage caused by oxidative stress (McPherson & Shepherd, 2006).

2.2.5.2 Cardiovascular Disease

Several decades ago, it was observed that individuals with homocysteinuria, a genetic disorder resulting in very high blood homocysteine concentrations, have premature vascular disease. This led to the theory that elevated homocysteine may be a risk factor for cardiovascular disease (McCully, 1969). Since this observation was made, there has been a vast amount of research into the relationship between homocysteine and cardiovascular disease.

To combine the results of the many observational studies performed in this area, a meta-analysis of 30 prospective or retrospective studies was completed to assess the relationship of homocysteine concentrations with ischemic heart disease and stroke (Homocysteine Studies Collaboration, 2002). The meta-analysis found that a 25% lower homocysteine level was associated with an 11% lower ischemic heart disease risk and about a 19% lower risk of stroke. There are currently several large clinical trials underway to examine the relationship between folic acid and vitamin B_{12} supplementation, homocysteine, and cardiovascular disease. A review by Clarke, Lewington, Sherliker, and Armitage (2007) summarized the results of four of these trials for which results are available. The trials were designed several years ago when the association of homocysteine with cardiovascular disease was believed to be stronger than what is currently thought. Because of this, even the combined results of these four trials had limited statistical power to detect a 10% difference in cardiovascular disease risk (Clarke et al., 2007). Therefore, more evidence is needed before recommendations

are made regarding folic acid and vitamin B_{12} supplementation to decrease homocysteine levels and the risk of cardiovascular disease.

2.2.5.3 Cognitive Function

Low levels of circulating folate and vitamin B_{12} or high levels of homocysteine may be associated with poor cognitive function in the elderly. Recent studies have examined the relationships between folate, vitamin B_{12}, homocysteine, and cognitive function and have found mixed results. Haan et al. (2007) examined the association between homocysteine, RBC folate, plasma vitamin B_{12}, and dementia and cognitive impairment in an elderly cohort of Mexican Americans. High homocysteine was associated with a greater risk of dementia or cognitive impairment suggesting that higher levels of plasma vitamin B_{12} may reduce the risk of dementia or cognitive impairment. In this study, RBC folate was not associated with dementia or cognitive impairment. A study by Feng, Ng, Chuah, Niti, and Kua (2006) examined the independent associations of homocysteine, folate, and vitamin B_{12} in high-functioning Chinese older adults. The results showed that homocysteine was associated with constructional ability and information processing speed, folate was associated with episodic memory and language ability, but vitamin B_{12} was not associated with any cognitive performance. McCracken, Hudson, Ellis, and McCaddon (2006) studied two markers of vitamin B_{12} status, serum MMA and holotranscobalamin, in relation to cognitive function and concluded that vitamin B_{12} deficiency in the elderly is associated with lower scores of language comprehension and expression. Homocysteine was not measured in this study. A three year randomised, double-blind trial, involving 818 subjects, examined the effect of 800 μg/d of oral folic acid on cognitive performance (Durga et al., 2007). The subjects had elevated homocysteine levels and were vitamin B_{12} replete. A sensitive tool to

assess cognitive functioning was used in the study instead of the commonly used Mini-Mental Examination. The results showed that folic acid supplementation provided a beneficial effect on global cognitive function, and specifically on memory and information processing (Durga et al., 2007).

Two Cochrane reviews have also been performed to examine the relationship between homocysteine, folic acid, vitamin B_{12}, and cognition. One of the reviews analyzed the association between vitamin B_{12} and cognitive impairment and concluded that, at the time of the review, there was insufficient evidence to suggest that vitamin B_{12} supplementation improves cognitive function (R. Malouf & Areosa Sastre, 2003). The other review examined the effect of folic acid, with or without vitamin B_{12}, on cognition and dementia. Again, the authors concluded that at the time of the review there was insufficient evidence to suggest that folic acid, with or without vitamin B_{12}, has a positive effect on cognitive function (M. Malouf, Grimley, & Areosa, 2003). Miller (2006) suggests that one possible reason for the inconsistent findings relating vitamin B_{12} to cognitive function is that more than one indicator needs to be measured in order to properly determine vitamin B_{12} status.

2.3 FORTIFICATION

2.3.1 Overview

Over the past century, food fortification has been used to combat several debilitating conditions: iodine has been added to table salt to prevent goiter; vitamin D has been added to milk to prevent rickets; and most recently, mandatory fortification of grain products with folic acid has been implemented to decrease the incidence of neural tube defects. These are all large-scale, public health strategies to combat specific

disorders. Fortification has also been used for people with specific dietary needs. For example, weight loss products are fortified with vitamins and minerals to ensure that people on low calorie diets still receive adequate amounts of micronutrients. Food fortification may also have the potential to improve the nutritional and health status of other subgroups in the population that are known to be at high nutritional risk, such as the elderly.

Although not yet studied, fortification of foods served at LTC facilities may be the most suitable way to improve the micronutrient intake of LTC residents, particularly for those requiring diet modification for dysphagia. As previously discussed, people consuming pureed diets have low energy intakes and thus are at risk of not receiving adequate amounts of nutrients. Fortification allows the provision of nutrient-dense foods to meet nutrient requirements without having to increase the volume of food consumed. Currently, planning a menu that offers the appropriate levels of nutrients results in the provision of large volumes of food and energy levels that are simply not feasible for this population, resulting in significant food wastage (Buchman, 1996). Providing foods that are fortified, yet indistinguishable from their unfortified counterparts, would ensure that acceptability of the overall diet will be optimized.

There have been a limited number of studies that have examined foods for the elderly that are fortified with multiple vitamins and/or minerals. The goal of a study by de Jong, Chin, de Graaf et al. (2001) was to study the acceptance of micronutrient-dense foods compared to regular foods among the frail elderly. The study included two enriched products which, if consumed twice per day, delivered ~100% of the Dutch RDA of several vitamins and ~25-100% of the RDA of several minerals. The results of this study demonstrated that the development of fortified products is feasible, but that it

is extremely important to develop fortified foods that taste identical to their unfortified counterparts. Another study by this group (de Jong, Chin, de Groot et al., 2001) looked at the impact of fortified foods and exercise on blood vitamins and their biomarkers, and neuropsychological functioning in the frail elderly. After the 17 week trial, the results showed a beneficial effect of the fortified foods on the blood vitamin, homocysteine, and methylmalonic acid concentrations in Dutch frail elderly. Overall, fortified foods are an acceptable alternative if they are formulated to taste the same as regular foods, and they appear to be capable of improving blood vitamin levels of the elderly.

2.3.2 Health Canada Guidelines

Health Canada has recently completed a review of its food fortification policy (Health Canada, 2005). The policy provides an opportunity for the development of commercially-prepared fortified foods under the classification of "special purpose foods". The policy defines special purpose foods as "foods that have been designed to perform a specific function, such as to replace a meal which necessitates a content of essential nutrients which cannot be achieved except by the addition of one or more of these nutrients. These foods include but are not limited to foods for special dietary use" (Health Canada, 2005, p. 18).

As outlined in the policy (Health Canada, 2005), in order to have fortified special purpose foods approved for use, rationale for fortifying a special purpose food for a specific group is necessary. Such rationale includes:

i. Evidence that the nutrition needs for the target group(s) are different from the general population, how and to what extent; this may require demonstration of inadequate intake or evidence of increased requirements. Intake data and /or

requirement data for the target group would provide the necessary evidence for nutrition needs.

ii. Evidence supporting that the proposed levels of fortification contribute to meeting the identified needs. Modeling data to show how the product, when used as intended, contributes to intakes in the context of the total diet would be appropriate evidence.

iii. Evidence that the proposed levels of fortification are safe and do not exceed the UL in the context of the total diet for the target group. This condition is an extension of the item ii) above in which upper levels of the intake distribution would be identified.

(Health Canada, 2005, p. 23-24).

2.3.3 Determination of Fortification Levels

The Dietary Reference Intakes provide valuable guidelines in assessing and planning dietary intakes of groups. For each nutrient, a set of reference values are determined. These are referred to as the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level (UL). These values are defined as follows:

- EAR: the average daily nutrient intake level that is estimated to meet the requirements of half of the healthy individuals in a particular life stage and gender group.
- RDA: the average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97 – 98 percent) healthy individuals in a specific life stage and gender group.

- AI: the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate; used when an RDA cannot be determined.
- UL: the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase. (Otten et al., 2006, p. 8)

A limitation to using the DRIs as reference values for planning nutrient intakes of elderly people living in LTC is that the values were developed for a healthy population. However, the DRIs provide the only evidence-based recommendations that can be used for dietary assessment and planning. Planning nutrient intakes for a group is challenging because the amount and selection of foods can vary greatly between individuals in the group, even when the same meal is offered in an institutional setting. Long-term care residents are a very heterogenous population with considerable variation in their age, health status, nutritional status, physical activity level, and dependency on others to carry out their activities of daily living (Akner & Floistrup, 2003). The energy and nutrient intakes of LTC residents can vary by as much as eight-fold when intakes are expressed based on the amount ingested per kilogram of body weight (Akner & Floistrup, 2003).

For nutrients that have an EAR and RDA, the EAR is used in conjunction with the usual intake distribution to develop a diet plan that provides an acceptably low prevalence of inadequate intakes within the group. The planning goal is to minimize the

prevalence of intakes below the EAR (Institute of Medicine, 2003). Using the EAR plus two standard deviations of group intake in the calculation of the target intake gives a value that would meet the needs of 97.5% of the group's requirements. In other words, only 2.5% of the group would be at risk for inadequacy. For nutrients with an AI, the goal is to increase the group's mean or median intake to the level of the AI. The UL, when available, can be used to plan a diet that provides a low prevalence of excessive intakes (Institute of Medicine, 2003).

Another approach for determining fortification levels is suggested in Health Canada's proposed policy for the addition of vitamins and minerals to food. The report suggests that special purpose foods being used as a meal replacement or nutritional supplement should be "formulated based on a defined nutrient contribution to the total daily intake, for example to provide 25% of the recommended daily intake in a serving of the food" (Health Canada, 2005, p. 19). Therefore, serving four fortified foods per day at 25% of the recommended daily intake in each serving will provide 100% of the RDA/AI per day from the fortified foods, assuming that the servings are completely consumed.

2.3.4 Sensory Testing

Sensory evaluation of foods may be defined as the "science that uses human panelists and their senses of sight, smell, taste, touch and hearing to measure the sensory characteristics and acceptability of food products" (Watts, Ylimaki, Jeffery, & Elias, 1989, p. 5). Sensory testing of fortified foods is important because it is critical to develop fortified foods that taste identical to their unfortified counterpart (de Jong, Chin, de Graaf et al., 2001). There are several types of sensory tests. Preference tests ask

panelists to express if one sample is preferred over another or if there is no preference. Acceptance tests are used to determine the degree of acceptance of a product. Hedonic tests measure the degree of liking for a product. Intensity ranking or scoring tests ask panelists to rank samples, or score them on line scales or category scales, based on the perceived intensity of a particular sensory characteristic. Discriminative tests are used to see if there is a difference between two samples (Watts et al., 1989). Thus, when testing to determine if there is a difference between fortified and unfortified foods, a discriminative test is necessary.

There are several different discriminative tests available. In the paired-comparison test, panelists are given two samples and asked to identify which sample has the greater intensity of a specific characteristic, for example, which product is sweeter (Watts et al., 1989). Another discriminative test is the duo-trio test. In this test, panelists are given three samples, one of which is labeled R for reference and two other samples labeled with a 3-digit random code. One of the coded samples is the same as the reference and the other is different. The panelists are asked to choose which sample is the same (or different) than the reference (Watts et al., 1989).

A third type of discriminative test is the triangle test. The primary advantage of the triangle test is that it is a simple test that determines whether there are detectable differences between samples (Watts et al., 1989). Therefore, the triangle test was a suitable choice for the present study in order to determine if there was a taste difference between the fortified and unfortified pureed foods. The triangle test does not determine the size or direction of the difference but this information was not necessary in this study.

To perform the triangle test, each panelist is given three coded samples and is told that two of the samples are the same and one is different. They are then asked to identify the odd sample (Poste, Mackie, Butler, & Larmond, 1991). It is common to use each sample as the duplicate for half of the tests and as the different sample for the other half (Poste et al., 1991). For example, when testing to see if there is a detectable taste difference between an unfortified food and the same food that has been fortified with vitamins, half of the panelists would receive two fortified samples and one unfortified sample, while the other half would receive two unfortified samples and one fortified sample.

To analyze the results of the triangle test, the number of correct identifications of the odd sample is compared to what you would expect by chance alone if there was no difference (i.e. one third of the time). Whether or not the number of the correct identifications is statistically significant can then be determined using statistical charts (Poste et al., 1991).

2.4 DIETARY ASSESSMENT

2.4.1 Methods of Dietary Assessment

There are several types of dietary assessment techniques available for use in research. Since no one method of dietary assessment is suitable in all situations, the researcher must decide which technique is most appropriate for the study being performed. This decision depends on factors such as the number of subjects, subject characteristics, and the method of data analysis. When choosing which assessment method to use it is important to be aware of the advantages and disadvantages of each method.

