CORRELATES AND CONSEQUENCES OF VITAMIN D STATUS IN OLDER PEOPLE

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ABSTRACT

It is well established that vitamin D contributes to bone health. New evidence from cross-sectional and prospective cohort studies and a few clinical trials suggest that vitamin D status may play a role in functional capacity declining with age; however, there are some gaps in these studies yet to be examined. Little is known about vitamin D status in healthy, free-living, older people in North America. Older individuals may be more vulnerable due to low vitamin D intake and limited endogenous synthesis. Thus, the objectives of this thesis were to (i) determine the distribution of serum 25 hydroxy vitamin D [25(OH)D] concentrations in healthy older people living in Québec and determine how season, age, sex and supplement consumption affect this distribution; (ii) determine to what extent vitamin D intake from foods, supplements and proxy measure of sunlight exposure explain the variation in serum 25(OH)D concentrations, controlling for the effects of age and sex; and determine the dietary predictors of optimal concentration of 25(OH)D; and (iii) examine whether vitamin D status can predict change in functional decline capacity over 1 and 2 years, controlling for season and other potential confounders. Data for this study have been obtained from a random sampling of 405 participants from the NuAge cohort study of 1793 independently-living men and women aged 68 to 82 years at baseline. The NuAge sample is a stratified sample of participants in three age categories 70 ± 2 years, 75 ± 2 years and 80 ± 2 years with approximately equivalent numbers of men and women in each group. For objective (i), a cross-sectional design was applied. Serum 25(OH)D was assessed using radioimmunoassay. Data were analyzed controlling for age, sex, season and other potential confounders. For objective (ii), six 24-hour recalls were obtained for the same

subjects as the first study also in a cross-sectional design. In addition to nutrient intake, foods were grouped into 6 food groupings and one group for vitamin/mineral supplements. Vitamin D intakes from foods and supplements were determined across the 3 vitamin D status categories. For objective (iii) we applied a longitudinal study design to measure the association of vitamin D status and change in physical decline expressed as a sum of scores of the 4 physical capacity tests over 1 and 2 years, controlling for a number of potential confounders. Results showed that healthy community-dwelling older people of Québec have a low prevalence of vitamin D deficiency. Season, vitamin D supplement consumption and vitamin D from food were clear predictors of vitamin D status, but age, sex and adiposity were not. Healthy older people with optimal vitamin D status had average intakes of 14.1 µg/ day from food and supplements. Fortified milk was the main food contributor to vitamin D intake, but food alone was not enough to meet Adequate Intakes recommended for vitamin D requirement. There was no association of vitamin D status with declines in functional status in this healthy older population.

RÉSUMÉ

Il est bien établi que la vitamine D contribue à la santé osseuse. Des données récentes d'études transversales, de cohortes prospectives et d'essais cliniques suggèrent que la vitamine D pourrait jouer un rôle dans la diminution des capacités fonctionnelles avec l'âge; toutefois, certaines lacunes de ces études n'ont toujours pas été étudiées. On sait peu de choses au sujet des niveaux de vitamine D chez les personnes âgées autonomes, en bonne santé, vivant en Amérique du Nord. Ces personnes peuvent être plus vulnérables aux carences d'une part à cause d'un faible apport en vitamine D et, d'autre part, à cause d'une synthèse endogène limitée. Ainsi, les objectifs de cette thèse étaient de (i) déterminer la distribution des concentrations sériques de 25 hydroxy vitamine D [25 (OH) D] chez les personnes âgées en bonne santé, vivant au Québec et de déterminer comment la saison, l'âge, le sexe et la consommation de suppléments affectent cette distribution; (ii) de déterminer dans quelle mesure l'apport alimentaire de vitamine D, l'apport de suppléments et la saison peuvent expliquer les variations des concentrations sériques de 25 (OH) D, en contrôlant pour les effets de l'âge et du sexe, ainsi que de déterminer l'apport alimentaire idéal de vitamine D pour obtenir un niveau optimal de 25 (OH) D; et, finalement (iii) d'évaluer l'effet de l'état nutritionnel en vitamine D sur la diminution des capacités fonctionnelles pendant 1 et 2 ans, tout en prenant en compte la saison et d'autres variables de confusion potentielles. Cette étude repose sur des données obtenues auprès d'un échantillon aléatoire de 405 participants de l'étude de cohorte NuAge, composée de 1793 hommes et femmes âgés entre 68 et 82 ans au départ, vivant de façon indépendante. Cet échantillon est stratifié selon trois catégories d'âge: 70 ± 2 ans, 75 ± 2 ans et 80 ± 2 ans, avec des nombres comparables d'hommes et de femmes

dans chaque groupe d'âge. Pour l'objectif (i), une étude transversale a été menée. Les taux sériques de 25 (OH) D ont été évalués à l'aide d'un test radio-immunologique, et ont été ajustés pour l'âge, le sexe, la saison et d'autres variables de confusion. Pour l'objectif (ii), six rappels de 24 heures ont été obtenus des mêmes sujets. En plus d'étudier l'apport de nutriments, les aliments ont été regroupés en 6 groupes alimentaires et un groupe pour les suppléments de vitamines et minéraux. L'apport de vitamine D provenant des aliments et des suppléments a été déterminé pour les 3 catégories de statut en vitamine D. Pour l'objectif (iii) nous avons mesuré l'association entre le statut en vitamine D et le déclin fonctionnel, exprimé par la somme de 4 tests de capacité physique, à l'aide d'analyses longitudinales de 1 et 2 ans ajustées pour plusieurs variables de confusion. Les résultats ont montré que la prévalence des carences en vitamine D est faible chez les individus âgées, en bonne santé et vivant dans la communauté au Québec. La saison, la consommation de suppléments et l'apport alimentaire sont de bons facteurs pour estimer l'état nutritionnel en vitamine D alors que l'âge, le sexe et le niveau d'adiposité ny'étaient pas associés. Les personnes âgées avec un taux optimal de vitamine D avaient un apport moyen de 14,1µg/jour, provenant de sources alimentaires et de suppléments. La principale source de vitamine D était le lait fortifié, mais les aliments seuls ne suffisaient pas pour atteindre les apports adéquats recommandés de vitamine D. Il n'y avait aucune association entre l'état nutritionnel en vitamine D et la diminution de l'état fonctionnel dans une population âgée en bonne santé.

ADVANCE OF SCHOLARLY KNOWLEDGE

1. Original contribution to knowledge

This doctoral dissertation is the first study in Canada to examine the free living healthy elderly, with precise and comprehensive dietary intake data, and functional performance measures.

We were able to establish the distribution of vitamin D status in a large population of healthy community-dwelling older men and women in Québec. This study also was able to capture habitual intakes accurately by means of using six 24-hour recalls. It was clearly shown that the intakes of those within optimal status are close to the Adequate Intake (AI) set by the DRI committee, but cannot be achieved without supplementation. Food groups that contribute most importantly to vitamin D intake were identified. Moreover, this thesis was able to demonstrate the lack of association between vitamin D status and physical functional decline in healthy older people.

2. Research publications in refereed scientific journals

Barake, R., Weiler, H., Payette, H., Gray-Donald, K. (2009). Vitamin D status in healthy free living elderly men and women living in Québec - Canada. *J Am Coll Nutr* (in press). (Manuscript 1)

3. Research Manuscript submitted to refereed journals

Barake, R., Weiler, H., Payette, H., Gray-Donald, K. Requirements to achieve optimal vitamin D status in the elderly. (Submitted to *J Nutr*) (Manuscript 2)

Barake, R., Weiler, H., Payette, H., Gray-Donald, K. Does vitamin D status predict decline in functional performance and muscle strength in healthy elderly population in Québec? (Submitted to *Eur J Clin Nutr*) (Manuscript 3)

5. Abstracts and presentations

Barake, R., Weiler, H., Payette, H., Gray-Donald, K. (2009). Does vitamin D status predict decline in functional status? *J Nut Health Aging*. The 19th International Association on Gerontology and Geriatrics (IAGG) - July 4 – 9 2009; Paris, France. (Oral presentation)

Barake, R., Weiler, H., Payette, H., Gray-Donald, K. (2009). Does vitamin D status predict decline in functional performance and muscle strength in a healthy elderly population in Québec? *Appl Physiol Nutr Metab*. 34 (3): 549. CSCN –CSSN Annual Scientific Meeting - May 30 – June 2 2009, Québec City, Canada. (Poster)

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Barake, R., Weiler, H., Payette, H., Gray-Donald, K. (2008). Predictors of vitamin D status in healthy elderly Quebecers. *Osteoporos Int.* 19 (Suppl 2): S271. International Osteoporosis Foundation (IOF), the world Congress on Osteoporosis - Dec 3 – 7 2008, Bangkok, Thailand. (Poster)

Barake, R. "Meeting with the NuAge team researcher" as part of "The NuAge Knowledge Translation Workshop" June 2008, Sherbrooke, Canada. (Oral presentation, invited speaker)

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Barake, R. "Vitamin D and the Elderly". April 2007, Sherbrooke, Canada. (Oral presentation, invited speaker)

CONTRIBUTION OF AUTHORS TO MANUSCRIPTS

For manuscript one, the candidate undertook the 25(OH)D analysis. The candidate analyzed all serum 25(OH)D samples in duplicate as well as assessed agreement and repeated analyses required. The candidate developed the research questions, verified the data, conducted all statistical analyses and wrote the manuscript. For manuscript 2, the candidate was involved in entering 24-hour recall data, cleaned data, updated information required to develop food groupings, performed statistical analyses, interpreted data, and wrote the manuscript. For the third manuscript, the candidate set the data ready by creating quintile cut-offs for 4 functional score measures and 3 muscle strength measures as well as a creating a total functional score in order to identify decliners from non decliners. The candidate performed all the statistical analyses, interpreted the data and wrote the manuscript.

Dr. Katherine Gray-Donald, the candidate's supervisor, a co – principal investigator on the NuAge longitudinal study, and the principal investigator (PI) on a grant secured from the Dairy Farmers of Canada (DFC) which funded work for this thesis, contributed to the development of the concepts and designs of the 3 studies. Dr. Gray-Donald supervised statistical analysis and interpretation of data for all manuscripts. She also helped in writing and edited the manuscripts presented in this thesis.

Dr. Hope Weiler, the candidate's committee member, co – investigator on a grant secured from DFC, was involved in training and supervising the candidates laboratory

work analyzing serum 25(OH)D. Dr. Weiler also helped with study design, interpretation of data as well as editing all manuscripts.

Dr. Hélène Payette, the candidate's committee member, PI for the NuAge Longitudinal Study and co – investigator on a DFC grant that funded this study, helped with study design, interpretation of data and editing all manuscripts.

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I dedicate this thesis to my parents Wijdan and Ismail Barake, my dear sister and brother Reem and Adib and my lovely niece and nephew Nada and Bader,

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LIST OF ABBREVIATIONS

25(OH)D: 25 hydroxy vitamin D

AI: Adequate Intake

BMD: Bone mineral density

BMI: Body Mass Index

CCHS: Canadian Community Health Survey

CHMS: Canadian Health Measures Survey

CDF: Canadian Dairy farmers

CI: Confidence Interval

CIHR: Canadian Institutes of Health Research

CPBA: Competitive protein binding assay

DBP: Vitamin D binding protein

DXA: Dual energy x - ray absorptiometry

DIN: Drug Identification Numbers

DRI: Dietary Requirement Intake

ELISA: Enzyme – linked immunosorbent assay

EU: European Union

FFQ: Food frequency questionnaire

GDS: Geriatric Depression Scale

HPLC: High performance liquid chromatography

IU: International Unit

kPa: Kilopascal

LASA: Longitudinal Aging Study Amsterdam

LC – MS/MS: Liquid chromatography – tandem mass spectrometry

µg: Microgram

mg: Milligram

MS: Multiple sclerosis

NHANES: National Health and Nutrition Examination Survey

OR: Odds ratio

OPRA: Osteoporosis Prospective Risk Assessment Study

PASE: Physical Activity Scale for the Elderly questionnaire

PTH: Parathyroid hormone

RAMQ: Québec health insurance registry

RIA: Radioimmunoassay

RCT: Randomized clinical trial

RR: Relative risk

SD: Standard deviation

SOF: Study of Osteoporotic Fractures

SPF: Specific protective factor

TUG: Timed "Up and Go"

UK: United Kingdom

UL: Upper limit

US: United States

UVB: Ultra violet blue

VDR: Vitamin D receptors

WHAS: Women's Health and Aging Study

CHAPTER 1. INTRODUCTION

1.1 Background and rationale

One out of every seven Canadians is a senior citizen. Seniors aged 65 years and over have surpassed the 4 million mark in Canada and comprise 13.7% of the total population, (Statistics Canada 2006). The rate of increase is projected to reach 21.2% in the year 2026. In 2001 only 2.2% of Canadian elderly aging from 65 - 74 years were institutionalized (Statistics Canada, 2006). The percentage rose to 22.6% in men and 35.4% in women ≥ 85 years, clearly showing that with longevity comes increased risk of several chronic diseases including osteoporosis as well as falls, frailty and increased risk of hospitalization. In the US approximately 30% of people > 65 years fall each year; causing moderate to severe injuries that may lead to disabilities, hospitalization, early nursing home admission, and death (CDC, 2001; Stevens et al., 2006). An estimated overall national saving of 138 million annually may be achieved by reducing falls by 20% (Public Health Agency of Canada, 2005). The cost of treating the 1.4 million Canadians suffering from osteoporosis, one of the main reasons for fall related injuries, may amount to 1.3 billion dollars yearly (Osteoporosis Canada, 2007).