In a 24 hour recall, subjects and/or their parents or caregivers are asked by a nutritionist who has been trained in interviewing techniques to recall the subject's exact food intake during the previous 24 hour period or preceding day. Recalls may be done once or repeated several times per individual (Gibson, 2005). One of the advantages of using the 24 hour method is that the questions are open-ended so the researcher can get as much or as little detail as needed (Willett, 1998). Since the recalls do not require the subject to be literate they can be used with young children or people with limited reading ability. The major limitation to using the 24 hour recall method is that it relies on the individual's short-term memory in order to be accurate. Therefore, this technique may not be suitable for certain populations such as the elderly (Willett, 1998).

A food frequency questionnaire aims to assess the frequency with which food items or food groups are consumed during a specified time period. Questionnaires may be quantitative, semi-quantitative or qualitative (Gibson, 2005). Qualitative questionnaires do not include serving sizes, semi-quantitative questionnaires include one serving size for each food, and quantitative questionnaires allow the serving size and the frequency of intake to be determined by the subject. Food frequency questionnaires are often used when assessing large groups, such as in epidemiological studies, because they are relatively easy to administer and computerized scanning makes coding much faster (Gibson, 2005). Food frequency questionnaires are also useful for assessing long-term dietary intake (Willett, 1998). A disadvantage of food frequency questionnaires is that they are not based on actual intake and are, therefore, most useful to assess relative, not absolute, food intake.

A diet history attempts to estimate the usual food intake and meal pattern of individuals over a relatively long period of time, often one month (Gibson, 2005). An

advantage of using a diet history is that information is obtained in a single interview instead of placing burden on subjects to record intake for several days (Sjoberg & Hulthen, 2004). However, there is no standardized protocol for taking a diet history, therefore the reliability and validity depends largely upon the techniques used by the person performing the interview. The interviewer must be highly trained in order to carry out the interviews in such a way that the questions asked do not bias the subject (R. D. Lee & Nieman, 2003).

In the duplicate portions technique, participants are asked to collect a duplicate portion of all foods and beverages consumed over a 24 hour period in a container provided by the investigator, often for several consecutive 24 hour periods. The duplicate diet composites are then homogenized and later chemically analyzed. In addition, the participants are asked to make a written food record of daily food intakes to ascertain the foods and quantities consumed (Gibson, 2005). This technique is useful when studying groups that are suspected to be at risk of ingesting high levels of dietary contaminants (Gibson, 2005). It is also useful when studying dietary components that are not found in nutrient databases. Two other advantages of this technique are that it does not involve an interviewer and does not rely on memory. However, there are two major limitations to using the duplicate portion technique. Firstly, participants may alter their usual food consumption patterns during the study to make collecting the food easier (Gibson, 2005). Secondly, this method creates a large subject burden so subjects must be highly motivated.

Food balance sheets provide a comprehensive picture of the pattern of a country's food supply during a specified reference period, calculated from the annual production of food, changes in stock, imports and exports, and distribution of food over various uses

within the country (Gibson, 2005). This technique may be used to make inter-country comparisons of food supplies and associations between nutrition and mortality at national levels. However, these associations should be made with caution because other lifestyle factors may be equally as important (Gibson, 2005).

Estimated food records are performed by having the subject use household measurements to estimate all foods and beverages consumed (Gibson, 2005). The advantages and disadvantages of estimated food records are similar to those of weighed food records, which are described in the following section. However, weighed food records are considered to be more accurate than an estimated food record (R. D. Lee & Nieman, 2003).

2.4.2 Weighed Food Records

Weighed food records are performed by having a research assistant weigh all foods and beverages prior to the subject consuming them (Gibson, 2005). Any leftovers are weighed by the research assistant after the subject is finished eating and the difference is calculated to determine the amount of food consumed. Having a research assistant perform the weighed food records helps to ensure that the measurements are done objectively and do not impact the accuracy of the intake measurements (Akner & Floistrup, 2003).

There are several advantages of using weighed food records in research studies. Weighed food records are useful when comparing nutrition intakes with specific dietary recommendations and because they are based on actual intake, they may be used to estimate absolute energy and nutrient intakes (Willett, 1998). Records are open-ended and therefore can provide as little or as much detail necessary to answer the research

questions and can accommodate cultural diversity within the study population (Willett, 1998). Weighed food records do not rely on memory and thus are a useful technique in elderly populations (Willett, 1998). Flexibility in analysis is another advantage of weighed food intakes. Data can be analyzed by nutrients, individual foods, any food grouping scheme, or by meals (Willett, 1998). Finally, a weighed food record is the most precise method available for estimating usual food and nutrient intakes of individuals and is often referred to as the "gold standard" against which other assessment methods are compared (Gibson, 2005).

However, there are also limitations to using weighed food records. A single day of intake is unlikely to be representative of usual intake. Recording multiple days of intake can be burdensome for the researcher and the subjects (Willett, 1998). The research assistant, equipped with a scale, must always be available whenever the subject is eating (Gibson, 2005).

In spite of these limitations, weighed food records performed by a research assistant are the most appropriate method of dietary assessment in an elderly LTC population. This method is accurate and does not require the subjects to perform any of the record keeping which would not be possible in an elderly population with a high prevalence of cognitive impairment.

CHAPTER 3

RESEARCH METHODS

3.1 STUDY DESIGN

There were two phases to the study. Phase I involved determining appropriate fortification levels for pureed foods, which included performing sensory testing of the pureed fortified foods to determine if there was a detectable difference in taste. Phase II involved feeding the fortified foods to long-term care residents to determine if the foods were capable of improving the nutritional status of the residents consuming them.

3.2 PHASE I - DETERMINATION OF FORTIFICATION LEVELS

3.2.1 Description of Pureed Foods

The pureed menu at Parkridge Centre, where the subsequent trial of the fortified foods was planned to take place, followed a set meal pattern with a pureed meat, pureed vegetable, and mashed potatoes served at both lunch and supper. It was decided that pureed meat products and starch products (either mashed potato or a commercial pureed bread product) would be fortified since these foods were always offered at lunch and supper at Parkridge Centre, resulting in a total of four fortified pureed foods offered per day. Calcium-fortified juices had become common in the marketplace and thus it was decided that calcium and magnesium would be added to the juices instead of to the starch and meat.

3.2.2 Methods to Determine Fortification Levels

There is no universally accepted method to determine fortification levels. Therefore, a combination of two methods was used in order to arrive at an appropriate level of fortification that would minimize inadequacies while preventing nutrient intakes above the UL. The first method used to calculate fortification levels was proposed by the Dietary Reference Intakes report on planning (Institute of Medicine, 2003). The report suggests using the formula "Estimated Average Requirement (EAR) plus 2 standard deviations (SD) of intake" for menu planning. This formula is used in menu planning with the goal of limiting the number of individuals within the group who are at risk for nutrient intake inadequacies. The value calculated by using this formula will herein be referred to as the "target intake". However, to use the formula, the SD of intake [SD_{in}] must be known. Fortunately we had this information from a previous study performed by our research group (Adolphe et al., 2006). The study included weighed food intakes for eight LTC residents consuming a pureed diet. Intakes were completed for the participants for a total of 6 days, 3 days prior to and 3 days after the intervention. Nutrient intake results from this study are shown in Appendix A. The mean nutrient intakes from the combined pre- and post-intervention weighed food records from the study were calculated since intake levels did not significantly change after the intervention. This provided a mean intake based on 6 days of food records for 8 subjects. Using these data, the target intake was then calculated for nutrients that have an EAR. This formula cannot be employed for nutrients without an established EAR. For these nutrients, the goal is to increase the group's mean or median intake to the level of the AI (Institute of Medicine, 2003).

A potential problem with using this calculation for our population was that the nutrient distribution was fairly large for some nutrients. This created a risk that some intakes would exceed the UL. Therefore, Health Canada's recommended method for determining fortification levels was used for comparison. The method suggests that special purpose foods being used as meal replacements or nutritional supplements should be "formulated based on a defined nutrient contribution to the total daily intake, for example to provide 25% of the recommended daily intake in a serving of the food" (Health Canada, 2005, p. 19). Since it was planned that the starch (bread product or mashed potatoes) and meat that were served at lunch and supper would be fortified, each resident would receive two fortified starch and two fortified meats per day. Therefore, 25% of the recommended daily intake per serving would provide 100% of the RDA/AI per day from the fortified foods, assuming that the servings are completely consumed.

Using a value of 25% of the recommended daily intake as per the Health Canada method and the EAR + 2SD formula for menu planning, the food intakes from our previous pureed food study were used to determine if various levels of fortification would increase nutrient intakes while not exceeding the UL. Using the food records from our previous study, the food record that had the lowest intake and the food record that had the highest intake for each nutrient were chosen. The fortified foods were then hypothetically substituted for the same food that was unfortified in the intake study to determine the nutrient intake level that would be achieved with fortification. A calculation was also performed using an "ideal" day of intake, i.e., if someone ate exactly what was provided to them on the menu. Again, fortified food substitutions were made, with both single and double portions of the fortified foods replacing the non-fortified equivalents. Therefore, even if someone were to consume double portions of

the four fortified foods provided per day, it could be determined if the fortification level would result in an intake exceeding the UL.

Fortification levels were not determined for vitamin A, vitamin K, and potassium because of the possible adverse effects. High intakes of vitamin A may increase the risk for low bone mineral content, fracture risk, and osteoporosis and intakes among the elderly in North America are generally adequate (Basu, 2006). Vitamin K and potassium have the potential to interact with medications prescribed for cardiovascular disease which are used by many elderly patients (Rohde, de Assis, & Rabelo, 2007; Sica, 2006).

3.2.3 Sensory Testing of Fortified Foods

After the desired fortification levels were determined, triangle sensory tests were performed to determine if the addition of fortification powder to pureed foods resulted in a detectable difference in taste. The triangle tests were coordinated by a summer research assistant, Shannon Leydon. Sensory panelists were recruited by word-of-mouth and included University of Saskatchewan staff and students with no known allergies. Ethical approval (Apprendix C) was obtained from the University of Saskatchewan Biomedical Research Ethics Board (Bio-REB) and consent (Appendix D) was received from each participant. Each panelist received a $5 bookstore gift certificate and an ice-cream treat as compensation for participation.

For each food item tested, each panelist received two sets of three samples that were coded with random three-digit numeric codes. One set contained two unfortified (control) samples and one fortified sample of the food item, and the other set contained one control sample and two fortified samples. The presentation order of the samples was rotated for each panelist. For each set, the panelists were instructed to taste each sample

using a different plastic spoon and rinse their mouth with water between each sample. The panelists were then asked to identify the odd sample by writing the code of the odd sample on the questionnaire form and to briefly describe why they felt the sample tasted differently. All sensory testing was performed in a controlled sensory evaluation laboratory with panelists sitting in individual booths. Statistical charts were used to determine the probability of the number of correct identifications of the odd sample (Poste et al., 1991). A *P*-value of > 0.05 indicated that there was not a detectable taste difference between the unfortified and fortified samples.

Six pureed foods were tested using three different fortification formulations. The first formulation tested contained vitamins and minerals (Table 3.1). The second formulation was exactly the same as that shown in Table 3.1, but iron was excluded. Lastly, a formulation was tested that contained only vitamins (Table 3.2).

Table 3.1 Daminade 1119 vitamin and mineral formulation (Nealanders International Inc.).

Vitamins and Minerals	Amount per 100 mg
Vitamin D_3	160 IU (4 µg)
Vitamin E	5.97 IU (4 mg)
Vitamin C (as Ascorbic Acid)	45 mg
Vitamin B_1 (as Thiamine Mononitrate)	0.4 mg
Vitamin B_2 (as Riboflavin)	0.5 mg
Vitamin B_3 (Niacinamide)	1 mg
Vitamin B_6 (as Pyridoxine HCl)	0.6 mg
Vitamin B_{12}	1 µg
Folate (as Folic Acid)	100 µg
Pantothenate (as Calcium Pantothenate)	1.25 mg
Biotin	7.5 µg
Iron (as Ferrous sulphate)	2 mg
Copper (as Copper gluconate)	0.3 mg
Zinc (as Zinc gluconate)	3 mg
Manganese (Manganese sulphate)	0.5 mg
Selenium (as Sodium selenite)	15 µg
Molybdenum (as Sodium molybdate)	11 µg
Chromium (as Chromium chloride)	7.5 µg
Maltodextrin	q.s. mg

Table 3.2 Daminade 1119 vitamin formulation (Nealanders International Inc.).

Vitamins	Amount per 100 mg
Vitamin D_3	160 IU (4 µg)
Vitamin E (dl-alpha tocopherol acetate)	5.97 IU (4 mg)
Vitamin C (as Ascorbic Acid)	45 mg
Vitamin B_1 (as Thiamine Mononitrate)	0.4 mg
Vitamin B_2 (as Riboflavin)	0.5 mg
Vitamin B_3 (Niacinamide)	1 mg
Vitamin B_6 (as Pyridoxine HCl)	0.6 mg
Vitamin B_{12}	1 µg
Folate (as Folic Acid)	100 µg
Pantothenate (as Calcium Pantothenate)	1.25 mg
Biotin	7.5 mcg
Maltodextrin	q.s. mg

3.3 PHASE II - FORTIFICATION TRIAL OF PUREED FOODS

3.3.1 Recruitment

Twelve subjects were recruited from three wards at Parkridge Centre, Saskatoon, Saskatchewan, Canada. The wards were chosen because they had the highest concentration of residents consuming a pureed diet. The number of wards was limited to three because of the logistical difficulties in performing weighed food intakes on multiple wards. Potential participants were identified by chart review. Subjects who were ≥ 50 years old and consumed a pureed diet daily were considered eligible to participate. Residents were excluded if they were deemed palliative, as determined by a note on the resident's chart or reported by the nursing staff. Consent was provided by the residents or their family members (Appendix D). If the resident was not competent to consent to participate in the study, the resident's next of kin was contacted by phone or mail. Ethical approval was obtained from the University of Saskatchewan, Biomedical Research Ethics Board (Appendix C). Operational approval was received from the Saskatoon Health Region, Strategic Health Information and Planning Services. Each participant, or the family member who consented on their behalf, received a $50 Hudson's Bay Company gift certificate as compensation for participation.

3.3.2 Pureed Food Fortification

Based on the results of the sensory testing, it was decided to proceed with fortification using only vitamins in order to minimize the flavour changes caused by the addition of the vitamin and mineral fortification powder to the pureed foods. Originally it was planned that the meat and starch (mashed potato or commercial pureed bread product) served at lunch and supper would be fortified. However, for logistical reasons,

it was decided instead to use only the pureed products already served at Parkridge Centre. Since they did not use a commercial bread product, it was decided that the pureed meat and vegetables served at lunch and supper would be fortified. To ensure that this change would not increase the fortification levels so that some residents would be at risk of exceeding the UL, the same methods were used as described in section 3.2.2 but instead of substituting the starch foods, the hypothetical fortified vegetables were substituted for the unfortified vegetables in the intake data from our previous study with this population. Since the focus of the study became only vitamin fortification, the beverages that were originally planned to be fortified with calcium and magnesium were not used in this study.

During the eight weeks of the fortification trial, a research assistant (Alanna Gibb) or graduate student (Jennifer Adolphe) visited Parkridge Centre every morning to add the fortification powder to the vegetables and meats that were served that day for lunch and supper. The vegetables were received from the supplier frozen. Approximately 8 kg of vegetables were used at both lunch and supper. The vegetables were cooked in a perforated pan in a commercial steamer until soft, and then placed into a food processor to be pureed. The fortification powder (100 mg powder/100 g vegetables) was sprinkled onto the vegetables prior to mixing in the food processor to achieve even distribution of the powder throughout the vegetables.

The meat was purchased from the supplier as a frozen pureed product that was previously cooked. Approximately 7 kg of pureed meat was used at both lunch and supper. The meat was pulled from the freezer 2-3 days prior to reheating and placed in metal insert pans. Reheating was done approximately two hours prior to serving in a covered metal insert pan in a commercial steamer. The pans were then placed in

warming carts to keep hot until serving. For the meats served at lunch, the pans were removed from the warming carts, the fortification powder was sprinkled on top of the meat (100 mg powder/100 g meat), and then the powder was mixed into the meat using a hand-held stick blender. For the meat served at supper, the fortification powder was added to the meat prior to reheating in the steamer. To do this, the thawing meat was removed from the refrigerator and placed in a commercial food processor bowl. The fortification powder was then added to the bowl and the meat was thoroughly mixed in the food processor. The meat was then re-portioned into the metal insert pans and placed in the refrigerator until it was reheated for supper. All meat products were fortified except for fish, which was served three times during the three week menu cycle. Fish was excluded from fortification because the additional blending of the product to add the fortification powder resulted in a consistency that was too thin.

The fortified pureed food was provided to all residents prescribed a pureed diet at Parkridge Centre, but food intakes and blood samples were only obtained from the study participants who provided consent. The fortified pureed foods were included in the menu for eight weeks, from the middle of April to the beginning of June, 2007. Initially the study was planned to begin in early February to prevent endogenous vitamin D synthesis if any of the participants were exposed to sunlight. Unfortunately, a flu outbreak at Parkridge Centre prevented the study from starting until later in the spring. However, since most of the study participants were immobile, the risk of vitamin D synthesis from sun exposure was minimal.

A chart review was performed for all participants to gather information regarding their age, body weight, height, primary diagnosis, supplement usage, medications, and length of stay. Eleven of the twelve subjects recruited completed the study; one

participant passed away prior to the study end. Three day weighed food intakes were performed for 10 participants. One participant was excluded because she lived on a different unit in the facility and it was too labour-intensive to perform weighed intakes for only one person. To detect if the fortified foods had an effect on nutritional status, blood samples were drawn from 11 participants at baseline and 8 weeks after the introduction of the fortified pureed foods to measure serum cobalamin, folate, and 25(OH)D levels. A sample of the fortification powder was analyzed using HPLC-UV to determine the actual content of vitamin D, vitamin B_{12}, and folate. The analysis was contracted through SunWest Food Laboratory Ltd., Saskatoon, Saskatchewan.

3.3.3 Analysis of Serum for Vitamin B_{12}, Vitamin D and Folate

Blood samples were taken from the participants by the Gamma-Dynacare Medical Laboratories mobile phlebotomy service to measure serum levels of vitamin B_{12}, vitamin D, and folate. The blood was collected by venipuncture using BD SST (Becton, Dickinson and Company Serum Separator Tube) vacutainers and BD Eclipse needles. The samples used to measure serum vitamin B_{12} were kept protected from light and analyzed the same day they were collected at St. Paul's Hospital, Department of Laboratory Medicine, Saskatoon, Saskatchewan using an ARCHITECT B_{12} assay, a chemiluminescent microparticle intrinsic factor assay. Serum folate and 25(OH)D analysis was not available in Saskatoon so the samples were protected from light, frozen at -24°C and shipped on dry ice for analysis. Serum folate was analyzed at St. Michael's Hospital, Toronto, Ontario using the Roche E170 electrochemiluminescence immunoassay. Serum 25(OH)D was measured using Liquid Chromatography – Mass Spectrometry at a provincial lab in Regina, Saskatchewan.

3.3.4 Statistical Analysis

Nutrient analysis of the weighed food records was performed using Food Processor version 8.8.0 for Windows by ESHA Research, Salem, Oregon. Statistics were performed using SPSS 14.0 (SPSS Inc. Chicago, IL). Descriptive statistics were calculated for subject characteristics, nutrient intakes and serum vitamin levels at baseline and 8 weeks post-intervention. A comparison of nutrient intake and serum vitamin levels at baseline and 8 weeks was performed using a Wilcoxon Signed Ranks test. This non-parametric test is appropriate since the sample size was small and the samples are related (i.e. before and after intervention). Prevalence of inadequacy was determined at baseline and after the intervention for vitamins with an EAR by using the EAR cut-point method (Otten et al., 2006). Scatterplots were made and Spearman's Rho correlation coefficients (the non-parametric equivalent of Pearson correlation coefficients) were calculated to determine if a linear relationship existed between vitamin B_{12}, folate, and vitamin D intakes and their corresponding serum levels at baseline and 8 weeks after the intervention.

CHAPTER 4

RESULTS

4.1 PHASE I - DETERMINATION OF FORTIFICATION LEVELS

4.1.1 Calculations for Determination of Fortification Levels

Table 4.1 shows the combined mean intake (± SD) for 6 days of weighed food intakes from the previous intake study performed at Parkridge Centre and the calculation of EAR + $2SD_{in}$ (i.e. target intake) for the nutrients that have an EAR. Table 4.2 uses the values for the target intake from Table 4.1 as well as the 25% calculation recommended by Health Canada to test what the actual nutrient intakes would be using the hypothetical fortified foods instead of the unfortified version.

Using the results in Table 4.2, appropriate fortification levels were determined and are shown in Table 4.3. The fortification levels for thiamine, riboflavin, vitamin B_6, vitamin B_{12}, vitamin C, vitamin E, zinc, selenium, and copper were the rounded values calculated by the target intake formula, EAR + $2SD_{in}$. The fortification levels for biotin, vitamin D, pantothenic acid, chromium, and manganese were determined using 25% of the AI because these nutrients do not have an EAR. The fortification levels for niacin, calcium, and magnesium were lower than the values calculated by either formula so that intakes would not risk exceeding the UL. The values for folate, iron, and molybdenum were determined using 25% of the RDA instead of the target intake formula. The target intake formula would have risked exceeding the UL for folate. A lower level of iron was desired due to the risk of iron changing the flavour of the food so the lower value

calculated by 25% of the RDA was chosen instead of the higher target intake value. The target intake formula could not be used for molybdenum because the SD_{in} could not be determined for this nutrient.

Table 4.1 Combined mean intake using 6 days of weighed food records per subject and EAR+2SD calculation.

Nutrient	Combined nutrient intake (mean ± SD)	Target Intake[1]
Thiamin (mg)	0.79 ± 0.34	1.67
Riboflavin (mg)	1.30 ± 0.51	2.11
Niacin (mg)	12 ± 4	21
Vitamin B_6 (mg)	1.07 ± 0.60	2.60
Vitamin B_{12} (µg)	1.91 ± 1.00	4.01
Biotin (µg)	29 ± 35	N/A
Vitamin C (mg)	92 ± 55	185
Vitamin A (µg RAE)	614 ± 927	Large SD prevents accurate calculation
Vitamin D (µg)	2.6 ± 2.7	N/A
Vitamin E (mg)	0.91 ± 2.23	16.45
Folate (µg DFE)	167 ± 104	527
Pantothenic Acid (mg)	3.02 ± 1.51	N/A
Calcium (mg)	732 ± 2.97	N/A
Iron (mg)	8.56 ± 4.15	14.3
Zinc (mg)	4.65 ± 2.32	14.0
Potassium (mg)	1909 ± 788	N/A
Magnesium (mg)	127 ± 63	476
Selenium (µg)	9.13 ± 11.17	67
Copper (µg)	456 ± 321	1343
Manganese (µg)	0.96 ± 0.69	N/A

Abbreviations: SD, standard deviation; N/A, not applicable because nutrient does not have an EAR.
[1]Calculated using the formula EAR + $2SD_{in}$

Table 4.2 Calculations for determination of pureed food fortification levels.

	RDA[1] (AI)	UL[1]	Health Canada method[2]	Target per day[3]	Target per serving[4]	Min. intake[5]	Max. intake[6]	"Ideal" single portion[7]	"Ideal" double portion[8]
Vitamins									
Thiamine (mg)	1.2	ND	0.3	1.7	0.4	0.1	3.2	2.5	4.6
Riboflavin (mg)	1.3	ND	0.3	2.1	0.5	0.5	5.5	3.1	5.6
Niacin (mg)	16	35	4	20	5.2	3.3	27	17	30
Vitamin B_6 (mg)	1.7	100	0.4	2.6	0.6	1.0	6.7	3.7	7.0
Vitamin B_{12} (g)	2.4	ND	0.6	4.0	1.0	3.3	5.1	4.7	9.4
Biotin (µg)	(30)	ND	7.5	AI		21	182	42	85
Vitamin C (mg)	90	2000	22.5	185	46	27	450	302	527
Vitamin D (µg)	(15)	50	3.8	AI		6	39	23	41
Vitamin E (mg)	15	1000	3.8	16	4.1	17	19	19	38
Folate (µg)	400	1000	100	527	132	21	617	563	1048
Pantothenic Acid (mg)	(5)	ND	1.25	AI		0.5	8.9	8.0	15
Minerals									
Calcium (mg)	(1200)	2500	300	AI		383	2290	659	736
Zinc (mg)	11	40	2.8	14	3.5	1.0	13	16	31
Magnesium (mg)	420	350	105	476	119	372	873	291	515
Selenium (µg)	55	400	14	67	17	0.6	152	76	147
Chromium (µg)	(30)	ND	7.5	AI		0.3	44	35	70
Copper (µg)	900	10000	225	1343	336	120	2090	2150	3850
Manganese (mg)	(2.3)	11	0.6	AI		0.03	4.5	3.4	6.0
Molybdenum (µg)	45	2000	11.25	n/a		0.44	65	51	103
Iron (mg)	8	45	2	14.3	3.6	1.1	34	16	27

Abbreviations: RDA, Recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level; EAR, estimated average requirement; SD, standard deviation; ND, not determined; n/a, not applicable due to incomplete food database for calculating SD_{in} from previous food records

[1]For adults >70 years

[2]25% of RDA/AI based on Health Canada's recommendation for using a defined nutrient contribution

[3]Calculation as per DRI recommendation for minimizing nutrient inadequacies ($EAR + 2SD_{in}$)

[4]Amount of fortification required per serving of protein and starch (4 servings per day, 2 of each) calculated as ($EAR+2SD_{in}$)/4

[5]Minimum nutrient intake achieved using food record with lowest intake for the nutrient and substituting fortified food for the equivalent non-fortified food

[6]Maximum nutrient intake achieved using food record with highest intake for the nutrient and substituting fortified food for the equivalent non-fortified food

[7]Ideal intake assuming all foods on pureed menu were eaten with substitution of one serving of fortified foods for non-fortified equivalents

[8]Ideal intake assuming all foods on pureed menu were eaten with substitution of two servings of fortified foods for non-fortified equivalents

Table 4.3 Fortification levels for pureed foods.