Vitamin D is becoming heavily targeted as a key nutrient in achievement of health and prevention of many chronic diseases including osteoporosis, cancer (colon, prostate, breast), diabetes, coronary heart disease and hypertension, and autoimmune diseases (Whiting & Calvo, 2005). Vitamin D status investigations are of concern due to the fact that vitamin D deficiency and insufficiency are becoming recognized as a global

pandemic affecting all sectors of the population, particularly in people residing in latitudes above 40° (Institute of Medicine, 1997). Older people are even more prone to develop vitamin D deficiency not only because of their challenged dermal synthesis of vitamin D (Lips, 2006; Maclaughlin & Holick, 1985), but also due to tendency of wearing concealing clothes and use of sunscreen lotions. Additionally, dietary intake of vitamin D may also be limited in older populations due to limited dietary sources, types of medications consumed that may interfere with vitamin D metabolism and reduced total food intake (Holick, 2006).

It is noteworthy to mention that dietary vitamin D sources are scarce. Vitamin D can be found in fatty fish such as salmon, herring as well as fish oil. Egg yolk and organ meats contain small amounts of vitamin D. Vitamin D fortified milk, yoghurt, butter, margarine and orange juice are considered good sources of vitamin D. However, fortification policies, if present, differ from one country to another making this route for vitamin D consumption an unreliable one. Vitamin D supplements are also considered a major source of vitamin D intake.

In Canada deficiency in vitamin D status is found in approximately 34% of Calgary residents ~ 64 years of age, mostly women recruited from the Canadian Multicenter Osteoporosis Study, who do not use supplements (Rucker et al., 2002). Some data exist for institutionalized elderly and are suggestive of low dietary intakes of vitamin D and low UVB light exposure (Lee et al., 2002; Liu et al., 1997) and hence poor vitamin D status despite a controlled health care environment. However, vitamin D status of community dwelling seniors is far less clear.

Aging is accompanied by changes in dietary patterns and reduced intake of nutrients due to medical, psychological, lifestyle and social factors leading to nutritional inadequacy (Hickson, 2006; Payette et al., 1995; Shciffman, 1997). Low levels of protein and energy intake were reported in 70% of frail elderly (Gray-Donald et al., 1994). Micronutrient deficiencies have also been described; 23%-72% of an elderly Canadian population had reported intakes of folate, vitamin D, calcium, magnesium and zinc of < 2/3 of recommended intakes (Payette et al. 1991). In terms of foods in Canada, low reported intakes of milk products have been reported (Keller & Hedley, 2002). For milk products, 79% of men and 84% of women of this age did not consume the minimum recommended number of servings. Average servings of milk products were 1.36 for men and 1.24 for women. Thus consuming less milk, a rich source of vitamin D, may lead to vitamin D deficiency.

Very little is known about vitamin D dietary intake patterns and consumption for the aging population over 70 years of age. The Canadian Osteoporosis Society recommends 20 μg (800 IU) of vitamin D_3 per day (Brown & Josse, 2002) and the Dietary Recommended Intake (DRI) value for vitamin D in men and women > 70 years (not including exposure to sunshine) is 15 μg (600 IU) of vitamin D_3 per day (Institute of Medicine, 1997). Over the last 9 years, nutritional scientists have acknowledged that the Adequate Intake value for vitamin D for all ages was set too low, as evidenced by dedication of the December 2004 *American Journal of Clinical Nutrition* supplement issue to this topic. Subsequently, in an editorial in *Osteoporosis International*, experts from the USA, Canada and Europe recommend an intake of 20 to 25 μg (800 to 1000 IU)

vitamin D per day to prevent fractures (Dawson-Hughes et al., 2005). Another editorial in the *American Journal of Clinical Nutrition* was in agreement with this recommendation (Vieth et al., 2007). Recently, Yetley and colleagues provided strong justification for reviewing the Dietary Reference Intake for vitamin D in the *American Journal of Clinical Nutrition* as well (Yetley et al., 2009).

Low vitamin D levels have been associated with impaired muscular function and increased propensity to falls and fractures (Janssen et al., 2002). Vitamin D receptors have been found in several tissues and organs implying a possible role of vitamin D in muscle strength and function (Campbell & Allain, 2006). Recent studies have shown an association between serum 25(OH)D concentrations and muscle strength and functions (Dhesi et al., 2002.; Mowe et al., 1999; Pfeifer et al., 2001; Shinchuk et al., 2007). Moreover, atrophy of type IIa muscle fiber has been reported in people with osteomalacia (Peacock, 1998). Although cross-sectional studies suggest an association between vitamin D and muscle strength and muscle function, evidence from cross-sectional studies is weak as low muscle strength may result in reduced sun exposure. Hence, the relationship between vitamin D status and muscle strength remains unclear when examining findings from cohort and vitamin D supplementation studies investigating this relationship.

1.2 Statement of purpose

The central hypothesis of this study is that lower dietary vitamin D intake leading to low vitamin D status in older people compromises muscle strength and function causing physical functional decline. On the basis of this hypothesis, the over all objectives set out for this study are:

- To conduct a comprehensive literature review of vitamin D status, vitamin D intake from food and supplements, and the association of vitamin D status with muscle function and muscle strength in the elderly
- To examine vitamin D status in healthy free-dwelling elderly population of Québec
- To examine the contribution of food and supplements to vitamin D intake in this population
- To determine whether vitamin D status can predict physical functional decline over one and two years in this healthy free-living elderly population of Québec

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CHAPTER 2. REVIEW OF LITERATURE

2.1 Introduction

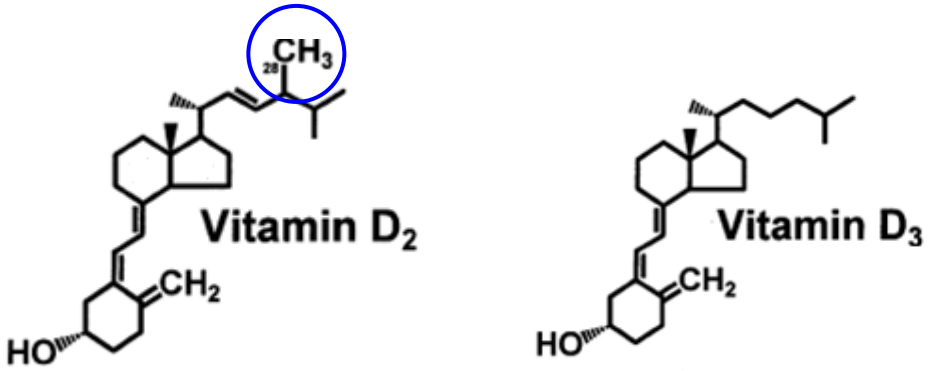
The purpose of this chapter is to review the literature, on vitamin D's important role in improving health in the growing number of older people. To achieve this target, it is essential to lay the foundation by introducing background information about vitamin D. After which, a comprehensive review of vitamin D deficiency prevalence globally, and in Canada specifically will be reviewed. Vitamin D intake requirements and habitual intakes will be reviewed to assess whether vitamin D requirement for this high risk group is attained. Finally, this chapter will review a possible proposed consequence, namely, physical functional decline, which may result from low vitamin D levels. It is also proposed that other possible negative health outcomes such as increased risk of developing some types of cancer, and increased risk of diabetes, hypertension and other immune system diseases may be associated with low vitamin D status (Holick, 2004); however, due to inconsistency in reported findings, this review will not discuss them in depth (Chung et al., 2009).

2.2 Vitamin D

2.2.1 Vitamin D structure

Vitamin D, one of the four fat-soluble vitamins A, D, E and K, is a seco-sterol that comes in many forms (Institute of Medicine, 1997). The two major physiologically relevant forms are D_2 (ergosterol) and D_3 (cholecalciferol) that are similar in structure with a difference in a side chain as seen in **Figure 2.1**, both are physiologically inert and require

two hydroxylations; one in the liver converting D_3 to 25 hydroxy vitamin D [25(OH)D] and a subsequent one in the kidney to arrive to the biologically active hormone 1,25 dihydroxy vitamin D [$1,25(OH)_2D$] (Lips, 2006).



(a) (b)

Figure 2.1. Major Forms of Vitamin D, (a) Vitamin D_2 and (b) Vitamin D_3**.** Adapted from (Institute of Medicine, 1997)

2.2.2 Vitamin D sources

Vitamin D can be acquired through two routes; dietary intake or dermal synthesis (Holick, 2006). Suprabasal layers of skin are able to synthesize vitamin D in the presence of UVB light in wavelengths ranging between 290 – 315 nm (Lehmann, 2005). Major dietary sources of vitamin D are demonstrated in **Table 2.1.** These sources are very limited; fatty fish and fish oil are the main natural sources of dietary vitamin D, egg yolk, and organ meats contain vitamin D to a lesser extent. In Canada, fortified food sources are limited to milk, and margarine; whereas in the United States more food items are optionally fortified

by vitamin D. Examples are yoghurt, cheese, cereals and orange juice (Calvo et al., 2004; Lamberg-Allardt, 2006; Tangpricha et al., 2003). Finnish researchers successfully tested the feasibility of fortifying bread with vitamin D_3 and were able to detect increased serum 25(OH)D in response to eating vitamin D fortified bread (Natri et al., 2006). In the United Kingdom (UK), margarine is vitamin D fortified in addition to some cereal products. Most of the other European countries and the rest of the world have no mandatory vitamin D food fortification policies. Thus, intake of vitamin D containing food sources is often challenged by scarcity of dietary sources, reduced consumption frequency in fear of food toxicity, as in consumption of fish, or avoidance of consumption of some sources such as organ meats owing to their high cholesterol content. Despite the fact that fortification remains a main source for dietary vitamin D intake, vitamin D levels may vary in retail milk. One Ontario study reported that only 20% of skimmed milk, 40% of 2% fat milk and 20% of whole milk contained the recommended levels of vitamin D (Faulkner et al., 2000). Samples tested were either over or under – fortified; under - fortification was observed in 27% of whole milk, and went up to 80% in some of the 2% milk samples (Faulkner et al., 2000). Similarly, findings reported from a study conducted in New York State revealed 48% of 648 milk samples were compliant with current regulation of vitamin D fortification of 10 – 15 μg/quart (400-600 IU/ quart), 46% fell below the 10 μg (400 IU) mark, and 6% of the samples were over fortified i.e. > 15 μg (600 IU) (Murphy et al., 2001). However, several randomized clinical trials (RCT) that offered vitamin D fortified milk to intervention groups *vs* non- vitamin D fortified milk to control groups have reported a significant increase in 25(OH)D concentrations in intervention groups (Chee et al., 2003; Keane et al., 1998; Lau et al., 2001). These findings support that under - fortification is not an issue and it may be balanced out with

over fortification. Milk intake in older people may be compromised because of gastrointestinal discomforts that may accompany milk consumption. Hence, vitamin D supplements remain to be the most reliable source of dietary vitamin D when compliance to consumption of 10 µg (400 IU) as recommended by Health Canada for older people is adhered to (Health Canada, 2009). In the US, vitamin D supplements in doses of 10 µg (400 IU), 20 µg (800 IU) and 25 µg (1000 IU) are available over the counter, and up to 125 µg (5000 IU) with prescription (Buhr & Bales, 2009). Multivitamin preparations have different doses of vitamin D ranging between 2.5 µg (200 IU) and 15 µg (600 IU). Calcium and vitamin D supplements are also available. Therapeutic doses of vitamin D may be obtained by prescription in oral, intramuscular or intravenous forms that can be administered daily, weekly, and monthly or every 6 months. A capsule of 1250 µg (50,000 IU) vitamin D_2, or 200 µg /ml (8000 IU /ml) are also available by prescription (Holick & Chen, 2008). Despite the fact that supplement consumption increased 25(OH)D concentrations in most cases, the magnitude of this increase is governed by baseline 25(OH)D status (Cranney et al., 2008; Heaney, 2006). A dose response has been reported in many vitamin D supplementation studies; however, in one study where a single oral dose of 2,500 µg (100,000 IU) was administered to the study population, 7 % of the subjects did not achieve 80 nmol/L at any point of the 4 month follow-up period (Ilahi et al., 2008). These subjects had 25(OH)D concentrations < 37.5 nmol/L at baseline.

Table 2.1. Major Dietary Sources of Vitamin D. from the Canadian Nutrient File

Dietary source of vitamin D	Amount	International Unites/ µg
Eel, mixed spiced, baked	100 g	23.3 µg
Herring, atlantic, baked or boiled	100 g	5.4. µg
Salmon, atlantic, wild, baked or boiled	100 g	8.2 µg
Cod liver oil	5 mls	10.6 µg
Tuna, skipjack, baked or boiled	100 g	12.7 µg
Egg yolk	17 g	3.7 µg
Milk, fluid, whole, pasteurized, homogenized, 3.3 % fat	250 ml	1.0 µg
Margarine, tub	14 g	1.9 µg
Orange juice, chilled, fortified with added calcium and vitamin D	250 ml	2.6 µg
Beef, liver, brsised	100 g	0.7 µg

2.2.3 Vitamin D physiology

Photolysis of 7-dehydrocholesterol, also known as provitamin D_3, to previtamin D_3 is triggered by the presence of UVB wavelengths ranging between 290-315 nm depending on several factors that may interfere with vitamin D synthesis. Body temperature isomerizes previtamin D_3 to vitamin D_3. Vitamin D from dermal synthesis and diet bind to vitamin D binding proteins (DBP) in the blood stream and are transported to the liver where vitamin D is hydroxylated to 25(OH)D. Subsequently, this inactive metabolite of vitamin D is hydroxylated to form the biologically active hormone 1, $25(OH)_2D$ in the kidney. Once transported to nuclei of target cells by intracellular binding protein, 1, $25(OH)_2D$ activates its nuclear receptor (VDR) inducing a heterodimerization between

the active VDR and a retinoic receptor (RXR). The formation of this heterodimer facilitates the interaction between the receptor's zinc finger region with DNA changing mRNA and inducing de novo protein synthesis (Montero-Odasso, 2005) The synthesis of $1,25(OH)_2D$ from 25(OH)D is stimulated by parathyroid hormone (PTH) and suppressed by calcium, phosphorus and $1,25(OH)_2D$ itself. The rate limiting step in catabolism is the degradation of 25(OH)D and $1,25(OH)_2D$ to $24,25(OH)_2D$ and $1,24(OH)_2D$ that are consequently excreted (Deeb et al., 2007).

2.2.4 Physiological effects of vitamin D

The major classical role of vitamin D is maintaining calcium homeostasis and being a major contributor in bone health (Calvo & Whiting, 2005; Heaney, 2007; Holick, 2007). Vitamin D is primarily responsible for regulating calcium absorption. Low vitamin D levels, compromises intestinal calcium absorption, and renal reabsorption of calcium. Additionally, low vitamin D intakes induce bone calcium resorption, leading to bone demineralization (Holick, 2006). Vitamin D receptors (VDR) have also been identified in muscle tissue implying a possible effect for vitamin D in muscle strength and function (Holick, 2006). Accumulating evidence suggests that vitamin D may affect immune cells and may be associated with higher risk of tumor development. Several epidemiological studies and some clinical trials have linked low 25(OH)D concentrations to hypertension (Rostand, 1997) and cardiovascular disease (Zittermann & Koerfer, 2008). Vitamin D is also believed to have an immunomodulatory effect and was linked to type I diabetes (Hypponen et al., 2001), psoriasis (Perez et al., 1996), multiple sclerosis (Munger et al, 2006) and rheumatoid arthritis (Merlino et al., 2004). In addition, vitamin D has also been shown to be associated with breast cancer (Bertone-Johnson et al., 2005), colon cancer

(Slattery et al., 2004) and prostate cancer (Chen & Holick, 2003; Ray et al., 2003) possibly due to its action on proliferation and differentiation of cells (van den Bemd & Chang, 2002; Ylikomi et al., 2002). A recently conducted comprehensive systematic review reported the inconsistent findings relating vitamin D with or without calcium to the different health outcomes (Chung et al, 2009). Findings were inconsistent across studies for prostate cancer, colorectal cancer and vitamin D intake, but the authors reported that calcium intake lowered systolic, but not diastolic blood pressure by 2-4 mm Hg, and that calcium intake was associated with decreased breast cancer risk in premenopausal women (Chung et al, 2009).

2.2.5 Factors that affect vitamin D availability

Several factors may impede dermal synthesis of vitamin D and may result in vitamin D deficiency. The elderly, in particular, may be exposed to a combination of the factors listed below, in addition to their reduced dermal synthesis, possible malabsorption, liver and kidney diseases; which put them at an additional risk.

2.2.5.1 Geographic/ seasonality factors

Dermal synthesis of vitamin D requires the presence of UVB light in specific wavelengths. People living in regions greater than latitudes of 37° North of the equator are at risk of deprivation of vitamin D dermal synthesis between the months of October through April due to larger solar zenith angle (SZA) (Webb, 2006). Similarly, time of day and poor weather conditions may interfere with vitamin D synthesis mediated by SZA or reduced UVB light atmospheric penetration (Institute of Medicine, 1997). Montreal lies

on latitude 45.7 N, which does not allow vitamin D dermal synthesis between the months of October/ November to March/ April.

2.2.5.2 Biological factors

Melanin, a pigmentation which gives skin its color, acts as a natural sun screen extending the time required for UVB light penetration, accordingly elongates the time needed to synthesis vitamin D (Webb, 2006). Age decreases the capacity of synthesizing vitamin D by 4-fold (MacLaughlin & Holick, 1985). Adiposity has also been associated with lower serum vitamin D concentrations in adults (Harris & Dawson-Hughes, 2007; Looker, 2005; Snijder et al., 2005). This inverse association was stronger in younger persons when compared to older people (Looker, 2005). Stronger inverse associations were reported when precisely assessing body fat by dual-energy x-ray absorptiometry (DXA) whereas weaker associations were reported when anthropometric measures were used (Snijder et al., 2005). The explanation of this inverse relation is not clear yet. Adipose tissue sequestering vitamin D, hence, making vitamin D less available is a possible explanation. Secondary elevation of PTH is also speculated to reduce hepatic hydroxylation of vitamin D to 25(OH)D (Liel et al., 1988). People with higher adiposity are less likely to get sun exposure and tend to cover up more as well (Looker A., 2005). Conversely, one short-term vitamin D supplementation RCT demonstrated that adiposity does not influence the elevation of 25(OH)D concentration (Canto – Costa et al., 2006). In this study 42 institutionalized older men and women were devided into 3 groups based on their % body fat as assessed by DXA. When supplemented with 175 µg (7000 IU) a week for 3 months, similar increase of 25(OH)D concentrations were reported across the 3 groups.

2.2.5.3 Behavioral factors

Sun screen in specific protective factor (SPF), the number that appears on sun screen's product indicating its ability to block out the UVB rays, of 15 can block UVB light; hence, it reduces vitamin D synthesis by 99% (Matsuoka et al., 1990). Two studies have shown sunscreens of SPF8 completely blocked cutaneous vitamin D synthesis when worn by subjects under investigation (Matsuoka et al., 1990; Matsuoka, et al., 1987). Inconsistent applications of sunscreens, and loss of sunscreen functionality after several immersions in water were responsible for similar 25(OH)D concentration values reported in those wearing and those not wearing sunscreen (Farrerons et al., 1998; Marks et al., 1995). Glass windows are as effective as wearing sunscreen in blocking UVB light (Holick, 1994). Clothing is another factor hindering vitamin D synthesis. Weave and color of fabric have a major effect on UVB protection, where darker colors and looser weaves result in more UVB light absorption (Berne & Fischer, 1980; McCarty, 2008). Cultural habits that involve covering large portions of skin also hinder vitamin D cutaneous synthesis of vitamin D. Milk intolerance or dietary preferences that invoke reduced milk intake, or reduced fatty fish intake and not consuming vitamin D supplements may also put people at additional risk of not meeting dietary vitamin D requirements.

2.2.5.4 Other factors

Medical conditions such as fat malabsorption, Crohn's disease and sprue will lead to increased risk of vitamin D deficiency (Holick, 2006). Some types of medication such as cholestyramine, corticosteroids, anticonvulsants and rifampin may also reduce the bioavailability of vitamin D (Di Munno et al., 2004; Walker-Bone et al., 2004). Thus these should be considered when examining vitamin D status.

2.2.6 Vitamin D assessment

More than 50 metabolites of vitamin D have been described to date. For the time being measurement of serum 25(OH)D concentration is the best clinical indicator for vitamin D status in individuals since it represents a summation of the total cutaneous production of vitamin D and the oral ingestion of either vitamin D_2 or vitamin D_3 (Institute of Medicine, 1997; Seamans & Cashman, 2009). The half – life of serum 25(OH)D is about 21 days, compared to 24 hours half-life for serum vitamin D and 4 hours for serum 1,25 $(OH)_2D$, representing accurate indication of vitamin D stores over a long period of time (Zerwekh, 2008). Additionally, hepatic production of 25(OH)D is dependent on substrate concentration and not significantly regulated. On the other hand, 1,25 $(OH)_2D$ production is tightly regulated by calcium homeastasis.

Several platforms are currently acceptable for vitamin D assessment. Competitive-protein binding assay (CPBA) has the advantage of being relatively inexpensive, co-specific for $25(OH)D_2$ and $25(OH)D_3$, but requires organic extraction / solvent evaporation, preparative chromatography and liquid scintillation counting. The ultraviolet (UV) detection assay following high performance liquid chromatography (HPLC) is considered the gold standard for the following reasons; first this method separates $25(OH)D_2$ and $25(OH)D_3$, secondly, it is a very stable and repeatable method. However, it does require a high level of expertise in addition to its costly equipment that requires larger sizes of serum or plasma samples (500 μL compared to 50 μL). Radioimmunoassay (RIA) is considered the most widely acceptable due to the overall simplicity of the assay, accuracy,

rapid application and strong correlation with values obtained from HPLC analysis. Enzyme-linked immunosorbent assay (ELISA) is a fourth method for detecting 25(OH)D and is available in 2 format assays. The most accurate method to date is the liquid chromatography-tandem mass spectrometry (LC-MS/MS) this method; requires deuterated internal standard. However, due to its need for high levels of expertise, it is best left to reference laboratories (Zerwekh, 2008). Finally, DiaSorin has developed a chemiluminescent 25(OH)D assay that is fully automated on the LIAISON analyzer. The advantage of using this method is the short assay time (40 minutes) where it can analyze 90 samples per hour. Moreover, when compared to DiaSorin RIA and LC-MS/MS a correlation coefficients of 0.94 and 0.95 were obtained respectively (Frenzel et al., 2006).

2.2.7 Vitamin D storage

Vitamin D metabolites may be stored as serum or plasma ≤ -20° C until assayed. Serum 25(OH)D is stable when frozen at the suggested temperatures for 2-3 years (Lips et al., 1999). 25(OH)D is also stable in uncentrifuged blood at 24° C for as long as 72 hours. Transporting samples frozen or by post, and in different storage conditions did not contribute significantly to the normal variation seen in measuring vitamin D metabolites (Berry et al., 2007). In a study conducted in the UK the coefficient of variation was 5.1% and 4.5% for stored serum 25(OH)D and controls, respectively. Serum 25(OH)D freezing – thawing cycles are relatively stable. In one study the correlation between baseline 25(OH)D concentration and after each freeze – thaw cycle up to four times was 0.99 (Antoniucci et al., 2005), and stability was maintained after 11 freezing - thawing cycles as reported by other researches (Zerwekh, 2008).

The main concern that rises from serum 25(OH)D assessment is the markedly different results reported based on using different assays. This results in difficulty of comparing findings from different studies, and calls for standardization. In one study researchers reported that when the same samples were sent to 6 laboratories using a variety of assays, 5 subjects out of 10 were classified as insufficient in some laboratories and normal in others (Binkley et al., 2004). In another study, when 104 serum 25(OH)D samples were measured in 3 different assays, the mean serum 25(OH)D level was 80% higher when measured by CPBA as compared to HPLC (Lips et al., 1999). When samples were assessed using RIA, in the previous study, intermediate values were yielded (Lips et al., 1999). Hence, unless a cross-calibration between laboratories is performed, values of 25(OH)D cannot be considered comparable between studies.

2.2.8 Vitamin D dietary recommendation for elderly people

In 1997 the Dietary Reference Intake (DRI) standing committee had set the adequate intake (AI) for vitamin D for people aged 51 -70 years at 10 μg (400 IU)/d and 15 μg/day (600 IU) for those > 70 years. The committee had set their estimation of vitamin D requirements based on bone loss studies (Institute of Medicine, 1997). In one randomized double blind 2 year vitamin D supplementation clinical trial which studied 247 postmenopausal women (mean age 64 ± 5 years), loss of bone mineral density (BMD) of the femoral neck was significantly less in those randomized to 17.5 μg (700IU) when compared to those randomized to 2.5 μg (100IU) (Dawson-Hughes et al., 1995). In a cross-sectional study of 333 women with mean age of 58 ± 6 years, no seasonal variation in PTH was observed in intakes > 5.5 μg (220 IU), whereas winter PTH was elevated in

those consuming < 5.5 μg (220 IU), which implies that intakes > 5.5 μg (220 IU) may be associated with better skeletal health (Krall et al., 1989).