	Fortification level per 65g starch serving[1]	Fortification level per 90 g meat serving[1]	Fortification level per 100g juice[2]
Vitamins			
Thiamine (mg)	0.4	0.4	-
Riboflavin (mg)	0.5	0.5	-
Niacin (mg)	1	1	-
Vitamin B_6 (mg)	0.6	0.6	-
Vitamin B_{12} (µg)	1	1	-
Biotin (µg)	7.5	7.5	-
Vitamin C (mg)	45	45	-
Vitamin D (µg)	4	4	-
Vitamin E (mg)	4	4	-
Folate (µg)	100	100	-
Pantothenic Acid (mg)	1.25	1.25	-
Minerals			
Calcium (mg)	-	-	100
Zinc (mg)	3	3	-
Magnesium (mg)	-	-	75
Selenium (µg)	15	15	-
Chromium (µg)	7.5	7.5	-
Copper (µg)	300	300	-
Manganese (mg)	0.5	0.5	-
Molybdenum (µg)	11	11	-
Iron (mg)	2	2	-

[1]Based on four fortified foods served per day (2 starch and 2 meat)
[2]Juices are served ad libitum

4.1.2 Sensory Testing

The results from six different triangle tests are summarized in Table 4.4. The table shows the type of vitamin and/or mineral formulation added to each puree, the number of panelists present at each session, and the number of panelists who could correctly distinguish the fortified sample in serving 1 (two control samples and one fortified sample) and the control sample in serving 2 (two fortified samples and one control sample). Percentages of correct choices are shown in parentheses. Tests that were not significant ($P > 0.05$) are shown in bold and indicate that panelists were not able to discriminate between the fortified and unfortified samples in these tests.

Panelists were able to discriminate between the fortified and unfortifed samples in both serving 1 and 2 when the tomato basil bread puree and savoury bread puree was fortified with vitamins and minerals with iron. Panelists were able to discriminate between the fortified and unfortified samples in serving 1 but not in serving 2 for the beef puree fortified with vitamins and minerals with iron, chicken puree with vitamins and minerals without iron, and the chicken sandwich and vegetarian pasta purees with vitamins only. Because the significance of the tests were different between serving 1 and 2, it cannot be said conclusively that the fortification did not change the flavour in these samples but that these food/micronutrient combinations most likely minimize flavour changes caused by the fortification powder. The effect fortification has on flavour appears to depend not only on the vitamin/mineral composition of the fortification powder but also on the food product to which the powder was added. For example, iron appears to have the greatest impact on changing the flavour except when added to beef. Thus, in order to minimize the chance of the fortification powder changing the flavour of the food, fortification was performed with vitamins only in the fortification trial.

Table 4.4 Sensory testing of fortified pureed foods using triangle tests.

			Serving 1 (1 Fortified Sample, 2 Controls)		Serving 2 (1 Control, 2 Fortified Samples)	
Pureed Food	Formulation Added	# of Panelists	# Correctly Identified Odd Sample (%)	Significance	# Correctly Identified Odd Sample (%)	Significance
Tomato Basil Bread Puree	Vitamin/Mineral blend with Iron	28	21 (75)	$P < 0.001$	20 (71)	$P < 0.001$
Beef Puree	Vitamin/Mineral blend with Iron	25	14 (56)	$P = 0.016$	7 (28)	**$P = 0.778$**
Savoury Bread Puree	Vitamin/Mineral blend with Iron	26	15 (58)	$P = 0.009$	16 (62)	$P = 0.003$
Chicken Puree	Vitamin/Mineral blend Without Iron	21	12 (57)	$P = 0.021$	10 (48)	**$P = 0.125$**
Chicken Sandwich/ Vegetarian Pasta Puree	Vitamin formulation only	21	12 (57)	$P = 0.021$	9 (43)	**$P = 0.240$**

Items in bold indicate that there was not a significant difference in taste between the fortified and unfortified samples.

4.2 PHASE II - FORTIFICATION TRIAL OF PUREED FOODS

To ensure that the decision to fortify the vegetables instead of the starch products still provided adequate nutrient levels without excessive intakes, the calculations completed in Table 4.2 were performed again, making the substitutions with the hypothetical fortified vegetables for the unfortified vegetables. The comparison of nutrient intakes using starch products versus vegetables is shown in Table 4.5 and shows that fortifying the vegetables instead of the starch would result in nutrient intakes at almost the same level.

Subject characteristics are shown in Table 4.6. Of the 11 subjects that completed the study, nine were female and two were male. Subject 6 was the subject for which weighed food intakes were not completed. Five of the subjects received vitamin and/or mineral supplements. The body mass index (BMI) range was quite wide at 19 – 33 kg/m^2. The ideal BMI range associated with maximum life expectancy appears to increase with age with the lower end of the range being 22 – 23 kg/m^2 and the upper end at 27 – 28 kg/m^2 (Chapman, 2006). Three of the eleven subjects had a BMI < 22 and three subjects had a BMI ≥ 28. Subject 1 received a 1200 µg/d vitamin B_{12} supplement which explains her very high serum vitamin B_{12} concentration. The composition of the multivitamin provided to some residents at Parkridge Centre is shown in Table 4.7. Table 4.8 shows the medications used by participants.

Analysis of the fortification powder found that the actual content per 100 mg was 4.1 µg vitamin D, 1.1 µg vitamin B_{12}, and 104 µg folic acid. These values were close to the labeled content as shown in Table 3.2. Table 4.9 shows baseline and intervention intakes for energy, protein, and vitamins from food (excluding supplements) as well as the EAR, RDA/AI, and UL for each nutrient for comparison of adequacy. Intakes for all

of the vitamins increased as a result of the fortification ($P < 0.05$); protein and energy intakes did not change. Prior to the intervention there was a prevalence of inadequacy of at least 10% for all vitamins except niacin. After the intervention, the prevalence of inadequacy was zero for all vitamins except vitamin B_6 (Table 4.10).

Table 4.11 shows the serum concentrations of vitamin B_{12}, folate, and 25(OH)D at baseline and 8 weeks after the fortified foods were included on the pureed menu. Prior to the intervention, all participants had serum vitamin B_{12} concentrations above the borderline deficiency cut-off value of 138 pmol/L. Serum vitamin B_{12} levels did not change after the intervention ($P = 0.386$). Serum folate concentrations significantly increased after the intervention ($P = 0.003$). At baseline, 64% of participants had a serum folate level below the deficiency cut-off value of 11.8 nmol/L. After the intervention, all participants had serum folate levels above 11.8 nmol/L. Serum 25(OH)D concentrations also significantly increased after the intervention ($P = 0.003$). Prevalence of serum 25(OH)D insufficiency at baseline was 55% (defined as serum 25(OH)D < 40 nmol/L), which was reduced to zero after the intervention.

Scatterplots for the relationships between vitamin intake and serum vitamin concentration are shown in figures 4.1 – 4.6. There was a significant correlation between vitamin D intake and serum 25(OH)D levels at baseline ($r_s = 0.857$, $P = 0.002$) but not after the intervention ($r_s = 0.444$, $P = 0.198$). Serum vitamin B_{12} and folate did not show a significant correlation with intake (Table 4.12). No side effects attributed to the fortification were reported to the research team by the residents receiving the fortified pureed foods, family members of the residents, or the staff at Parkridge Centre.

Table 4.5 Comparison of nutrient intakes resulting from fortification of starch servings vs. vegetable servings.

	Nutrient intakes with *starch* servings fortified				Nutrient intakes with *vegetable* servings fortified			
	Min. intake[1]	Max. intake[2]	"Ideal" 1 svg[3]	"Ideal" 2 svg[4]	Min. intake[1]	Max. intake[2]	"Ideal" 1 svg[3]	"Ideal" 2 svg[4]
Vitamins								
Thiamine (mg)	0.1	3.2	2.5	4.6	0.1	3.2	2.2	3.9
Riboflavin (mg)	0.5	5.5	3.1	5.6	0.5	5.4	2.8	4.9
Niacin (mg)	3.3	27.5	17.1	30.5	2.4	27.3	16.4	27.1
Vitamin B_6 (mg)	1.0	6.7	3.7	7.0	1.0	6.6	3.3	5.8
Vitamin B_{12} (µg)	3.3	5.1	4.7	9.4	2.1	4.7	4.0	8.0
Biotin (µg)	21	181	42	85	21	180	42	84
Vitamin C (mg)	27	450	302	527	26	450	271	462
Vitamin D (µg)	6	39	23	41	9	37	20	36
Vitamin E (mg)	17	19	20	39	10	16	17	33
Folate (µg)	21	617	563	1048	21	601	495	914
Pantothenic Acid (mg)	0.5	8.9	8.0	15.1	0.5	8.7	7.2	12.3
Minerals								
Calcium (mg)	383	2290	659	736	383	2290	659	736
Zinc (mg)	1.0	12.9	16.0	30.6	1.0	12.4	13.9	26.3
Magnesium (mg)	372	873	291	515	372	873	291	515
Selenium (µg)	0.6	152	76	147	0.6	148	66	126
Chromium (µg)	0.3	44	35	70	0.3	42	23	45
Copper (µg)	120	2090	2150	3850	120	2050	1950	3220
Manganese (mg)	0.03	4.5	3.4	6.0	0.03	4.4	3.0	5.3
Molybdenum (µg)	0.4	65	51	103	0.4	62	33	66
Iron (mg)	1.1	34.0	16.0	27.5	1.1	33.4	14.6	25.4

[1]Minimum nutrient intake achieved using food record with lowest intake for the nutrient and substituting fortified food for the equivalent non-fortified food
[2]Maximum nutrient intake achieved using food record with highest intake for the nutrient and substituting fortified food for the equivalent non-fortified food
[3]Ideal intake assuming all foods on pureed menu were eaten with substitution of one serving of fortified foods for non-fortified equivalents
[4]Ideal intake assuming all foods on pureed menu were eaten with substitution of two serving of fortified foods for non-fortified equivalents

Table 4.6 Subject characteristics.

Subject	Age (years)	BMI (kg/m^2)	Length of Stay (years)	Primary Diagnoses	Daily Supplement Use
1	80	19	7	Schizophrenia, Alzheimer's Disease	Calcium 1300 mg Multivitamin Vitamin B_{12} 1200 μg
2	69	21	2	Alzheimer's Disease	-
3	70	30	3	Vascular Dementia / Alzheimer's Disease	-
4	81	23	7	Dementia	-
5	81	28	7	Alzheimer's Dementia	Calcium 500 mg
6	81	33	2	Cerebral Vascular Accident, Congestive Heart Failure	-
7	62	21	8	Alzheimer's Disease	Calcium 500 mg
8	78	25	5	Alzheimer's Disease	-
9	70	27	3	Schizophrenia, Paranoid	-
10	54	26	3	Huntington's Chorea, Depression	Multivitamin
11	79	25	6	Dementia, Parkinson's, Angina	Multivitamin
Mean ± SD	73 ± 9	25 ± 4	5 ± 2		
Range	54 - 81	19 - 33	2 - 8		

Abbreviations: BMI, body mass index

Table 4.7 Composition of multivitamin provided to residents.

Vitamin	Amount
Vitamin A	1500 µg RAE (5000 IU)
Vitamin C	50 mg
Vitamin B_1 (Thiamin)	3 mg
Vitamin B_2 (Riboflavin)	2.5 mg
Vitamin B_6	1 mg
Vitamin B_{12}	3 µg
Vitamin D_3	10 µg
Niacinamide	20 mg

Abbreviations: RAE, retinol activity equivalent; IU, International Units

Table 4.8 Prescription[1] medication use by residents.

Subject	Daily Prescription Medications		
1	Clonazepam	Seroquel	Hydroxyzine
2	Carbamazepine Lorazepam (Ativan)	Risperidone Seroquel	Sertraline
3	Eltroxin	Mirtazapine	Risperidone
4	Citalopram Clonazepam	Eltroxin Lorazepam (Ativan)	Seroquel
5	Lorazepam (Ativan) Citalopram Glyburide	Indapamide **Metformin** **Ranitidine**	Seroquel
6	Citalopram Lipitor	Metoprolol Pariet	Warfarin
7	Baclofen Carbamazepine Clonazepam	Fentanyl	Venlafaxine
8	Hydromorphone (Dilaudid) Lorazepam (Ativan)	Seroquel	Trazodone **Ranitidine**
9	Seroquel Ramipril Plavix Lasix	Viaderm Salbutamol ventalin Hydroxyzine	Clopixol Flonase Lotriderm Benztropine
10	Paroxitine Risperidone	Seroquel	Lorazepam (Ativan)
11	Lorazepam (Ativan) Selegiline	Seroquel	

[1]Does not include over-the-counter medications or laxatives.
Drugs in **bold** indicate those that potentially interact with folate and/or vitamin B_{12}

Table 4.9 Energy, protein and vitamin intakes from food of long-term care residents consuming a pureed diet (n=10).