Since then, an outburst of vitamin D research had been conducted. Since vitamin D intake recommendations were based on bone health, and currently vitamin D researchers have reasons to believe that vitamin D's role extends beyond its role in bone health to disease – prevention as described earlier. Accordingly, some vitamin D researchers suggest higher dietary intakes of 20 – 25 μg (800 – 1000 IU) to obtain 25(OH)D concentrations of at least 75 nmol/L, the suggested cut-off for optimal vitamin D status, based on several end points (Bischoff-Ferrari et al., 2006). Impaired calcium absorption at 25(OH)D concentrations < 80 nmol/L (Heaney et al., 2003) and elevated PTH that levels at < 80 nmol/L were the main parameters for estimating vitamin D requirements (Vieth et al., 2003). Other end points have been under investigation as well, in one study, where BMD was used as an end point, a significant positive association between 25(OH)D concentrations and total hip BMD was seen in a multiethnic young and older adults from the NHANES III survey; BMD was 4.8 % higher in people with 25(OH)D ≥ 98 nmol/L compared to those with 25(OH)D ≤ 53 nmol/L (Bischoff-Ferrari et al., 2004). Findings from a recent meta-analysis investigating fracture reduction as an end point, showed that non-vertebral fracture prevention was dose - dependant on vitamin D consumption, and a 20% reduction of fractures was observed with higher doses of vitamin D > 10 μg (400 IU) when compared to doses < 10 μg (400 IU) in people ≥ 65 years (Bischoff-Ferrari et al., 2009). When investigating lower extremity function as an end point, a positive association between higher concentrations of serum 25(OH)D (best at 90-100 nmol/L)

and lower-extremity strength was seen in a population based study investigating 4100 participants ≥ 60 years assessed by an 8-foot-walk test and chair-stand test (Bischoff-Ferrari et al., 2004). Epidemiological studies have also shown an inverse association between 25(OH)D and colorectal cancers and colorectal adenoma. In the Nurses' Health Study a relative risk (RR) of 0.53 (95% CI: 0.27 - 1.04) was reported for the highest 25(OH)D concentration quintile (median: 88nmol/L) when compared to the lowest quintile (median 38 nmol/L) (Feskanich et al., 2004).

Since most of the studies discussed above were observational studies, that may show associations but do not necessarily demonstrate causality, clinical trials were carried out in an attempt to quantify the relation of vitamin D intake and 25(OH)D concentrations. Heaney and colleagues conducted a dose-response study investigating 67 men (mean age= 38.7 ± 11.2 years) (Heaney et al., 2003). The subjects were randomized to 0, 25, 125 and 250 µg (0, 1000, 5000, 10,000) IU vitamin D_3 for 20 weeks during winter. Findings estimated a 0.7 nmol/L increase in 25(OH)D concentrations for every 1 µg (40 IU) of vitamin D consumed. Extrapolating, the authors estimated a need for 12.5 µg (500 IU) of vitamin D to maintain 25(OH)D at 80 nmol/L (at equilibrium) and higher values are required for those who start at a lower baseline level (Heaney et al., 2003). Similarly, in another randomized placebo controlled supplementation study investigating 225 older subjects aged ≥ 64 years supplemented with 0, 5, 10 and 15 µg (0, 200, 400, 600) IU for 22 weeks, 8.6 µg (344 IU) were required to maintain 25(OH)D concentrations above 25 nmol/L for 97.5% of the study sample (Cashman et al., 2009). Also by extrapolating, the authors estimated a requirement of 38.7 µg (1,548 IU) of vitamin D to maintain serum 25(OH)D concentrations of > 80 nmol/L for 97.5% of the participants. Simply put, some

researchers believe that healthy non-sun shy individuals such as construction workers, lifeguards and farmers who have serum 25(OH)D ranging from 135 – 225 nmol/L should be considered the normal reference for adequate vitamin D status.

Noteworthy, there is no agreement among different Canadian agencies on vitamin D intake recommendations; however, the majority is in favor of higher intakes than those set by the DRI summarized in **Table 2.2.** (Dietitians of Canada May 2008).

Table 2.2. Current Recommendations on Vitamin D Intake by Various Canadian Agencies. Adapted from "Dietitians of Canada: Current Issues May 2008".

Agency	Age group	Recommended vitamin D intake, µg/ IU
Health Canada **http://www.hc-sc.gc.ca/fn-an/nutrition/child-enfant/infant-nourisson/vita_d_supp-eng.php**	Adult > 50 yr	10/ 400
DRI (Institute of Medicine) **http://www.hc-sc.gc.ca/fn-an/nutrition/reference/table/index_e.html**	Adult 50 – 70 yr Adult > 70 yr	10/ 400 15/ 600
Canadian Cancer Society **http://www.cancer.ca/Canada-wide/Prevention/Use%20SunSense/Vitamin%20D.aspx?sc_lang=en**	Adults	25/ 1000 as supplements
Canadian Dermatology Society **http://www.dermatology.ca/latest_news/latest_news.html**	Adults	25 / 1000 as supplements
Osteoporosis Society of Canada **http://www.osteoporosis.ca/**	Adult > 50 yr	20/ 800

2.2.9 Vitamin D status cut-offs

There is no consensus on levels set for vitamin D adequacy and inadequacy. Terms like sub-optimal, inadequate and insufficient status have been often used interchangeably, but 25(OH)D concentrations were not uniform across the studies. Insufficiency was defined as 25(OH)D concentrations < 50 and < 75 nmol/L (Holick et al., 2005; Linnebur et al., 2007). On the other hand, the DRI standing committee has defined vitamin D deficiency for the elderly at < 37.5 nmol/L; despite that, deficiency has been defined as < 50, < 40, < 37.5, < 30 and < 25 nmol/L across different studies (Bischoff-Ferrari et al., 2008; Gloth et al., 1995; Liu et al., 1997; Ramel et al., 2009; Thomas et al., 1998).

2.2.10 Consequences of vitamin D inadequacy

Chronic vitamin D inadequacy may cause hyperparathyrodism leading to increased bone turnover, reduced BMD leading to fractures in adults (Lips, 2001). Osteoporosis may also result from lower 25(OH)D concentrations (Eriksen & Glerup, 2002). Clinical signs may be manifested in the form of deep diffused skeletal pain and very tender bony structures may be felt upon palpitation (Malabanan et al., 1998). True or overt deficiency in older people is defined by 25(OH)D concentrations < 37.5 nmol/L as set by the DRI committee. While rickets is a major outcome of vitamin D deficiency in children, osteomalacia is the adult consequence of vitamin D deficiency. Additionally, proximal muscle may accompany severe vitamin D deficiency (Holick, 2006).

2.2.11 Vitamin D safety

Elevated plasma 25(OH)D characterizes hypervitaminosis D. Serum 25(OH)D can go up to 1,250 nmol/L causing hypercalcemia that results in kidney stone formation and soft

tissue calcification (Institute of Medicine, 1997). Hypercalciuria, anorexia, nausea and vomiting are also signs of hypervitaminosis D (Institute of Medicine, 1997). The Upper Tolerable Level (UL) was set at 50 μg (2000 IU)/ day based on a study conducted by Narang and colleagues (1984). In this study 30 men and women < 60 years were supplemented with 10, 20, 30, 60 and 95 μg/ day (400, 800, 1200, 2400 and 3800 IU) for 3 months, mean serum calcium increased from 2.43 to 2.6 mmol/L in those consuming 60 μg (2,400 IU) but was insignificantly slightly higher than the normal range (2.1 - 2.5 mmol/L), however, for those consuming 95 μg (3,800 IU) increase in serum calcium increased from 2.46 to 2.83 mmol/L (Narang et al., 1984). Some researchers believe that this UL set by the DRI committee is too low. In one supplementation study 61 young men and women were randomized to 25 μg (1000 IU) or 100 μg (4000 IU) for 5 months (Vieth et al., 2001). The 25 μg/ day (1000 IU) dosage was effective at ensuring 25(OH)D concentrations of ≥75 nmol/L in 35% of subjects and 100-μg/ day (4,000 IU) dosage was effective at ensuring 25(OH)D concentrations of ≥75 nmol/L in 88% subjects. Mean serum calcium remained within reference limits and the number of treated subjects with hypercalciuria was not different between the two dose groups. No vitamin D toxicity was reported (Vieth et al., 2001). In another similarly designed 5 month vitamin D supplementation randomized study investigating adults (mean age 53 ± 14 years) and (55 ± 9 years), similar findings were in agreement with the previous study and no vitamin D toxicity was reported (Vieth et al., 2004). In a third study Barger-Lux and colleagues (1998) did not report any incidence of vitamin D toxicity when 37 young men were randomized to 25, 250 or 1250 μg/ day (1000, 10,000, 50,000) vitamin D_3 for 8 weeks as part of a clinical trial (Barger-Lux et al., 1998). Toxicity needs to be studied in much

larger samples, of different age ranges; both genders as may be only some people may be prone to experience adverse effects. Additionally, the previous studies were mostly of short duration, some participants may need these high doses to replete their baseline 25(OH)D concentration. Although the studies reviewed above imply that higher vitamin D intakes than currently recommended may be relatively safe, long term effects of higher vitamin D intakes have not yet been investigated.

2.3 Prevalence of vitamin D deficiency

A large number of studies showed high prevalence of vitamin D insufficiency and deficiency across younger adults ≤ 50 years in North America (Binkley et al., 2007; Looker et al., 2002; Nesby-O'Dell et al., 2002; Rucker et al., 2002; Tangpricha et al., 2002; Vieth, et al., 2001) and in older people ≥ 50 years as well (Glowacki et al., 2003; Harris et al., 2000; Liu et al., 1997; Thomas et al., 1998; Weiler et al., 2007). However, most of these studies in ≥ 50 years assessed vitamin D status in institutionalized, home bound and elderly people with poor health conditions, while very few examined vitamin D status in healthy free living community-dwelling older people. The following sections will review these studies.

2.3.1 Prevalence of hypovitaminosis D in institutionalized inactive elderly

Many vitamin D insufficiency and deficiency studies were carried out in hospitalized and institutionalized older people; **table 2.3** presents some of these studies. Vitamin D status was examined in relatively large numbers of older men and women; despite the use of different 25(OH)D concentrations cut – offs to define vitamin D deficiency, the

prevalence was high in all but one study (Ramel et al., 2009). Taking a closer look at this study that reported low vitamin D deficiency, it was apparent that providing a daily cod liver oil dose as part of breakfast in a long term stay facility in Iceland was sufficient to demonstrate lower prevalence of deficiency in this group when compared to other studies. The listed studies were conducted in cities at different latitudes, all above 39°; hence, vitamin D deficiency was higher in winter and spring as shown in studies that tested in different seasons (Bischoff-Ferrari et al., 2008; Liu et al., 1997; Thomas et al., 1998). Some studies did not report supplement consumption; others showed low supplement consumption, which may offer an explanation for high prevalence of vitamin D deficiency (Bischoff-Ferrari et al., 2008; DeLappe et al., 2006; Shinchuk et al., 2006; Thomas et al., 1998).

Table 2.3. Studies on Vitamin D Deficiency Prevalence in Institutionalized Inactive Elderly People

Author	City, Country (Latitude)	Setting	n	Mean age years	Sex	Season or month	Deficiency cut – off 25(OH)D nmol/L	Deficiency	Assay	Supplement users
Gloth et al., 1995	Baltimore, US (39.2°)	64 Nursing - homes 52 Home care facilities	244	81	F: 85 M: 31	NR Sun-deprived	< 25	48 %	RIA	NR
Liu et al., 1997	Toronto, Canada (43.4°)	3 long-term care facilities	155	81	F: 73 M: 82	March September	< 40 < 40	60 % 38 %	CPBA	NR
Thomas et al, 1998	Massachusetts, US (42.5°)	Hospitalized in medical wards	150 140	62	F: 138 M: 152	March September	< 37 < 37	63 % 49 %	CPBA	21 %
DeLappe et al., 2006	Galway, Ireland (53.1°)	Hospitalized in medical wards	114	80	F: 100	Fall	< 50	75 %	RIA	25 %

Table 2.3. Continued

Author	City, Country (Latitude)	Setting	n	Mean age years	Sex	Season or month	Deficiency cut – off 25(OH)D nmol/L	Deficiency	Assay	Supplement users
Shinchuk et al., 2006	Boston, US (42.5°)	Rehabilitation facility	53	60	F: 23 M: 30	February - June	< 50	49 %	CPBA	12 %
Bischoff – Ferrari, 2008	Zurich, Switzerland (47.2°)	Hip fractured hospitalized patients	222	87	F: 171 M: 51	Spring Summer Fall Winter	< 30	70% 54 % 49 % 64 %	RIA	10 %
Ramal, 2009	Reykjavik, Iceland (64.4°)	Long stay facility	60	83	F: 38 M: 22	Summer Winter	< 25	12 %	RIA	NR Daily cod liver oil

F = female, M = male, RIA = radioimmunoassay, CPBA = competitive protein binding assay, NR = not reported

2.3.2 Prevalence of vitamin D in clinical groups affected by osteoporosis

Several studies have investigated prevalence of vitamin D deficiency in older people with health problems such as osteoporosis, vertebral or hip fractures, or in those with low incomes, which may result in poor nutrition, thus, poor health conditions (**Table 2.4).** Only two of the listed studies reported supplement consumption in these clinical groups who are in high need of supplementation (Beringer et al., 2006; Harris et al., 2000). Seasonal effect on vitamin D status was clearly demonstrated, vitamin D deficiency was more pronounced in those tested in winter as seen in subjects tested in Italy and Montreal (Delvin et al., 1988; Isaia et al., 2003). Harris and colleagues have demonstrated that ethnicity can be a predictor of vitamin D deficiency, where prevalence of vitamin D deficiency in black older people living in Boston was double that of white older people (Harris et al., 2000). Using different 25(OH)D concentration cut-offs makes it difficult to compare studies, prevalence of vitamin D deficiency was lower in those studies that used the DRI criteria of 25(OH)D concentrations < 37.5 nmol/L (Glowacki et al., 2003; Guardia et al., 2008) when compared to other cut-offs, with the exception of one study were mean 25(OH)D concentration was below 37.5 nmol/L in all men in 2 seasons, the study population had low income recruited from hospital visits which may suggest poor health conditions (Delvin et al., 1988).