	Baseline (mean ± SD)	Intervention (mean ± SD)	P-Value	EAR[1]	RDA (AI)[1]	UL[1]
Energy (kcal)	1272 ± 257	1265 ± 253	0.5	ND	ND	ND
Protein	46 ± 12	46 ± 10	0.65	45 g/d (0.66 g/kg/d)	54 g/d (0.80 g/kg/d)	ND
Thiamin (mg)	0.8 ± 0.3	1.7 ± 0.3	0.005	1.0	1.2	ND
Riboflavin (mg)	1.2 ± 0.4	2.2 ± 0.4	0.005	1.1	1.3	ND
Niacin (NE)	19 ± 5	24 ± 6	0.007	12	16	35
Vitamin B_6 (mg)	1.6 ± 0.6	2.7 ± 0.6	0.007	1.4	1.7	100
Vitamin B_{12} (µg)	3.4 ± 1.2	5.1 ± 1.4	0.007	2.0	2.4	ND
Vitamin C (mg)	151 ± 78	228 ± 67	0.007	75	90	2000
Vitamin D (µg)	2.1 ± 2.2	12.2 ± 3.3	0.005	--	(15)	50
Folate (µg DFE)	114 ±58	505 ± 86	0.005	320	400	1000[2]
Pantothenic Acid (mg)	3.1 ± 1.1	5.7 ± 1.0	0.005	--	(5)	ND

Abbreviations: DFE, dietary folate equivalent; NE, niacin equivalents; ND, not determined
[1]Highest value for males and females >70 years.
[2]Applies only to synthetic forms from supplements and/or fortified foods.

Table 4.10 Prevalence of inadequacy[1] at baseline and after intervention for vitamins with an EAR (n=10).

Vitamin	Prevalence of inadequacy at baseline (%)	Prevalence of inadequacy after intervention (%)
Thiamin	80	0
Riboflavin	30	0
Niacin	0	0
Vitamin B_6	20	10
Vitamin B_{12}	10	0
Vitamin C	10	0
Folate	100	0

[1]Prevalence of inadequacy = # of participants with vitamin intake below EAR/total # of participants

Table 4.11 Serum concentrations of vitamin B_{12}, folate and 25(OH)D at baseline and 8 weeks after intervention.

Subject	Serum Vitamin B_{12} (pmol/L)		Serum Folate (nmol/L)		Serum 25(OH)D (nmol/L)	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
1	>1476	>1476	21.2	37.9	71	75
2	371	562	7.4	19.4	29	71
3	205	271	8.0	20.6	44	59
4	382	545	15.0	32.2	51	76
5	548	508	10.7	20.4	21	63
6	861	508	15.4	26.3	33	56
7	273	350	6.5	25.5	16	58
8	290	319	13.6	32.7	14	52
9	359	352	6.2	21.6	34	58
10	578	522	7.6	18.9	71	80
11	492	545	6.4	21.2	64	82
Mean ± SD	436 ± 192[2]	448 ± 111[2]	10.7 ± 4.9	25.2 ± 6.4	41 ± 21	66 ± 11
P-value[3]	0.386		0.003		0.003	
Range	205 - 1476	271 - 1476	6.2 – 21.2	18.9 – 37.9	14 – 71	52 - 82
% Deficient/ Insufficient	0	0	64	0	55	0
Normal Values[1]	Deficient: <116 Borderline: 116-138 Normal: 138-781		>11.8		Deficient: <25 Insufficient: <40 Toxic: >250	

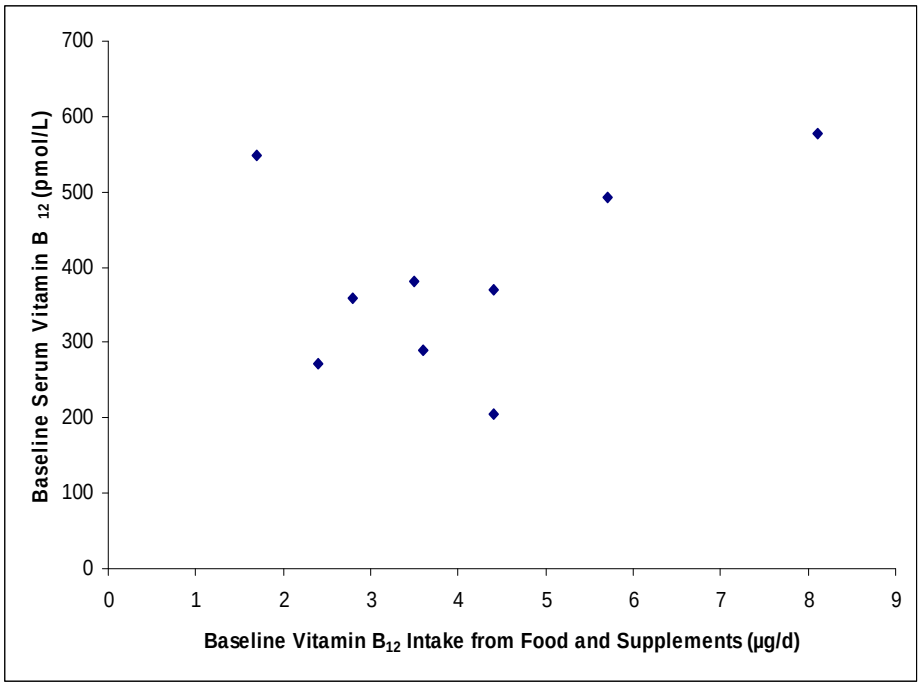
[1]Normal values are those used by the medical laboratories where the samples were analyzed.
[2]Subject 1 excluded from calculation since value is an outlier.
[3]Comparison of baseline vs. intervention using Wilcoxon Signed Ranks test.

Table 4.12 Correlation coefficients for serum vitamin levels vs. vitamin intakes from food and supplements (n=10)[1].

	Correlation coefficient (r_s)	P-value
Baseline		
Vitamin B_{12} intake – Serum vitamin B_{12}	0.234	0.544
Folate intake – Serum folate	0.158	0.663
Vitamin D intake – Serum vitamin D	0.857	0.002
Intervention		
Vitamin B_{12} intake – Serum vitamin B_{12}	0.117	0.764
Folate intake – Serum folate	0.164	0.651
Vitamin D intake – Serum vitamin D	0.444	0.198

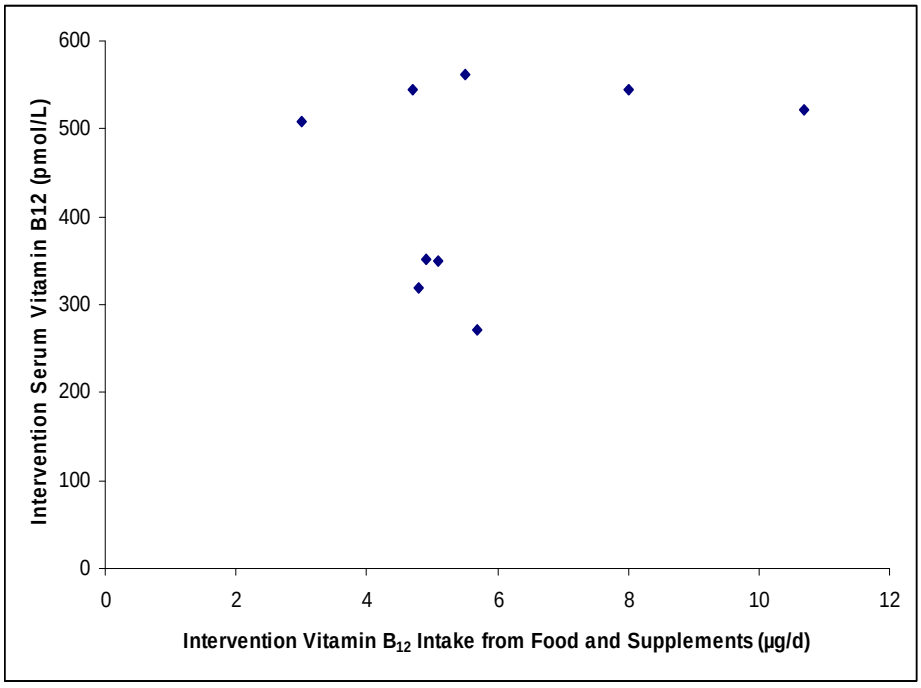
[1] n = 9 for vitamin B_{12} correlations due to removal of outlier

Figure 4.1 Association of baseline vitamin B_{12} intake from food and supplements with baseline serum vitamin B_{12} levels[1].



[1]Outlier excluded (serum vitamin B_{12} >1476 pmol/L; vitamin B_{12} intake 1206 µg/day)

Figure 4.2 Association of intervention vitamin B_{12} intake from food and supplements with intervention serum vitamin B_{12} levels[1].



[1]Outlier excluded (serum vitamin B_{12} >1476 pmol/L; vitamin B_{12} intake 1207 µg/day)

Figure 4.3 Association of baseline folate intake from food and supplements with baseline serum folate levels.

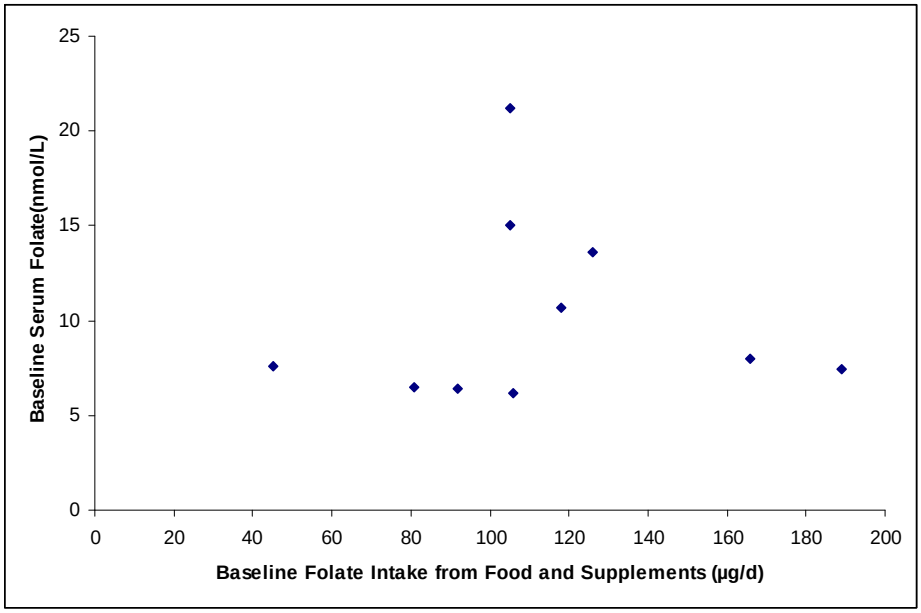


Figure 4.4 Association of intervention folate intake from food and supplements with intervention serum folate levels.

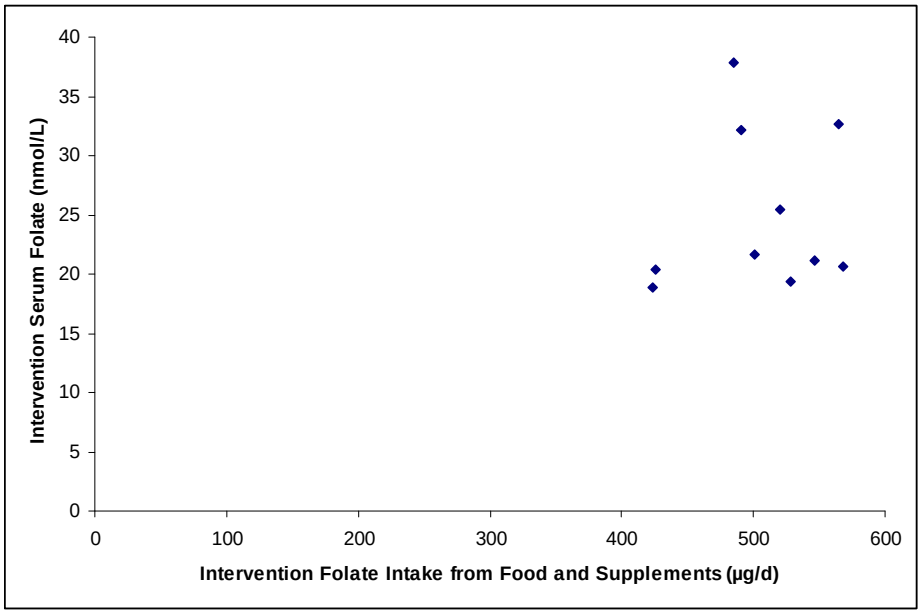


Figure 4.5 Association of baseline vitamin D intake from food and supplements with baseline serum 25(OH)D levels.