Table 2.4. Studies on Vitamin D Deficiency Prevalence in Clinical Groups Affected by Osteoporosis / Fractures and Underprivileged Elderly People.

Author	City, Country (Latitude)	Setting	n	Mean age years	Sex	Season or month	Deficiency cut – off 25(OH)D nmol/L	Deficiency	Assay	Supplement users
Glowacki et al., 2003	Boston, US (42.3°)	Osteoarthritis	68	67	F	NR	< 37.5	22%	RIA	NR
Isia et al., 2003	Italy (37 - 45°)	Osteoporotic	800	68	F	Winter	< 30	78 %	RIA	NR
Guardia et al., 2008	Detroit, US (42.3°)	Recruited from bone and mineral clinic	2924	68	F: 90 % M: 10 %	All seasons	< 37.5	15%	RIA	NR
Neuprez et al., 2007	Belgium (50.5°)	At least one fracture	1195	77	F	All	< 50	43	RIA	_
Harris et al., 2002	Boston, US (42.3°)	Osteoporotic, low income	B:136 W:110	75	F: 66 % M: 34 %	Winter	< 50 < 50	73 % 35 %	CPBA	43 %
Delvin et al., 1998	Montréal, Canada (45.7°)	Low income	188	65	F: 74 % M: 26 %	Winter Summer Winter Summer	< 37 < 37 < 37 <37	F: 100% F: 43% M: 100% M: 100%	RIA	NR
Beringer et al., 2006	Belfast, Ireland (54.4°)	Fractures	86	63	F: 70 % M: 30 %	All	< 50	56 %	_	73 %

B: black, W: white, F = female, M = male, RIA = radioimmunoassay, CPBA = competitive protein binding assay, NR = not reported

2.3.3 Prevalence of vitamin D deficiency in healthy elderly subjects

Very few studies were conducted to assess vitamin D status in free living healthy elderly people. In a study recruiting 339 elderly subjects (mean age = 71 years) from 7 cities in Argentina mean serum 25(OH)D was 35.3 nmol/L for those residing in Southern latitudes between 41 – 55° south, *vs* mean serum 25(OH)D of 50 nmol/L for those residing in cities of 26° south, vitamin D status was assessed using RIA (Oliveri et al., 2004). When vitamin D status was evaluated using CPB assay in 538 free-living white Dutch men and women ≥ 60 years, 51% had serum 25(OH)D concentrations < 50 nmol/L (van Dam et al., 2007). Vitamin D status was investigated in 1,179 rural postmenopausal women living in Nebraska; although, 59% of the women were vitamin D supplement consumers, only 1/3 of the study population had optimal vitamin D status at a cut-off > 80 nmol/L as assessed by RIA (Lappe, Davies, Travers-Gustafson, & Heaney, 2006). No specific information was provided regarding the doses of supplements used; it is possible that either supplements used were not high in vitamin D content or not consumed frequently. A newly released publications of the Canadian Health Measures Survey reported a mean 25(OH)D concentration of 73.5 nmo/L for a representative sample of 516 Canadian men and women aged 60-79 years; close to optimal status (Statistics Canada, 2009a).

In Canada, few studies assessed vitamin D status. One study measured 25(OH)D concentration in a wider range of ages in rural and urban Aboriginal women as well as urban white women living in Manitoba; vitamin D deficiency was more prevalent in rural and urban Aboriginal than urban white women (Weiler et al., 2007). In Montréal, prevalence of vitamin D deficiency was 36.4 % in a predominantly older women

population using 25(OH)D concentration of 50 nmol/L cut – off to define deficiency (Vecino-Vecino et al., 2006).

2.4 Vitamin D intakes in elderly subjects

The importance of good nutrition over the life span and its contribution to health and quality of life cannot be overestimated. Physiological, social and emotional factors which accompany the aging process may lead to reduced food and nutrient intakes; hence, causing nutritional inadequacy (Robnett & Chop, 2010). Knowing that dietary sources of vitamin D are limited; several studies have attempted to estimate dietary vitamin D intake in older hospitalized and healthy older people; however, estimates of these intakes are not generalizable to healthy community-dwelling older people. Data estimating dietary vitamin D intake in free living healthy older Canadians are very limited. The following section reviews some of these studies.

2.4.1 Vitamin D intake in hospitalized and unhealthy elderly

In one study, 41 post surgical women ≥ 60 years who were hospitalized for total hip replacement or fractured neck of femur, had median intakes of 1.4 µg (56 IU) as assessed by three 24-hour dietary recalls after 5 days post surgery; since vitamin D is stored, this reflects poor vitamin D intake (Murphy et al., 2000). In another study carried out in the US, dietary vitamin D intake was assessed in 345 homebound meal-recipients ≥ 60 residing in North Carolina by three 24-hour recalls (Sharkey et al., 2002). Vitamin D intake from foods and supplements in all men was below AI for vitamin D with a mean of 5.6 µg (224 IU), and all women but one were also below the recommended AI with a

mean intake of 4.0 µg (160 IU). Consistent with the previous study, mean dietary vitamin D intake of 244 homebound elderly aged ≥ 60 years living in Baltimore was 3.0 µg (120 IU) (Gloth et al., 1995). No data regarding vitamin D supplement consumption was reported in this study; however, this group of older people was described as sun deprived.

2.4.2 Vitamin D intake in healthy free-living elderly

The European Nutrition and Health Report (2004) reported data on the nutritional status from a number of countries of the European Union (EU). Data regarding vitamin D intake was available for 9 countries; Austria, Denmark, Germany, Hungary, Italy, Norway, Portugal, Spain and the United Kingdom. Mean dietary vitamin D intakes for men and women aged 55 to ≥ 85 years ranged from 2.5 to 5.8 µg (100 to 232 IU) and 2.5 to 4.7 µg/ day (100 to 188 IU), respectively (Fabian & Elmadfa, 2008). Results clearly demonstrate that reduced dietary vitamin D intakes when compared to the EU recommendations set at 10 µg/ day (400 IU). In another study, dietary data for 832 men and women ≥ 70 years recruited from a population – based elderly cohort study in southern France (POLANUT), with a latitude above 43°, was assessed by a 165-item semi-quantitative food frequency questionnaire (FFQ) (Carriere et al., 2007). Median intake of vitamin D was reported to be 1.1 µg (44 IU) /1000 Kcal for men and 1.0 µg (40 IU) /1000 kcal for women, not meeting the recommended dietary allowance of 10 µg (400 IU) /day. It was not clear whether the food frequency questionnaires included consumption from supplement; however, it is noteworthy to mention that FFQ may only estimate and not quantify vitamin D intakes. In Spain, vitamin D intake of 53 elderly women (age = 72 ± 1.6 years) leading an autonomous life style was investigated in winter and summer (Rodríguez et al., 2008). Mean dietary intakes of vitamin D from food and supplements were 4.7 ± 4.7 µg

(188 IU ± 188 IU) in winter and 5.2 ± 4.8 µg (208 ± 192 IU) in summer. Fish was the main contributor to vitamin D intake; however, the consumption was not enough to reach the EU recommendations for vitamin D. In a cross-sectional study investigating prevalence of vitamin D deficiency in 95 healthy community-dwelling Irish women aged 51-75 years, researchers reported a mean vitamin D intake of 2.9 µg (116 IU) from food alone and 3.5 µg (140 IU) from supplements assessed by 14 - day diet history. Serum 25(OH)D concentrations ≤ 25 nmol/L were reported in 48% of the study population (Hill et al., 2006). In another recent study in Ireland, 4.4 µg (176 IU) of habitual intake of vitamin D was reported in 216 community dwelling men and women as assessed by a FFQ (Cashman et al., 2009). In North America, data of 1,255 men and 1,368 women aged ≥ 70 years analyzed from the Third Health and Nutrition Examination Survey (NHANES III) reported that 90% of the elderly did not consume adequate intakes of vitamin D (Moore et al., 2004). Food alone contributed about 5.9 µg (236 IU) and 4.5 µg (180 IU) in men and women respectively, and food and supplements together was 8.3 µg (332 IU) and 8.1 µg (324 IU). Researchers in Denver investigated dietary vitamin D intake and its association with vitamin D status in 80 ambulatory men and women with a mean age of 77.8 years (Linnebur et al., 2007). Dietary intake was assessed by FFQ and 25(OH)D concentrations were assessed by RIA. Findings demonstrated that dietary intake from food sources only did not differ significantly among the 3 vitamin D status categories 6.4 µg (256 IU), 7.0 µg (280 IU) and 5.3 µg (212 IU) in sufficient, insufficient and deficient groups, respectively. However, a significant difference for vitamin D supplement consumption, 15.5 µg (620 IU), 11.0 µg (440 IU) and 1.6 µg (64 IU) across the 3 vitamin D status groups was reported.

2.4.3 Vitamin D intake in Canada

In younger Canadians ≤ 51 years of age, vitamin D intakes from food and supplements were estimated to be 5.6 μg (224 IU) in men and 4.8 μg (193 IU) in women as reported from data of 9433 participants the Longitudinal Study on Osteoporosis assessed by FFQ (Polliquin et al., 2009). This intake was nearing the 5 μg (200 IU) AI recommendation for people < 50 years; however, when examining studies from older age groups the picture is not very optimistic. Several studies estimated vitamin D intakes using FFQ in older people, hospitalized, or residents of long term care facilities; intakes never exceeded 5 μg (200 IU) from food alone or 8 μg (320 IU) from supplements and food (Lee et al., 2008; Lengyel et al., 2008). Mean vitamin D intake for the province of Québec estimated from a single 24 - hour recall as reported by the CCHS was 5.6 μg (224 IU) and 6.9 μg (276 IU) in women (n = 215) and men (n = 132) over 70 years of age (Statistics Canada, 2009b). In this survey, participants were asked to report any vitamin supplementation consumed in the past month.

It is apparent that dietary data collection was conducted by different methods; however, 24 – hour recalls have the ability of capturing food and nutrient intakes more precisely for several reasons: they are open ended so they can accommodate any food combination reported by participants, food preparation method, and food processing method. Moreover, multiple 24 – hour recalls account for day to day variation in intake within the subject. If a week end is included in a 24 – hour recall this accounts for changes in eating behaviour that may occur with a person's intake. If several 24 – hour recalls are collected,

at least 6 months apart, this may better capture intakes according to the seasonal availability of foods and patterns of their consumption (Willett, 1998).

Despite the variation of data collection methods (24 - hour recalls, personal interviews, food frequency questionnaires, food records and household budget surveys) which requires caution in interpreting results, it is clear that mean dietary vitamin D intake in elderly populations falls below the recommended AI for the majority of this age group worldwide.

2.5 Vitamin D and physical functional decline

The incidence of falls is a common and serious problem due to consequential injury and cost (Krauss et al., 2005; Nadkarni, et al., 2005); the incidence rises in people from about 30% per year at age 65 years to about 50% per year at ages of 80 years and above (Boonen et al., 2006; Campbell, 1981; Tinetti et al., 1988; Tinetti & Williams, 1998). Several factors may contribute to the increased prevalence of falls in the elderly, such as skeletal muscle weakness, balance impairment, poor diet, cognitive impairment, reduced visual acuity, physical performance and foot problems (Tinetti et al., 1988; Tinetti & Williams, 1997). Falls are not a perfect measure of the problem of decline and are non – specific to deficiency, as the less one walks the less opportunity one has to fall.