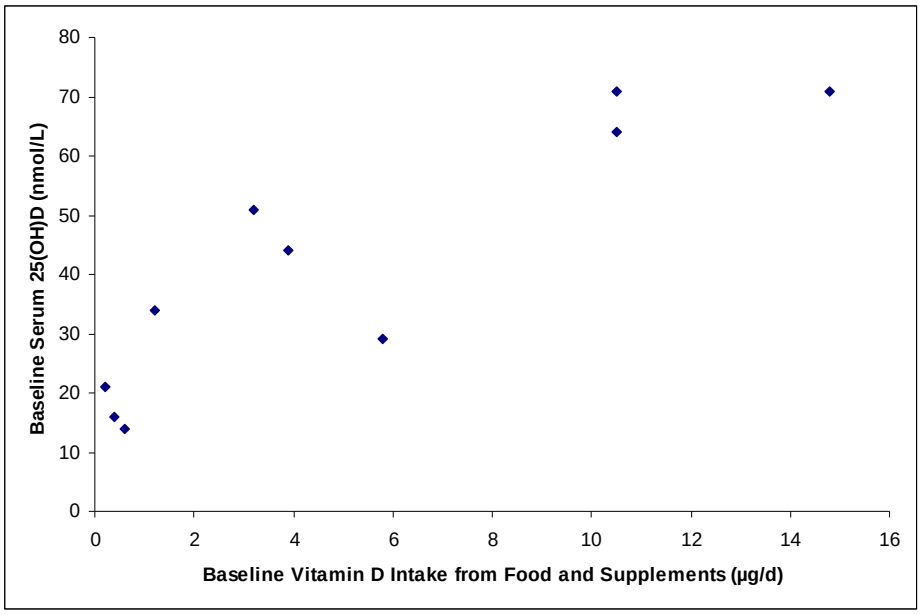
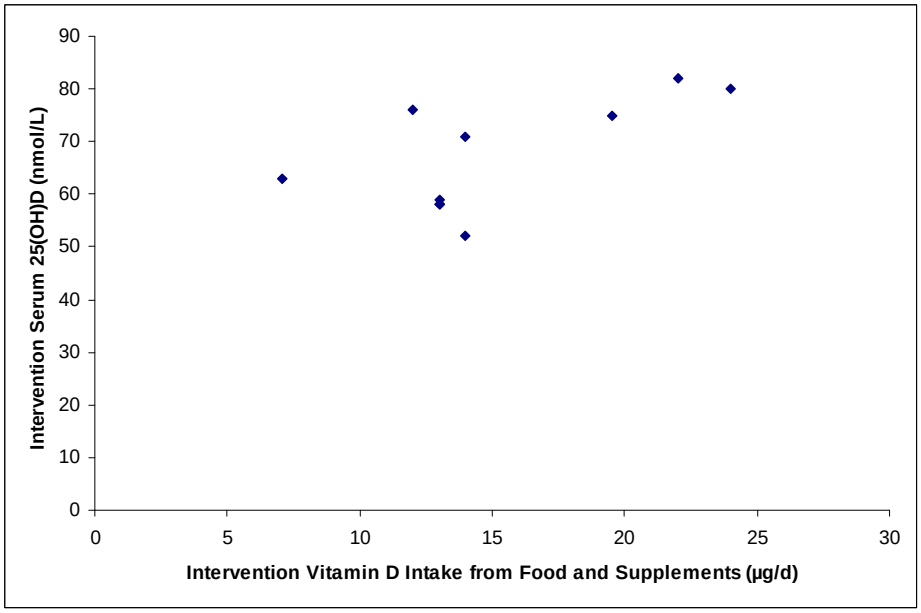


Figure 4.6 Association of intervention vitamin D intake from food and supplements with intervention serum 25(OH)D levels.



CHAPTER 5

DISCUSSION

5.1 DETERMINATION OF FORTIFICATION LEVELS

This study demonstrates that the DRI menu planning calculation for target intake ($EAR + 2SD_{in}$) in combination with the Health Canada fortification guidelines (Health Canada, 2005) are useful methods for determining appropriate fortification levels. Vitamin intakes of residents consuming a pureed diet in a LTC facility increased as a result of the fortification and the mean group intakes increased above the EAR for all nutrients after the intervention, but did not exceed the UL. One of the disadvantages of the DRI menu planning method is that it can only be used for nutrients with an EAR, not for those with an AI. In addition to using the DRI menu planning method, we used a defined nutrient contribution of 25% to calculate the fortification level based on the Health Canada guidelines. For several of the vitamins (thiamine, riboflavin, vitamin B_6, vitamin B_{12} and vitamin C) the final fortification level was the value calculated from the DRI equation. If only the Health Canada method was used to determine fortification levels, the fortification may not have raised nutrient intakes sufficiently to decrease the prevalence of inadequacy. On the contrary, if only the DRI equation had been used, the fortification levels may have resulted in excessive intakes for some vitamins (e.g. niacin), which is why using a combination of both methods is beneficial. This study

shows the value of the DRI menu planning calculation and emphasizes the need for developing EAR/RDAs for the nutrients for which only an AI is currently available.

The results of the sensory testing allowed the decision to be made to use vitamins only in the fortification powder in order to minimize flavour changes. Meat products appeared to mask the flavour changes best. Of interest is that sensory panelists were less likely to detect a difference between the control and fortified samples when given one control and two fortified samples instead of two controls and one fortified sample.

5.2 FORTIFICATION TRIAL

The principal finding of this phase of the study was that vitamin fortification of pureed foods for residents in long-term care is a promising method for improving nutritional status. Serum folate and 25(OH)D concentrations increased for all participants, from a mean (± SD) concentration of 10.7 ± 4.9 to 25.2 ± 6.4 nmol/L for serum folate ($P = 0.003$), and 41 ± 21 to 66 ± 11 nmol/L for 25(OH)D ($P = 0.003$). Serum vitamin B_{12} levels did not change ($P = 0.386$) which may be due to a threshold effect since none of the participants had insufficient levels at baseline.

An advantage of using food fortification to treat micronutrient malnutrition is that compliance with the treatment is guaranteed, as long as the residents consume the food, whereas compliance with using vitamin/mineral supplements can be low. A two year double-blind, randomized control trial examined whether vitamin D supplementation can reduce the incidence of falls and fractures in elderly LTC residents (Flicker, MacInnis, Stein, Scherer, Mead, & Nowson, 2005). Institutional staff was responsible for providing the supplements to the study participants. The results of the study showed that 14% of the subjects had a compliance of 50% or less, which

demonstrates that even when supplements are included as part of the medical care of LTC residents, compliance can be low.

Using weighed food records performed by research assistants for dietary assessment was appropriate for this study since all of the participants except one were cognitively impaired, which would have made it very difficult to use any other dietary assessment method. The results of the dietary assessment showed that energy and protein intakes did not change while nutrient intakes significantly increased (Table 4.9). Energy intakes appeared to be quite consistent in this population at about 1200 kcal/day based on the current study and our previous study (Adolphe et al., 2006). Because elderly people in LTC consuming a pureed diet are often inactive, they may have lower energy requirements than those who are more mobile, but it is still important that their micronutrient needs are met. Since energy intakes did not change after the intervention, it appears that the fortified foods were as acceptable as the unfortified foods. However, the fortification trial was not designed to specifically look at food acceptability. Residents in the study were served consistent portions of food and were usually fed by a caregiver. These factors may have affected the amount that they ate so a definite conclusion about food acceptability cannot be made. Nevertheless, fortification appears to be a good solution to micronutrient malnutrition for this population.

Although dietary assessment is an important tool to use to determine if individuals and groups are ingesting sufficient levels of micronutrients, biochemical measurements are needed to determine the micronutrient status of an individual. Serum 25(OH)D, vitamin B_{12}, and folate were chosen as indicators of nutritional status in this study because not only are they vitamins of concern in this population, but there are biochemical tests available to determine their status (Health Canada, 2006). These

vitamins appear to be good indicators of nutritional status in this population and the increase in serum levels found in this study suggest that the bioavailability of vitamin D and folate is good from pureed meat and vegetables. Although an increase in serum vitamin B_{12} was not observed, likely due to adequate levels at baseline, the bioavailability of vitamin B_{12} from these foods should also be satisfactory.

There are several methods available to quantify serum folate, including microbiological methods, assays using folate-binding proteins, and chromatographic techniques, but chemiluminescence detection methods are currently the most commonly used (Owen & Roberts, 2003). The samples obtained in the present study were analyzed by a medical laboratory that used a Roche E170 electrochemiluminescence immunoassay. This system is based on the technology used by the older Roche Elecsys 2010 system (Wan, Augustin, Chan, Leblond, Verjee, & Adeli, 2005). A study by Owen & Roberts (2003) showed that of five automated folate analysis methods, the Elecsys 2010 and ARCHITECT I2000 demonstrated the best linearity. However, the study also showed that the Elecsys 2010 may have a higher degree of imprecision (16%) than some of the other methods (10%) at low folate concentrations. This could have possibly affected our baseline results since some of the baseline samples had low serum folate concentrations. However, the serum folate levels found at baseline are not surprising given the very low dietary folate intake in our sample at baseline (114 ± 58 μg/d).

Several methods are available to determine 25(OH)D concentrations. High-performance liquid chromatography (HPLC) is generally considered to be the gold standard method, but it is a cumbersome procedure that is not suited for high output clinical laboratories (Hollis & Horst, 2007). However, liquid chromatography-mass spectrometry (LC-MS), the method used in the present study, is a suitable alternative to

HPLC. When properly performed, LC-MS is a very accurate method and the fact that 25(OH)D is highly hydrophobic is not a major factor when using this technique (Hollis & Horst, 2007). One of the problems that may affect LC-MS results is that the method is unable to discriminate between 25(OH)D_3 and the inactive isomer 3-epi-25(OH)D_3. However, this is a problem primarily in newborns (Hollis & Horst, 2007) and thus should not have affected the results from our elderly study population.

A possible disadvantage of using chemiluminescence to determine serum vitamin B_{12} levels is that levels can be overestimated if the samples are not handled properly. One study found that using serum separator tubes resulted in elevated serum vitamin B_{12} levels if additional sample preparation (i.e. centrifugation) did not occur prior to analysis using a Bayer Centaur chemiluminescence method (Lowrey & Smith, 2003). However, in the study by Lowrey & Smith (2003), the samples were transported from distant locations which could result in changes in the sample from time of withdrawal to analysis, while in our study the samples were analyzed within the same city.

There is currently not a definitive cut-off value for vitamin B_{12}, folate, and vitamin D deficiencies. Current research is suggesting that cut-off values for these vitamins may be higher than previously thought. Optimal vitamin D status has recently been defined as a serum 25(OH)D value of $\geq$ 90 nmol/L (Bischoff-Ferrari et al., 2006; Heaney, 2006; Whiting et al., Submitted June 1, 2007). The lower limit for vitamin B_{12} may be as high as 258 pmol/L (L. H. Allen & Casterline, 1994) and for folate a value of 10-15 nmol/L may be ideal (Hultberg et al., 2002). Because the serum samples in the present study were analyzed in off-site medical laboratories, the cut-off values used to determine the participants' deficiency status were those provided by the laboratories. The reference value from the lab for serum folate (> 11.8 nmol/L) was within the ideal

range suggested in the literature but the lab reference values for serum vitamin B_{12} (normal ≥ 138 pmol/L) and 25(OH)D (normal ≥ 40 nmol/L) were lower than those reported in the literature. Using the values provided by the lab, the deficiency rate at baseline was zero for serum vitamin B_{12} and 55% for serum 25(OH)D and none of the participants were considered deficient for either vitamin after the intervention. However, if the higher cut-off values of 258 pmol/L for vitamin B_{12} and 90 nmol/L for 25(OH)D suggested in the literature were used to determine deficiency rates, the deficiency rates in the present study would have been 9% for serum vitamin B_{12} and 100% for serum 25(OH)D at baseline. Still using the higher cut-off values, the prevalence of serum vitamin B_{12} deficiency after the intervention would have decreased to zero while the prevalence of serum 25(OH)D deficiency would have remained at 100%. This demonstrates that determining the prevalence of deficiency using serum vitamin levels can be highly dependent on the reference values used.

Even when the higher cut-off value suggested in the literature is used for determining vitamin B_{12} deficiency, the prevalence of deficiency in the present study was low. This was surprising since the literature indicates a relatively high prevalence of cobalamin deficiency in the institutionalized elderly (Andres et al., 2004). There is no obvious explanation for this other than that our sample size was relatively small. One of the subjects had very high serum cobalamin levels due to a large daily oral dose of vitamin B_{12}. This may indicate a heightened awareness among the geriatricians, who care for the residents in this facility, that testing and subsequent treatment for cobalamin deficiency is important for this population. There is also the possibility that the methods used to analyze the serum samples overestimated the concentrations, as discussed above.

Although an improvement in serum 25(OH)D status was observed after the intervention, the fortification level was not high enough to achieve an optimal 25(OH)D status of ≥ 90 nmol/L in any of the participants. An additional 10 µg of vitamin D was added daily to the food. The mean difference in vitamin D intake from baseline to after the intervention (from fortification powder and other food sources) was 10.1 µg/day. This increased vitamin D intake resulted in a mean serum 25(OH)D level of 66 nmol/L after the intervention, an increase of 25 nmol/L from baseline. Other studies have also reported large increases in serum 25(OH)D in response to 10 µg of vitamin D supplementation. Ooms, Roos, Bezemer, van der Vijgh, Bouter, and Lips (1995) found an increase of 35 nmol/L (baseline 27 nmol/L; intervention 62 nmol/L) after one year of supplementation. A recent study by Holvik, Madar, Meyer, Lofthus, and Stene (2007) also found a similar response after four weeks of supplementation with 10 µg of vitamin D from a multivitamin tablet or fish oil capsule in subjects aged 19-49 years. Baseline serum 25(OH)D was 40.3 nmol/L in the multivitamin group and 48.5 nmol/L in the fish oil group. The mean 25(OH)D concentration increased by 36 nmol/L in the multivitamin group and 32 nmol/L in the fish oil group.

However, some studies have not shown such a large increase in 25(OH)D in response to modest vitamin D supplementation. A study by Viljakainen et al. (2006) found that a 10 µg vitamin D supplement increased serum 25(OH)D levels from 46.5 to 60 nmol/L in six weeks, a difference of only 13.5 nmol/L. This is likely not due to the short duration of the study since the authors reported that 25(OH)D levels reached a plateau at 6 weeks. Pfeifer, Begerow, Minne, Nachtigall, and Hansen (2001) found an increase of 39 nmol/L (baseline 26 nmol/L; intervention 65 nmol/L) after eight weeks of supplementation, but this resulted from supplementation with 20 µg of vitamin D. The

results from our study showed a 2.5 nmol/L increase of 25(OH)D per 1 μg of vitamin D. Heaney et al. (2003) reported on several studies for which the relationship between 25(OH)D and vitamin D supplementation could be calculated and found a range from 0.56 nmol/L per 1 μg of vitamin D to as high as 5.5 nmol/L per 1 μg of vitamin D. It is uncertain exactly what factors impact this relationship, though baseline 25(OH)D status appears to have an effect. Nevertheless, the results from the present study are situated within the range reported by Heaney et al. (2003).

Mean folate intake increased from 114 μg DFE/day at baseline to 505 μg DFE/day after the intervention, a difference of 391 μg DFE/day. Baseline mean serum folate concentration was 10.7 nmol/L which increased to 25.2 nmol/L after the intervention, a difference of 14.5 nmol/L. Other studies have found similar dose-responses to folic acid fortification. A study by van Vliet (2007) found similar responses to 400 μg of folic acid which was provided via a fortified spread that was consumed daily for six weeks. In males, serum folate concentrations increased from 15.8 nmol/L to 29.8 nmol/L, a rise of 14 nmol/L. Females showed an increase of 16.5 nmol/L, from a mean baseline level of 13.7 nmol/L to 30.2 nmol/L after the intervention. Venn et al. (2002) found that after four weeks of consuming a breakfast cereal fortified with 300 μg of folic acid, serum folate levels increased by 15 nmol/L, from a baseline concentration of 18 nmol/L to 33 nmol/L after the intervention.

There was a correlation between vitamin D intake and serum 25(OH)D at baseline (r_s = 0.857; P = 0.002) but not after the intervention (r_s = 0.444; P = 0.198). Although this study was originally planned to take place during the winter but was delayed due to unforeseen circumstances until the spring, to the best of our knowledge participants were not exposed to sunlight during the study. The correlations for serum

folate and folate intake, and serum vitamin B_{12} and vitamin B_{12} intake were not significant which may indicate confounding factors that affect these relationships. One possible confounder in this study was drug-nutrient interactions. As shown in Table 4.8, metformin, which was prescribed to one participant, is known to decrease vitamin B_{12} and folate status (Wulffele et al., 2003). Two participants were prescribed ranitidine, one of which was the participant also taking metformin, which is known to decrease vitamin B_{12} absorption (Pronsky, 2000).

5.3 IMPLICATIONS FOR DIETETIC PRACTICE

The feasibility of on-site fortification was assessed as part of this study. During the eight weeks of the fortification trial, a graduate student or research assistant visited the facility daily to add the fortification powder to the food. It took approximately one hour each day to fortify the four pureed foods. It is not feasible for foodservice staff to fortify the pureed foods because of the extra time required. It is also important, from a safety perspective, to ensure that the correct amount of fortification powder is added to the food. This can be difficult to guarantee in large institutional kitchens where different workers are continuously filling different work shifts. Figuring out the logistics of how and when to add the fortification powder during production can also be a challenge. It would be ideal for the food to be fortified during manufacturing. However, this would require facilities to purchase commercially-prepared pureed foods, and facilities that still make their own pureed foods may be resistant to this change.

The multivitamin offered to residents at Parkridge did not contain all vitamins and did not even contain folate. This was surprising to the investigators considering the wide variety of broad spectrum multivitamins currently available on the market.

Multivitamins may be used by physicians as reassurance that their patients are receiving adequate micronutrients. It is important for physicians and other health professionals to be aware that not all multivitamin preparations are the same. In addition, unless a multivitamin/mineral preparation is specifically requested in the physician's orders, minerals were not included in the micronutrient supplement offered at Parkridge Centre.

A community meal service model is used at Parkridge Centre whereby heated foodservice carts are used on each ward to serve the residents. This method offers several advantages over tray service. It allows residents to choose what they want to eat at the time of the meal instead of days, or even weeks, beforehand. It also allows residents to request an additional serving of food if desired. Observations made while the weighed food records were performed on two of the facility's wards was that many of the study participants were consuming all of their meals, yet the caregiver who was assisting the resident with eating did not return to the foodservice cart for an additional serving. Because the participants were unable to communicate their desire for more food, it is unknown whether or not the amount that they were receiving was adequate to satisfy their hunger, or if they would eat more if given the chance. Second helpings could increase their energy, protein, and micronutrient intakes.

5.4 LIMITATIONS

There are limitations to this study. Subjects were recruited from only one long-term care facility and the sample size was small. It was difficult to recruit a large sample size for this study for several reasons. Only one of the participants was able to consent for herself; family consented for the other participants. In some cases, it was difficult to get in contact with the family, and several family members did not want to provide

consent for various reasons. Another factor that resulted in the small sample size was the limitation of trying to recruit from as few wards as possible in order to make it feasible to perform the weighed food intakes.

Originally it was planned that meats and starches would be fortified so these were the products used in the sensory testing. However, for logistical reasons, vegetables were fortified in the fortification trial instead of starches. It is not anticipated that this would have affected the acceptance of the foods because vegetables tend to be more flavourful than starches and would likely mask any flavour changes due to fortification better than starches.

5.5 CONCLUSION

This study has provided the first evidence that vitamin fortification of pureed foods for long-term care residents is an effective method for improving nutritional status in a population that is at high risk for malnutrition. The positive results of this study provide evidence for food manufacturers to develop and seek regulatory approval for fortified pureed foods.

5.6 FUTURE RESEARCH

The fortification trial was a pilot study and a larger, multi-centre study needs to be performed. Since the pilot study determined that on-site fortification of pureed foods is quite labour intensive and not very practical, the larger study should use foods that have been fortified during the manufacturing process.

This study measured three biomarkers of nutritional status: serum 25(OH)D; serum vitamin B_{12}; and serum folate. Future studies should assess the effect of

fortification on other health indicators that are relevant to improving the health and quality of life of the elderly. Such indicators could include homocysteine, cognitive function, incidence of cardiovascular disease and cancer, quality of life, incidence of decubitus ulcers, and functional ability.

Based on the results of sensory testing, the fortification trial only used vitamins to fortify the pureed food. Since food intakes are low in this population, it would be ideal to be able to fortify using a broad spectrum of vitamins and minerals that does not result in detectable flavour changes. One possible way to achieve this is by using microencapsulated minerals. It may be especially important to use microencapsulated iron since it seems to negatively affect the flavour. However, microencapsulation would increase the cost of fortification and a cost feasibility study of using microencapsulated nutrients should be performed.

In the present study, vitamin K was excluded from the fortification powder. However, in future studies it may be valuable to consider adding vitamin K. It is important that the level of vitamin K consumed is consistent in people prescribed drugs such as warfarin (Rohde, de Assis, & Rabelo, 2007). Vitamin K supplementation in patients prescribed warfarin may help to improve anticoagulation control in patients with an unstable response to warfarin (Sconce, Avery, Wynne, & Kamali, 2007).

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APPENDIX A

Dietary Intake Data from Previous Long-Term Care Study

Table A1 Nutrient intake of long-term care residents consuming a pureed diet at baseline and at week eight.

	Nutrient Intake at Baseline (mean ± SD)	Nutrient Intake at Week Eight (mean ± SD)
Energy (kcal)	1340 ± 402	1244 ± 443
Protein (g)	49 ± 21	43 ± 15
Thiamin (mg)	*0.7 ± 0.3	*0.9 ± 0.4
Riboflavin (mg)	1.3 ± 0.5	1.3 ± 0.6
Niacin (mg)	*13 ± 5	*12 ± 4
Vitamin B_6 (mg)	*1.2 ± 0.7	*1.0 ± 0.5
Vitamin B_{12} (µg)	*2.0 ± 1.1	*1.8 ± 0.9
Vitamin C (mg)	95 ± 60	*89 ± 51
Vitamin A (µg RAE)	*874 ± 1250	*354 ± 227
Vitamin D (µg)	*5 ± 7	*6 ± 4
Vitamin E (mg)	*1 ± 1	*1 ± 3
Folate (µg DFE)	*164 ± 103	*170 ± 103
Pantothenic Acid (mg)	*3 ± 2	*3 ± 2
Calcium (mg)	*719 ± 313	*745 ± 288
Iron (mg)	8 ± 5	9± 4
Zinc (mg)	*4.7 ± 2.6	*4.6 ± 2.0
Potassium (mg)	*2108 ± 859	*1711 ± 668
Magnesium (mg)	*122 ± 64	*122 ± 63

Abbreviations: RAE, retinol activity equivalent; DFE, dietary folate equivalent.
*Indicates mean vitamin/mineral intake is below the Recommended Dietary Allowance or Adequate Intake (highest value for males and females >70 years).

APPENDIX B

Nutrient Intake from Food and Supplements for Individual Participants

Table B1(a) Mean (±SD) vitamin intakes at baseline and 8 weeks after intervention from food and daily vitamin supplement intake per subject (subjects 1-6).

	1	2	3	4	5	6
Thiamin (mg)						
Baseline	0.7 ± 0.2	1.3 ± 0.5	1.0 ± 0.1	0.7 ± 0.1	0.8 ± 0.8	ND
Intervention	1.6 ± 0.2	1.9 ± 0.3	1.9 ± 0.2	1.6 ± 0.2	1.5 ± 0.4	ND
Supplement	3	-	-	-	-	-
Riboflavin (mg)						
Baseline	1.2 ± 0.2	1.7 ± 0.2	1.7 ± 0.1	1.2 ± 0.1	0.7 ± 0.3	ND
Intervention	2.0 ± 0.6	2.6 ± 0.5	2.8 ± 0.2	2.2 ± 0.1	1.5 ± 0.2	ND
Supplement	2.5	-	-	-	-	-
Niacin (NE)						
Baseline	18 ± 1.0	24 ± 2.9	22 ± 2.0	20 ± 1.2	13 ± 2.9	ND
Intervention	23 ± 9	26 ± 7	25 ± 2	23 ± 5	17 ± 6	ND
Supplement	20	-	-	-	-	-
Vitamin B_6 (mg)						
Baseline	1.3 ± 0.2	1.5 ± 0.3	1.6 ± 0.5	1.5 ± 0.3	1.9 ± 1.5	ND
Intervention	3.1 ± 0.6	2.5 ± 0.6	3.3 ± 0.5	2.5 ± 0.4	1.8 ± 0.2	ND
Supplement	1	-	-	-	-	-
Vitamin B_{12} (µg)						
Baseline	3.5 ± 0.5	4.4 ± 0.4	4.4 ± 0.3	3.5 ± 0.5	1.7 ± 0.7	ND
Intervention	4.3 ± 0.4	5.5 ± 0.7	5.7 ± 0.8	4.7 ± 0.7	3.0 ± 0.4	ND
Supplement	3(MV) + 1200	-	-	-	-	-
Vitamin C (mg)						
Baseline	139 ± 113	209 ± 72	208 ± 53	183 ± 105	226 ± 92	ND
Intervention	229 ± 28	318 ± 52	267 ± 25	208 ± 69	177 ± 24	ND
Supplement	50	-	-	-	-	-
Vitamin D (µg)						
Baseline	0.5 ± 0.4	5.8 ± 0.7	3.9 ± 2.2	3.2 ± 0.7	0.2 ± 0.04	ND
Intervention	9.5 ± 3.1	14 ± 2.8	13 ± 1.8	12 ± 0.7	7.1 ± 1.3	ND
Supplement	10	-	-	-	-	-
Folate (µg DFE)						
Baseline	105 ± 26	189 ± 63	166 ± 15	105 ± 14	118 ± 104	ND
Intervention	485 ± 110	528 ± 126	568 ± 50	491 ± 43	426 ± 74	ND
Supplement	-	-	-	-	-	-
Pantothenic Acid (mg)						
Baseline	2.9 ± 0.2	4.7 ± 1.0	4.5 ± 0.5	3.3 ± 0.3	2.2 ± 1.2	ND
Intervention	5.0 ± 1.0	6.7 ± 1.1	6.7 ± 0.7	5.6 ± 0.5	4.2 ± 0.5	ND
Supplement	-	-	-	-	-	-

Abbreviations: DFE, dietary folate equivalent; NE, niacin equivalents; MV, multivitamin; ND, not determined.

Table B1(b) Mean (±SD) vitamin intakes at baseline and 8 weeks after intervention from food and daily vitamin supplement intake per subject (subjects 7-10).