2.5.1 Vitamin D and functional decline, possible mechanisms

It is postulated that the association between vitamin D deficiency and functional decline may be attributed to vitamin D receptors (VDR) and type IIa muscle fibers. Two different vitamin D receptors (VDR) have been identified in human muscle tissue, one located at the nucleus and one located at the membrane (Bischoff et al., 2001; Campbell & Allain,

2006; Norman, 1998). In one study, the absence of VDR in knock out mice resulted in lack of proper muscle development, implying a major role for vitamin D in healthy muscle growth and development (Kato et al., 1999). It is also known that type IIa muscle fibers are the fast twitch fibers; hence, they are the first to be recruited to avoid falling (Montero-Odasso & Duque, 2005). Muscle biopsies have shown a reduction of type IIa muscle fibers as well as muscle atrophy in severely vitamin D deficient subjects (Yoshikawa et al., 1979). Interestingly, some studies showed a relative increase in number of type IIa muscle fibers after vitamin D repletion (Sato et al., 2005; Sorensen et al., 1979). There is a decrease in number of both VDR and type IIa muscle fiber with aging, which implies deterioration in muscle health (Bischoff-Ferrari et al., 2004). Accordingly, it is proposed that low vitamin D status may lead to impaired muscular function and increased propensity to falls and fractures (Jensen et al., 2002). Three cross-sectional studies and one clinical trial have shown an association between serum 25(OH)D concentrations and muscle strength and function (Dhesi et al., 2004; Mowe et al., 1999; Pfeifer et al., 2001). Vitamin D has also been associated with myalgia and proximal muscle weakness (Muhlebach & Bischoff-Ferrari, 2009; Prabhala et al., 2000). Vitamin D supplementation has proven effective in treatment of proximal myopathy in vitamin D deficient individuals by reversing the condition; however, no clear mechanism of action is yet determined (Prabhala et al., 2000). Although studies suggest an association between vitamin D and muscle function, this relationship remains unclear. The diagram below, **Figure 2.2**, illustrates the different possible pathways by which vitamin D deficiency may influence falls and fractures.

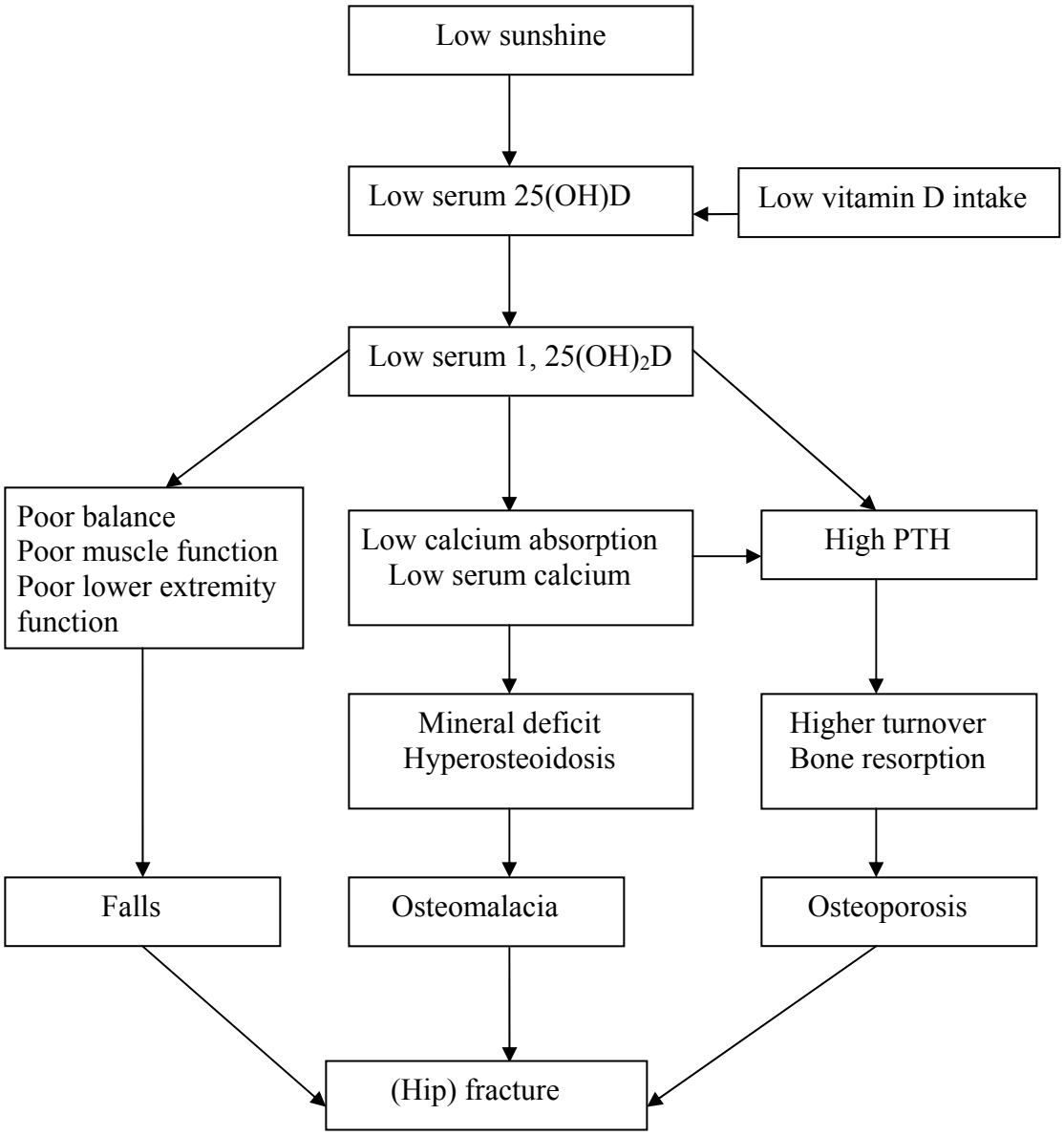


Figure 2.2 Vitamin D Deficiency, Falls and Fractures

The previous diagram demonstrates that low vitamin D levels may lead to fractures by directly influencing bones, or possibly through its indirect effect on muscle strength, function or balance leading to increase risk of falls. Active research has been conducted to examine these associations, however, no conclusive outcomes have been agreed on as demonstrated by the following studies.

2.5.2 Vitamin D and fractures

Cohort studies have examined the association between vitamin D status and incidence of fractures. Additionally, meta-analyses and one systematic review evaluated studies that investigated vitamin D supplementation with vitamin D alone, vitamin D and calcium, calcium alone and placebo on fracture risk reduction (Bischoff-Ferrari et al., 2007; Bischoff-Ferrari et al., 2005; Bischoff-Ferrari et al., 2009; Boonen et al., 2007; Cranney et al., 2008). In one study conducted on 1, 311 community-dwelling older men and women of the Longitudinal Aging Study Amsterdam (LASA), an increased risk of fractures was observed in persons aged 65 – 75 years with 25(OH)D concentrations < 30 nmol/L, when followed up for 6 years and controlling for a number of important confounders (van Schoor et al., 2008). In a meta-analysis that was assessing the effect of supplementation on hip fractures and non-vertebral fractures (from 5 and 7 RCTs). Results demonstrated that supplementing with 17.5 – 20 µg (700 - 800 IU) vitamin D with or without calcium reduced the relative risk of hip fracture by 26%, and reduced the relative risk of non-vertebral fractures by 23% when compared to calcium or placebo (Bischoff-Ferrari et al., 2005). On the other hand, Boonen and colleagues (2007) conducted another meta-analysis comparing RCTs of supplementing with only vitamin D and comparing the findings with pooled RCTs of supplementing with vitamin D and

calcium. The investigators reported that oral vitamin D appeared to reduce hip fracture only when combined with calcium (Boonen et al., 2007). In another meta-analysis that investigated supplementing with calcium only, data from 8 prospective cohorts and 5 clinical trials (800 – 1600 mg Ca/ day) were analyzed, calcium was not significantly associated with hip fractures in men and women from these studies (Bischoff-Ferrari et al., 2007). Findings were reported as follows: cohort studies pooled RR for additional 300 mg Ca/ day intake was 1.01 (95 % CI: 0.97; 1.05), and RCTs pooled RR was 0.95 (95 % CI: 0.81; 1.05) between calcium and placebo. In one calcium supplementation study, 930 healthy participants (mean age = 61) were randomized to 1200 mg Ca or placebo for duration of 4 years and followed up for 10.8 years (Bischoff-Ferrari et al., 2008). Forty-six incident fractures *vs* 56 were reported in the treatment and control groups respectively. Apparently, calcium supplements reduced the risk of fractures significantly among healthy individuals; however, this beneficial effect seemed to disappear after the treatment was stopped. Findings from another systematic review evaluating 14 RCTs were inconsistent; the authors reported that vitamin D may reduce risk of fracture only when consumed in large doses with calcium in institutionalized elderly people (Cranney et al., 2008). In the latest meta-analysis conducted on vitamin D supplementation studies, the authors examined 12 and 8 (nonvertebral and hip fracture trials), this time incorporating both dose and adherence to supplement intake (Bischoff-Ferrari et al., 2009). The authors pooled trials with vitamin D doses > 10 μg (400 IU) per day. A "received dose" was calculated by multiplying dose taken by adherence (dose X adherence). The "received dose" allowed better assessment as opposed to "treatment dose". The authors concluded that nonvertebral fractures are dose dependent with vitamin

D consumption. When adherence is taken into consideration. higher doses of vitamin D may reduce fractures by 20% in people > 65 years.

2.5.3 Vitamin D and functional performance measures

Some cross-sectional studies have demonstrated a positive association between low 25(OH)D concentrations and low physical functional status assessed by different functional test batteries, or muscle strength tests; however, cohort studies and supplementation RCTs gave conflicting findings, the following section describes these studies in detail.

2.5.3.1 Association of 25(OH)D and functional status in cross-sectional studies

Many cross-sectional studies have investigated the relationship between vitamin D status and muscular functional capacity, but the direction of effects is unclear. In these studies the strength of associations differed with different muscle strength or muscle function tests and depended on gender and 25(OH)D concentrations. In one cross-sectional study, Zamboni and colleagues (2002) reported an association between vitamin D and muscle strength (isometric knee and arm extensors tested by a hand held dynamometer) as well as self reported physical function (n = 269, mean age = 72 years); increased disability in women but not men was associated with hypovitaminosis (< 37.5 nmol/L), after adjusting for BMI, albumin, appendicular fat-free mass and season; however, this study had not controlled for physical activity which is an important confounder. In another study Bischoff-Ferrari and colleagues (2004) showed a significant association between vitamin D status and lower-extremity strength in 4100 ambulatory men and women > 60 years old. A 5.6% lower 8-foot walk time for participants (n = 820) in the highest 25(OH)D quintile

(86.1 – 400.1 nmol/L) and a 3.9% lower sit-to-stand time were shown compared to participants (n = 821) from the lowest quintile (8.7 – 43.4 nmol/L) (Bischoff-Ferrari et al., 2004). Houston and colleagues (2006) reported in a cross-sectional study investigating 976 community-dwelling men and women (age 78.4 years) that serum 25(OH)D < 25 nmol/L was significantly associated with lower performance levels on short physical performance battery scores (walking speed, ability to stand from a chair, ability to maintain balance) and handgrip strength test in men. In women, only handgrip strength was associated with vitamin D status (Houston et al., 2006). It is worth mentioning that participants with missing measurements were excluded and were more likely to be older female subjects. These findings were not in agreement with a Japanese study that reported no association of vitamin D concentrations of less than 40 nmol/L and handgrip strength in 75 women with a mean age of 74.3 years (Nakamura et al., 2006). Different findings between men and women were reported in a Japanese study investigating the association of both serum 25(OH)D and serum albumin with several functional status and muscle strength tests in 1094 men and women with average age of 77 years (Kwon et al., 2007). Knee extension power (muscle strength) (67.1 Nm *vs* 83.9Nm) and time up and go (muscle function) (7.4 *vs* 6.0 seconds) were significantly different between the lower serum 25(OH)D and albumin mg/dl and the higher 25(OH)D group in men. While in women, handgrip (muscle strength) (14.1 *vs* 18.1 kg) and functional reach (balance) (29.3 *vs* 33.5 cm) differed significantly between the highest and lowest serum 25(OH)D and albumin groups, when adjusting for age and BMI. In another Dutch study where 48% of the participants had serum 25(OH)D < 50 nmol/L, investigators reported an association between serum 25(OH)D and physical performance measures assessed as the sum of scores of walking test, chair stands, and a tandem test. In the 1234 men and women with

mean age of 75.3 years, the strength of the association leveled off for 25(OH)D concentrations > 50 nmol/L (Wicherts et al., 2007) indicating the potential importance of very low levels of vitamin D. These studies have not investigated the nutritional status of the participants or any nutrients consumed, with the exception of one study (Bischoff-Ferrari et al., 2004) in which dietary calcium consumption was adjusted for as reported from 24 hour recalls.

2.5.3.2 Association of 25(OH)D and functional status in cohort studies

Cohort studies investigating participants in residential care as well as community dwelling elderly yield conflicting findings. Four studies reported no association between 25(OH)D concentrations and functional decline, while the other two studies showed an association of vitamin D status and decline in muscle function or muscle strength. The strength of the association was attenuated as the 25(OH)D approached optimal concentration. A four - year prospective multi-center cohort study (Faulkner et al., 2006) investigated 398 community-dwelling women participating in the Study of Osteoporotic Fractures. There was no association between 25(OH)D concentration and change in neuromuscular function (chair stand, walking speed and balance - walk time) or muscle strength (handgrip, and quadriceps strength) when adjusted for age, BMI, height, clinical site, season of serum collection, education, ethnic origin, physical activity, smoking, dietary calcium intake and osteoporosis. In a 2 - year study that followed 80 frail Japanese elderly, no association of handgrip strength with 25(OH)D concentration was found (Nakamura et al., 2007). In this study the participants were frail elderly with a mean age of 82.1 years with a mean baseline 25(OH)D concentration of 54.6 nmol/L. Data from 1002 disabled community-dwelling women from the Women's Health and Aging Study

(WHAS) were analyzed to examine the relation between vitamin D status and muscle strength and function in a 3 year follow-up (Verreault et al., 2002). All muscle strength (knee extensor, hip flexor, and handgrip) and muscle function (walking speed, repeated chair stands) measures were not associated with vitamin D status. The study excluded women on vitamin D supplements, which is unfortunate, as the investigators cannot rule out that high levels are better. Despite the fact that the women participating in this study were moderately to severely disabled, the proportion of women with severe vitamin D deficiency was low. In Sweden, 986 ambulatory elderly females aged 75 years recruited form the Malmo Osteoporosis Prospective Risk Assessment Study (OPRA) were followed up for 3 years to examine the association between vitamin D levels, functional performance (Gerdhem et al., 2005). The difference in gait speed was not significantly different from the higher 25(OH)D concentarions of 80 – 222 nmol/L. In another study, 979 Dutch males and females recruited from the Longitudinal Aging Study of Amsterdam, with a mean age of 75.3 years (Wicherts et al., 2007), investigators reported a 16 % decline in functional performance when participants were followed-up for 3 year. This was the only study that measured the physical performance parameters as the main outcome. Participants with serum 25(OH)D concentrations below 25 nmol/L, comprised 11% of the study population, showed the highest risk of performance decline in the 3 year follow-up (OR=2.21). This study did not provide data on dietary intakes of vitamin D from food and supplements or out door activity, the study adjusted however for age, gender, number of chronic diseases, degree of urbanization, BMI, alcohol consumption and physical activity and smoking. In another Dutch study, 1008 participants aged between 55 - 85 years were followed up for 3 years (Visser et al., 2003). Participants with 25(OH)D concentrations < 25 nmol/L were more likely to have declines in handgrip

strength (OR= 2.56) when compared to those with 25(OH)D concentrations > 50 nmol/L after adjusting for age, sex, smoking, serum creatinine concentrations, chronic disease, season, BMI and activity level reported in a questionnaire, however, no data regarding nutritional status and intake were investigated.

2.5.3.3 Association of 25(OH)D and functional status in supplementation clinical trials

Equally conflicting findings regarding the association of vitamin D with functional decline were reported in randomized clinical trials. Two studies conducted in institutionalized or participants with poor health conditions indicated a positive association between groups supplemented with vitamin D and functional decline reported in comparison to groups with no supplementation. No such association was seen in healthy community dwelling men when supplemented with vitamin D and calcium. Details of these important studies follow: In one randomized double blinded clinical trial, 139 males and females recruited from clinics servicing chronic fallers, with serum 25(OH)D concentrations < 30 nmol/L and a mean age of 77 years were randomized to receive a single 15,000 µg (600, 000 IU) intra muscular injection of ergocalciferol or a placebo of 2 ml normal saline injection (Dhesi et al., 2004). After six months, significant improvement in functional performance and balance was observed in the treatment group as compared to placebo, however, muscle strength did not improve. In a double blinded randomized controlled study, 122 elderly women with a mean age of 85.3 years, residing in a geriatric care facility were investigated (Bischoff et al., 2003). Participants were randomized to either 1200 mg calcium/day or to 1200 mg calcium plus 20 µg (800 IU) vitamin D/day for 12 weeks with number of falls measured as the primary outcome and muscle strength and one measure of muscle function TUG as secondary outcomes. An

overall musculoskeletal function was assessed by a summed score of one muscle function (TUG) and 3 muscle strength tests (knee flexor strength, knee extensor strength and hand grip strength). Although differences between supplemented and non-supplemented groups were not significant for each test independently, a significant improvement in the overall musculoskeletal function was significant between the treatment and control group ($p = 0.0094$). In the only study to examine the impact of vitamin D supplementation in healthy elderly examined 65 community-dwelling healthy men with a mean age of 76 years, subjects were provided with 500 mg of calcium daily and randomized to 25 μg (1000 IU/day) of vitamin D or placebo for 6 months. No significant difference between groups was reported in muscle strength and physical performance (Kenny et al., 2003). Given the inconsistent results in the three previous studies, the relation between supplementation with vitamin D and muscle strength remains equivocal.

2.5.4 Vitamin D and falls

There is a lack of consensus on a clear effect of vitamin D supplementation on reducing risk of falls or fallers. In one clinical trial where 64 institutionalized women (age range = 65 - 97 years) were randomized to 1200 mg calcium plus 20 μg (800 IU) vitamin D *vs* 1200 mg calcium alone, calcium and vitamin D reduced falls by 60 % (Bischoff-Ferrari et al., 2006). When a regression analysis was undertaken, investigators reported that 22% of the treatment effect was explained by a change in postural balance; investigators believe that the prevention of falls by vitamin D may be mediated by a change in postural balance. In another vitamin D supplementation study, 124 nursing home residents (mean age = 89 years) were randomized to 0, 5, 10, 15, 20 μg (0, 200, 400, 600, 800 IU) /day vitamin D for 5 months, those who consumed 20 μg (800 IU) of vitamin D has a 72% lower

adjusted incidence rate ratio of falls compared to the placebo, no significant differences were observed among the other treatments *vs* placebo, or among the 5 treatments (Broe et al., 2007). On the other hand, when 243 frail hospitalized older people (mean age = 79 years) were randomized to a single dose of vitamin D 7,500 μg (300,000 IU) or placebo and 10 week quadriceps resistance exercise and assessed after 3 and 6 month follow up, no effect of either intervention was detected on falls (Latham et al., 2003). Although, Cranney and colleagues found the association between vitamin D intake and reduction in number of falls to be fair from reviewing 14 RCTs, the authors also cautioned from the different falls ascertainment methods across the different studies, and the different methods of vitamin D administration and doses (Cranney et al., 2008). In a recently conducted meta-analysis, authors reported that unlike fractures, vitamin D consumption may not have an effect on fall reduction (Bischoff-Ferrari et al., 2010 in press).

Although many of the previous studies examining 25(OH)D concentrations and muscle strength, function, balance, fracture and fall occurrence suggest an association; it is not yet clear at which 25(OH)D concentrations will vitamin D repletion be effective in improving the outcomes in question.

2.6 Conclusion

Despite the considerable number of studies conducted to assess prevalence of vitamin D status in hospitalized and in clinical groups globally, very little information is known about prevalence of vitamin D deficiency in health free living populations worldwide and in Canada specifically. The literature is equally lacking on data regarding dietary vitamin D intakes from foods and supplements in healthy older people in Canada. Data suggesting

an association of vitamin D deficiency and functional capacity come from cross-sectional studies that cannot prove causality, and findings from longitudinal cohort studies are equivocal, especially in healthy older people. Thus, these outstanding questions remain:

- How is vitamin D status distributed in a healthy population of older people as opposed to the often described status in institutionalized/ hospitalized or those with clinical health problems related to bone health?
- Are the current vitamin D AIs set for older people appropriate to achieve optimal vitamin D status? Can these requirements be met by habitual dietary intakes?
- What foods contribute to most vitamin D intake in older people?
- Does vitamin D status predict physical functional decline in healthy older people?

The following chapters of this dissertation will attempt to answer these questions.

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Bridge 1

From reviewing the literature, little is known about vitamin D status in ambulatory, healthy, free living; community dwelling elderly in North America. Consumption of vitamin D from its limited dietary sources may be problematic especially for the elderly population. Additionally, vitamin D synthesis is challenged in this age group (MacLaughlin and Holick, 1985). Knowing how important vitamin D is for bone health, and knowing that vitamin D status may play a role in functional capacity declining with age, we are compelled to examine vitamin D status in an older population. The NuAge cohort study is a population-based longitudinal study examining nutrition as a determinant of successful aging. The NuAge study sample of 1793 older men and women was recruited from Québec health insurance registry (RAMQ), and stratified by sex, season, and 3 age strata; 70 ± 2 years, 75 ± 2 years and 80 ± 2 years. Biological, dietary, anthropometric, physical performance measures and other socio-demographic measurements were collected at baseline and annually for 5 years. The availability of data and measurements from the NuAge study gave us the opportunity to design our study and enabled us to test the hypothesis for this dissertation.

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CHAPTER 3. MANUSCRIPT 1

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Vitamin D Status in Healthy Free Living Elderly Men and Women Living in Québec – Canada

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3.1 Abstract

Objectives: To assess vitamin D status in relation to age, sex, season, adiposity, physical activity level and supplement use in healthy elderly Canadians living in Québec.

Design: Cross-sectional survey

Setting: Data for 405 healthy free-living elderly Quebecers aged between 68 – 82 years with no major health problems were examined.

Subjects: Men and women in age groups 68 - 72, 73 - 77 and 78 - 82 years in two seasons participating in the NuAge cohort. Measurements: Serum 25(OH)D assessed by radioimmunoassay (RIA), weight, height, smoking status, disease status, education, perception of income, Physical Activity Scale for the Elderly questionnaire (PASE) and vitamin D supplement consumption.

Results: The prevalence of vitamin D deficiency (25(OH)D < 37.5 nmol/L) was 12.6 % and 5.7% for men in winter and summer, and 8.7 % and 1.9 % for women, respectively. Over 50 % had 25(OH)D concentrations < 75 nmol/L. Correlates of Vitamin D status by multiple regression analysis indicate that the vitamin D level in summer was 13.8 nmol/L higher than those measured in winter and 17.2 nmol/L higher for those using supplements. Age, sex, body mass index, and physical activity were not associated with 25(OH)D concentration.

Conclusion: Prevalence of vitamin D deficiency is low in healthy elderly men and women, especially in summer, but over 50% of the participants had sub-optimal vitamin D concentrations. Vitamin D supplement consumption increased 25(OH)D concentrations substantially, particularly in the winter months and should be encouraged.

Key words: Community–dwelling elderly, vitamin D status, Québec population

3.2 Introduction

The contribution of vitamin D to bone health across all age groups is well established (Calvo et al., 2005; Heaney, 2007; Holick, 2006). Several epidemiological studies and few clinical trials have linked vitamin D status to hypertension (Hintzpeter et al., 2008; Krause et al., 1998; Rostand, 1997) and cardiovascular disease (Pilz et al., 2008; Zittermann et al., 2003). Vitamin D is also believed to have an immunomodulatory effect and there are reported links to type I diabetes (Hypponen et al., 2001), psoriasis (Perez et al., 1996), multiple sclerosis (Losy & Michalowska – Winder, 2002; Munger et al., 2004) and rheumatoid arthritis (Merlino et al., 2004). In addition, there are reports that vitamin D is associated with breast cancer (Bertone – Johnson et al., 2005), colon cancer (DeLuca, 2004; Slattery et al., 2004) and prostate cancer (Chen & Holick, 2003). These may be due to the action of vitamin D on the proliferation and differentiation of cells (Van den Bemd & Chang, 2002).

Despite the fact that vitamin D can be synthesized dermally, this route for acquiring vitamin D is challenged for a substantial part of the year in people residing in countries that are located on latitudes over 40º in the northern or southern hemispheres (Institute of Medicine, 1997). Pollution, cloud cover and sunscreen use also interfere with the dermal synthesis vitamin D (Lips, 2006). Older age is an additional risk factor owing to the reduced efficiency of the dermal conversion of 7-dehydrocholesterol in the presence of UVB-light (MacLaughlin & Holick, 1985; Webb et al., 1988). Additionally, meeting the adequate intake (AI) for vitamin D for the elderly, 10 µg (400 IU)/day for ages 51 – 70 years and 15 µg (600 IU)/day for > 70 years (Institute of Medicine, 1997), is challenging to achieve without additional supplementation. The recommendations of the Canadian

Food Guide to Healthy Eating , includes 10 μg (400 IU) of vitamin D consumed as a supplement and the 2005 Dietary Guidelines for Americans also suggest the use of vitamin D fortified food and vitamin D supplements (Health Canada, 2005; USDA, 2005).

Vitamin D status has been examined in a broad range of ages in North America; two studies assessed vitamin D status in free living men and women exclusively in low income populations (Delvin et al., 1988; Harris et al., 2000) while clinical population groups (Vecino – Vecino et al., 2006) and institutionalized and home-bound elderly populations have been studied more extensively (Gloth et al., 1995; Golray et al., 1089; Omadahl, 1989; Thomas et al., 1998; Vaqueiro et al., 2006). In Sweden 78 % of men and women of ages 79-96 years had sub-optimal levels of vitamin D, defined as serum 25(OH)D concentrations < 75 nmol/L (Melin et al., 2001). In Dutch free-living elderly men and women, 51 % of the participants had serum 25(OH)D concentrations < 50 nmol/L. Greater adiposity and less time spent on outdoor activities were associated with worse vitamin D status (Van Dam et al., 2007). In a study investigating vitamin D status in 759 men and women from the Framingham Heart Study cohort, only 6.2% of men and 14.5% of women had 25(OH)D concentrations < 37.5 nmol/L (Jacques et al., 1997).