	7	8	9	10	11
Thiamin (mg)					
Baseline	0.6 ± 0.28	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.04	0.7 ± 0.07
Intervention	1.6 ± 0.1	1.7 ± 0.3	1.7 ± 0.01	1.3 ± 0.4	1.9 ± 0.3
Supplement	-	-	-	3	3
Riboflavin (mg)					
Baseline	0.8 ± 0.08	1.4 ± 0.4	1.1 ± 0.3	1.1 ± 0.2	0.9 ± 0.2
Intervention	2.2 ± 0.2	2.1 ± 0.2	2.2 ± 0.3	2.2 ± 0.3	2.1 ± 0.3
Supplement	-	-	-	2.5	2.5
Niacin (NE)					
Baseline	14 ± 1.8	18 ± 0.9	17 ± 3.3	26 ± 4.9	14 ± 2.0
Intervention	23 ± 2.8	23 ± 4	22 ± 4.0	31 ± 2.7	21 ± 2.8
Supplement	-	-	-	20	20
Vitamin B_6 (mg)					
Baseline	1.2 ± 0.5	1.9 ± 1.1	1.5 ± 0.5	1.4 ± 0.4	1.8 ± 0.9
Intervention	2.7 ± 0.2	2.9 ± 0.4	3.0 ± 0.6	2.6 ± 0.5	2.8 ± 0.4
Supplement	-	-	-	1	1
Vitamin B_{12} (µg)					
Baseline	2.4 ± 0.9	3.6 ± 1.3	2.8 ± 0.3	5.1 ± 0.8	2.7 ± 0.2
Intervention	5.1 ± 1.1	4.8 ± 0.9	4.9 ± 1.1	7.7 ± 0.9	5.0 ± 1.4
Supplement	-	-	-	3	3
Vitamin C (mg)					
Baseline	94 ± 53	112 ± 30	171 ± 36	63 ± 39	110 ± 10
Intervention	177 ± 24	231 ± 76	279 ± 82	148 ± 74	200 ± 47
Supplement	-	-	-	50	50
Vitamin D (µg)					
Baseline	0.4 ± 0.1	0.6 ± 0.3	1.2 ± 1.1	4.8 ± 0.7	0.5 ± 0.3
Intervention	13 ± 3.9	14 ± 3.9	13 ± 3.3	14 ± 2.5	12 ± 4.2
Supplement	-	-	-	10	10
Folate (µg DFE)					
Baseline	81 ± 43	126 ± 27	106 ± 34	45 ± 20	92 ± 77
Intervention	520 ± 64	565 ± 36	501 ± 54	424 ± 146	547 ± 59
Supplement	-	-	-	-	-
Pantothenic Acid (mg)					
Baseline	2.1 ± 0.3	3.3 ± 0.3	2.8 ± 0.9	3.2 ± 1.1	2.0 ± 0.1
Intervention	5.7 ± 0.2	6.0 ± 0.9	5.7 ± 0.7	6.3 ± 0.7	5.2 ± 1.0
Supplement	-	-	-	-	-

Abbreviations: DFE, dietary folate equivalent; NE, niacin equivalents; ND, not determined.

APPENDIX C

Research Ethics Board and Saskatoon Health Region Confirmation of Approval

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB)

13-Mar-2006

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Wendy J. Dahl	Pharmacy and Nutrition	05-196

INSTITUTION (S) WHERE RESEARCH WILL BE CARRIED OUT

University of Saskatchewan

Saskatoon SK

SUB-INVESTIGATOR(S)

R. T. Tyler

SPONSORING AGENCIES

SASKATCHEWAN PULSE GROWERS

TITLE:

Development and Commercialization of Pulse-Based and Nutrient-fortified Pureed Foods

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
13-Mar-2006	01-Mar-2007	Revised Researcher's Summary, inclusive of Appendix A (28-Feb-2006) Revised Participant Information and Consent Form, University of Saskatchewan Staff and Students (09-Mar-2006) Revised Participant Information and Consent Form, Parkridge residents (09-Mar-2006)

CERTIFICATION

The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION

In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon, SK S7N 5C8
Phone: (306) 966-4053 Fax: (306) 966-2069

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 08-Dec-2006

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Wendy J. Dahl	Pharmacy	06-194

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Parkridge Centre
110 Gropper Cresent
Saskatoon SK S7M 5N9

SUB-INVESTIGATOR(S)
Susan J. Whiting

STUDENT RESEARCHER(S)
Jennifer L. Dunne

SPONSORING AGENCIES
LEISUREWORLD AND PRIVATE RECIPES LTD.
UNIVERSITY OF SASKATCHEWAN

TITLE
Effect of Fortrified Pureed Foods on Nutrient Intakes and Serum Vitamin Levels in Long-Term Care Residents

APPROVAL DATE	STUDY APPROVAL EXPIRY	APPROVAL OF
08-Dec-2006	07-Dec-2007	Amended Researcher's Summary (28-Nov-2006) Amended Participant Information and Consent Form (28-Nov-2006) Change in Research Site

Full Board Meeting ☐
Delegated Review ☒

Date of Full Board Meeting:

CERTIFICATION
The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION
In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

Michel Desautels, Ph.D., Chair
University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:

Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon SK S7N 5C8
Telephone (306) 966-4053 Fax: (306) 966-2069

Saskatoon Health Region

Research Services Unit
Strategic Health Information & Planning Services (SHIPS)
Joanne Franko, Manager
Suite 300 Saskatoon Square
410 22nd St E
Saskatoon, SK S7K 5T6
Phone: 306.655.3356 Fax: 306.655.3373

DATE: **January 4, 2007**

TO: **Dr. Wendy Dahl, Food and Nutrition Services, RUH**

FROM: **Joanne Franko**
Manager, Research Services Unit

RE: **RESEARCH PROJECT ETHICS COMMITTEE (EC)#: 2006-194**
PROJECT NAME: Effect of Fortified Pureed Foods on Nutrient Intakes and Serum Vitamin Levels in Long-Term Care Residents
PROTOCOL #: N/A

Saskatoon Health Region is pleased to provide you with operational approval of the above-mentioned research project.

Please advise me when the data collection phase of the research project is completed. I would also appreciate receiving a summary of the results for this research project. As well, any publications or presentations that result from this research should include a statement acknowledging the assistance of Saskatoon Health Region.

I would like to wish you every success with your project. If you have any questions, please contact our office at 655-3351.

Yours truly,

Joanne Franko

Joanne Franko, M.Sc.
Manager, Research Services Unit

cc: Karen Nogier, Laboratory Medicine, RUH
Maureen Beisel, Manager, Resident Care Services, PRC
Noella Leydon, Director, Food and Nutrition Services, Corp

APPENDIX D

Participant Consent Forms for Sensory Testing and Fortification Trial

Participant Information and Consent Form (University Staff and Students)
March 09, 2006

Study Title: Development and Commercialization of Pulse-based and Nutrient -Fortified Pureed Foods

Principle Investigator: Dr. W. Dahl, Adjunct Professor, College of Pharmacy and Nutrition

Sub-investigator: Dr. R. Tyler, Professor and Associate Dean (Academics), College of Agriculture.

Funded by: Saskatchewan Pulse Growers and the NRCC Industrial Research Assistance Program.

Assisting with this study may help to develop new, nutrient-dense pureed foods. If you are a student, staff or faculty member at the University of Saskatchewan, and you decide not to participate or to later withdraw your consent, your decision will not affect your academic standing, employment, promotion or the services you could otherwise expect to receive at the University.

Participation in this study is voluntary and you have the right to refuse participation or if you volunteer, to withdraw from the study at anytime. If you do not wish to participate, you do not have to provide any reason for your decision nor will you lose the benefit of any medical care to which you are entitled or are presently receiving.

Purpose of the Study:

The purpose of this study is to determine the sensory acceptability of pulse-based and nutrient-fortified pureed foods.

Benefits: There may be no direct benefit for participating in this study.

Procedures:

1. You will be asked to participate in a maximum of five, 20 minute sensory (taste-testing) sessions where you will sample a variety of pulse-based or nutrient-fortified pureed foods on several different days. You will be asked to taste three foods per session. If there are any foods that you do not want to sample, you can decline to do so.

2. You will be asked evaluate the aroma, taste, mouth-feel, ease of swallowing and general acceptability of the pureed foods. A written questionnaire will be used to record sensory characteristics.

Sensory evaluation will be carried out in the Sensory Laboratory, College of Agriculture.

Confidentiality:

While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team.

Participation is Voluntary:

Your participation in this study is entirely voluntary. You have the right to refuse participation and to withdraw from the study at any time, for any reason. Early withdrawal from the study will not result in any sort of penalty.

Potential Risks:

There are no foreseen risks associated with study. Individuals with food allergies will not be able to participate in this study. As with any intervention, there may be unforeseen risks.

Research Related Injury:

There will be no costs to you for your participation in this study. You will not be charged for any research procedures. In the event that you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no cost to you.

Compensation

Sensory participants will be provided with $5.00 University of Saskatchewan Bookstore gift certificates for each sensory session.

Contacts:

If you have any questions with regards to this research project, please do not hesitate to contact the researchers below:

Dr. Wendy Dahl 655 1310
Dr. Robert Tyler 966 4064

This study has been approved, on ethical grounds, by the Biomedical Research Ethics Board (Bio-REB) of the University of Saskatchewan. If you have any questions about your rights as a research subject, you may contact the chair of the Biomedical Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966 4053.

The contents of this consent form have been explained to me. I have been able to ask questions about the study and these questions have been answered to my satisfaction. I have received a copy of the consent form for my own records. I freely consent to participate in this study. I am not waiving any of my legal rights by signing this consent form.

Do you have a food allergy? ____ yes ____no

SIGNATURES

Study Volunteer: _____Date: _____

Research Coordinator: _____ Date: _____

Participant Information and Consent Form **November 28, 2006**

Study Title: Effect of fortified pureed foods on nutrient intakes and serum vitamin levels in long-term care residents.

Principal Investigator: Dr. Wendy J. Dahl is investigating the effect of vitamin fortification of pureed food on nutrient intakes and serum levels of vitamins in long-term care residents on a pureed diet.

Wendy Dahl RD PhD, Adjunct Professor
College of Pharmacy and Nutrition, University of Saskatchewan
110 Science Place, Saskatoon, SK, S7N 5C9

Sub-investigators: Dr. Susan Whiting, Professor and Jennifer Dunne, Graduate Student, College of Pharmacy and Nutrition, University of Saskatchewan.

You are being asked to take part in this research because you are a resident in a long term care home and on a pureed diet. Participation in this study is voluntary and you have the right to refuse to participate. If you volunteer, you can withdraw from the study at anytime. If you do not wish to participate, you do not have to provide any reason for your decision, nor will you lose the benefit of any care to which you are entitled or are at present receiving.

In this consent form "you" always refers to the patient. If you are an authorized representative, please remember that 'you' refers to the patient. An authorized representative in this study would be the person who is entrusted by a competent patient/subject to make all the decisions around the study for the patient provided the patient gives consent to the general understanding of the clinical trial and authorizes the representative to make ongoing decisions when he/she (the patient) cannot.

Purpose of the Study:

Residents of long term care homes have high nutritional needs, but often consume small amounts of food, particularly if they have swallowing problems and are on a pureed diet. The purpose of this study is to determine whether adding vitamins to usual pureed foods improves intake of vitamins as well as improving the nutritional status of those consuming the pureed foods.

Benefits: There may not be direct benefits to you for participating in this study. Knowledge gained from this study may help to improve nutritional care for long term care residents with swallowing disorders.

Procedures:

1. You will be required to provide 5 ml venous blood samples, two at the beginning of the study and two at the end of the study. A Certified Laboratory Technologist will collect the blood samples. Your blood samples will be tested for the levels of vitamin B_{12}, folate and vitamin D.

2. Your facility medical chart will be reviewed to determine what vitamin and mineral supplements you are currently prescribed. The chart review will be carried out by the research coordinator.

3. You will consume your regular pureed diet for eight weeks. However, some of the pureed foods will be have added vitamins.

4. Your food intake will be measured for three days at the beginning of the study and three days at the end of the study. Food placed on your plate will be weighed before it is served. After you have eaten, any food remaining on your plate will be weighed once your plate is removed from the table.

Care will be taken throughout the three days of the food intake weighing, that your meals will notbe delayed. Your time commitment for this study is estimated at about two hours, as it is expected that only the venous blood sample will take your time.

Confidentiality:

While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that theinformation you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available toanyone except the research team. It is possible that that the research team may wish to presentthe results of this study in scientific journals or at conferences and workshops, but your identity will not be revealed.

Participation is Voluntary:

Your participation in this study is entirely voluntary. You have the right to refuse to participate and to withdraw from the study at any time, for any reason. Early withdrawal from the study will not result in any sort of penalty.

Potential Risks:

The risk of drawing blood include: temporary discomfort from the needle stick, bruising and rarely, infection.

Research Related Injury:

There will be no costs to you for participation in this study. You will not be charged for any research procedures and participating in this study will not affect your care. In the event that you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you.

Compensation:

You will be provided $50 for participation in this research study.

Contacts:

If you have any questions with regards to this research project, please do not hesitate to contact the researchers below:

Jennifer Dunne	(306) 717 1848
Wendy Dahl	(306) 655 1310
Susan Whiting	(306) 966 5837

This study has been approved, on ethical grounds, by the Biomedical Research Ethics Board (Bio-REB) of the University of Saskatchewan. If you have any questions about your rights as a research subject, you may contact the chair of the Biomedical Ethics Board, c/o the Ethics Office, University of Saskatchewan at (306) 966 4053.

The contents of this consent form have been explained to me. I have been able to ask questions about the study and these questions have been answered to my satisfaction. I have received a copy of the consent form for my own records. I freely consent to participate in this study. I am not waiving any of my legal rights by signing this consent form.

SIGNATURES

Participant: _____ Date: _____

Authorized Representative _____ Date: _____

Relationship of the Authorized Representative to the Participant: _____

Research Coordinator: _____ Date: _____