As little is known about vitamin D status in healthy aging Canadians, our objective was to assess vitamin D status among healthy elderly of different ages and examine vitamin D status in relation to season, adiposity, physical activity and supplement use. The population studied was sampled from the NuAge cohort study, which provided stored blood samples, anthropometric measurements, and data from questionnaires on physical activity, education and other variables.

3.3 Materials and methods

3.3.1 Subjects

A sample of 405 men and women from the NuAge cohort study of 1793 independently-living French or English speaking Canadians (98.4 % Caucasian), aged 68 to 82 years was studied. The NuAge sample was recruited from a stratified sample from three age-sex categories. Seventy ± 2 years, 75 ± 2 years and 80 ± 2 years, these groups were chosen as those at 65 may be still working, and healthy participants of 85 years become increasingly difficult to recruit. The NuAge sample was obtained from a random sample within each age-sex stratum defined above from the Québec health insurance registry (RAMQ) which provides health care for the entire population. Subjects were sent an invitation letter and telephoned to assess eligibility and willingness to participate. Only community dwelling people were contacted. Inclusion and exclusion criteria were assessed in two phases; a telephone screening survey and a clinical examination. Participants who spoke English or French, were free of disabilities in activities of daily living, had no cognitive impairment, were able to walk one block or to climb one flight of stairs without rest, and were willing to commit to a 5-year study period were recruited. The sample examined in a cross-sectional design (n = 405) was randomly selected from all interviews conducted in the NuAge cohort between January and March (period of no dermal synthesis of vitamin D) and between June and September inclusively after stratification by age, sex and season. The data for the two seasons were obtained from different people. In the NuAge cohort study each participant was interviewed annually at the same time of year, so that there was no opportunity to re-measure the same individual in different seasons. Frozen serum samples which had been stored at -80ºC were originally collected after a 12-hour fast between January 2005 and March 2006. The research protocol was approved by the ethics

committees of the Institut universitaire de gériatrie de Montréal, University Institute of Geriatrics of Sherbrooke and McGill University. All participants provided written informed consent after being fully informed of the study objectives and procedures and their right to withdraw from the study at any time.

3.3.2 Data collections and measurements

Serum 25(OH)D concentration was measured by radioimmunoassay (RIA) using a commercial kit (DiaSorin, REF: 68100E, Stillwater, MN, USA) at McGill University, with an inter-assay CV < 8%. The assay measures both $25(OH)D_2$ and $25(OH)D_3$. All samples were analyzed in duplicate and 76% had agreement of ≥ 90 %. Aliquots with < 80 % agreement were discarded; two new samples were tested until agreement of at least 80% was reached and the mean of the two repeated measures was used. Optimal vitamin D status was defined as > 75 nmol/L (Dawson–Hughes & Heaney, 2004), sub-optimal status between 37.5 and 79.4 nmol/L (Dawson–Hughes & Heaney, 2004) and vitamin D deficiency as < 37.5 nmol/L (Institute of Medicine, 1997). In terms of accuracy, the low control on average was 52.9 nmol/L and was within the manufacturer specification 23.3 – 57.5 nmol/L, and the high control on average was 119.5 nmol/L and within manufacturer specification 85.0 – 202.0 nmol/L.

Demographic and socioeconomic information, smoking status and supplement use, disease status and the Physical Activity Scale for the Elderly questionnaire (PASE) were collected through a structured computer-assisted interview carried out the same day as the blood draw. Standing height and weight were measured on the same day with subjects dressed in light indoor clothing without shoes, using a stadiometer and a beam balance.

3.3.3 Sample size and statistical analysis

We sampled 405 subjects and analyzed their 25(OH)D concentration levels. Based on a mean 25(OH)D concentration of 74 nmol/L, with SD of 30 from our data, this sample provides 80% power to detect a difference in 25(OH)D concentration between men and women of 8.3 nmol/L. The detectable difference by age group is limited to differences of at least 10.5 nmol/L.

We examined the distribution of 25(OH)D concentrations presented as means and confidence intervals and examined differences by age-sex group using one-way ANOVA. The chi-square test was used to test differences in percentages; Fisher's exact test was used where applicable. Correlations were estimated using Pearson's correlation. Independent correlates of serum 25(OH)D were examined using multiple regression analysis. Statistical inferences were made based on a two-sided significance level of $p < 0.05$. All statistical analyses were performed using SAS version 9.1 (SAS institute Inc, Cary, NC, USA).

3.4 Results

Characteristics of the sample stratified by sex are shown in **Table 3.1.** The participants were generally healthy elderly community dwelling Quebecers. Questionnaire data revealed that 58.0 % of the participants self-reported arthritis, 55.0 % hypertension, 12.8 % diabetes, 33.8 % gastric problems, 4.6% liver ailments, 5.7 % kidney ailments and 4.1 % thyroid problems. Serum 25(OH)D concentrations ranged from 17 – 175 nmol/L for men and 26 – 185 nmol/L for women. Seasonal differences in means for 25(OH)D in each age group for both sexes are presented in **Table 3.2.** The subjects tested in the

different seasons did not differ in any of the characteristics listed in **Table 3.1** with the exception of PASE where participants were more active in summer ($p = 0.037$). Mean serum 25(OH)D concentrations were significantly higher in June through September relative to January through March in both men and women ($p < 0.001$). When stratified by age, the trend in the seasonal effect was similar across all age sex groups, but owing to reduced statistical power with stratification in 6 age sex groups, a significant difference was observed only in men aged 73 - 77 years. Within each season and sex group, age was not associated with 25(OH)D concentration.

The percentages of men and women at different serum 25(OH)D cut-off levels by season and supplement use are presented graphically in **Figures 3.1 and 3.2** for men and women, respectively. Those without supplements were most likely to be deficient. In winter the differences between supplement users and non-users were statistically significant whereas in summer the trend was also for supplement users to be more likely to have optimal status but the difference was not statistically different. In winter, no vitamin D deficiency was observed among vitamin D supplement users and in summer there was only one man who had deficient levels.

Age, sex, BMI, education, perception of income, smoking and PASE score were not associated with 25(OH)D concentrations. The reporting of arthritis, diabetes, gastric problems or hypertension was also not related to 25(OH)D concentration. Using multiple linear regression, independent correlates of serum 25(OH)D, age, sex, season and vitamin D supplement intake were examined. Summer season and intake of vitamin D containing supplements (vitamin D, cod liver oil and multivitamins), were strong independent

correlates of 25(OH)D concentration in this multiple regression analysis (**Table 3.3).** Those tested in summer or early fall had 25(OH)D concentration 13.8.nmol/L higher than those tested in winter, whereas, those consuming supplements containing vitamin D had 25(OH)D concentrations 17.2 nmol/L higher than those who were not. There was no effect of age or sex in the multivariate model.

Patterns of consumption of vitamin D containing supplements among users were as follows: 47% of participants consumed only calcium/vitamin D supplements (varying concentrations of calcium with 400 IU of vitamin D); 17 % consumed only multivitamin tablets; 10% consumed vitamin D alone (usually 400 IU). The remaining supplement consumers (26%) consumed 2 or more of the previous types of supplements.

3.5 Discussion

This is the first study in Canada to assess vitamin D status in elderly free-living healthy men and women in relation to age, supplement use and season. In our study serum 25(OH)D concentrations for men and women of all ages averaged 66.7 nmol/L in winter and 80.8 nmol/L in summer. The prevalence of suboptimal status in elderly Quebecers was relatively high, particularly in winter. Sunlight exposure in summer and vitamin D containing supplements contributed independently to the adequacy of vitamin D status. Within the 15 year age range studied, there were no effects of sex or age on vitamin D level.

In our study, the mean 25(OH)D concentrations in men and women were consistent with those reported from the Framingham Heart Study cohort (Jacques et al., 1997), but tended

to be higher than those reported for an elderly community dwelling cohort (Vecino – Vecino et al., 2006) recruited from hospital clinics in Québec, where researchers reported average serum 25(OH)D to be 64.3 nmol/L. Subjects with poor health status may have been included. Among osteoporotic women in 18 countries, the prevalence of serum 25(OH)D concentrations < 75 nmol/L, ranged from 37 % to 92 % (Lips et al., 2006). In hospitalized elderly populations in Minnesota (Simonelli et al., 2005) the prevalence of 25(OH)D < 75 nmol/L, was > 90 %. It would appear that health status is related to vitamin D sufficiency.

The lower levels of deficiency and sub-optimal levels observed in summer as compared to winter in our study were in agreement with other studies (Lips et al., 2006; Tangpricha et al., 2002). The percentage deficient was clearly reduced in the summer months. In our study, summer was associated with an average 13.8 nmol/L higher concentration of serum 25(OH)D. Also, independently of season, vitamin D supplement use showed a clear effect, a 17.2 nmol/L increase in serum 25(OH)D. Sex had no effect on vitamin D status in our study which is consistent with a Swedish study investigating vitamin D status in community-dwelling elderly (Melin et al., 2001). In contrast with two other studies (Lips et al., 2006; Simonelli et al., 2005), however, age was not a predictor of low vitamin D status in our sample of generally healthy elderly over a 15 year age range. However, in these two studies, participants' age ranges were much broader than in our study.

Although vitamin D status may be a problem in an institutionalized group of elderly people, particularly if they are not receiving supplements or exposed to UVB light, this was less evident in our study of an essentially healthy elderly group. Studies of vitamin D

status in individuals as they age have not been performed to date and it may be that the reported decline with age is more due to illness than aging *per se.* This distinction remains to be elucidated. Despite the wide range of BMI values in our sample, adiposity, measured as BMI and was not a correlate of vitamin D status. This was in contrast to other studies of younger subjects (Lappe et al., 2006; Rucker et al., 2002). Within older subjects the association of 25(OH)D with BMI, weight (Dawson – Hughes et al., 1997), or fat percentage (Bolland et al., 2006) were present and only detectable when measured by more precise measures such as dual-energy x- ray absorptiometry (Lucas et al., 2005; Snijder et al., 2005;). Looker et al. confirmed this interaction by comparing younger and older subjects and found the inverse association of body fat and 25(OH)D was weaker in the older age group when compared to younger subjects (Looker, 2005). Our study was not able to address the issue of possible differences by skin color as a small minority of French or English speaking Quebecers are of non – European ancestry.

3.6 Conclusion

In conclusion, although vitamin D deficiency was not frequent in this sample of healthy elderly participants, a large percentage had concentrations of 25(OH)D below optimal levels of vitamin D, particularly among those assessed in winter. Those taking supplements had a substantially higher concentration of 25(OH)D (17.2 nmol/L) reinforcing the recommendation of obtaining vitamin D from both food and supplements for all older healthy individuals. Supplement use is particularly important during the winter months.

3.7 Acknowledgements

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3.8 Titles and legends to figures

Titles:

Figure 3.1. Percentage of optimal, sub-optimal and deficient concentrations of 25(OH)D in men by season and supplement use

Figure 3.2. Percentage of optimal, sub-optimal and deficient concentrations of 25(OH)D in women by season and supplement use

Legends:

Figure 3.1. Black represents 25(OH)D < 37.5 nmol/L, white represents 25(OH)D between 37.5 – 75 nmol/L, gray represents 25(OH)D >75 nmol/L

Figure 3.2. Black represents 25(OH)D < 37.5 nmol/L, white represents 25(OH)D between 37.5 – 75 nmol/L, gray represents 25(OH)D >75 nmol/L

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Table 3.1. Baseline Characteristics of a Sample of Men (n = 208) and Women (n = 197) Analyzed for Vitamin D Status in Québec / Canada

	Men		Women	
	n	%	n	%
Age				
68 - 72 years	70	34	70	36
73 - 77 years	69	33	57	29
78 – 82 years	69	33	70	36
Site				
Sherbrooke	122	59	116	59
Montreal	86	41	81	41
Season				
January - March	103	50	92	47
June - September	105	50	105	53
Non-smoking	192	92	191	97
Perception of income				
Very adequate	94	45	91	46
Adequate	109	52	101	51
Not adequate	5	2	4	2
Not adequate at all	-	-	1	1
Vitamin D supplement users	35	17	88	45

Table 3.1. Continued

	Men		Women	
	n	Mean (SD)	n	Mean (SD)
Weight, kg	207	78.5 (14.2)	197	66.1 (11.3)
Height, m	206	1.70 (0.10)	197	1.60 (0.10)
Waist circumference, cm	208	100.5 (12.0)	196	90.1 (10.9)
Body mass index, kg/m²	206	27.7 (4.4)	197	27.7 (4.7)
Education, years	208	11.7 (4.9)	197	11.0 (3.7)
PASE*	206	102.3 (50.8)	193	71.7 (38.3)

* PASE "Physical Activity Scale for the Elderly" questionnaire, score range (0-277), higher scores indicate higher levels of activity.

Table 3.2. Serum 25(OH)D Means (CI) in nmol/L for Men and Women by Age and Season

	January - March		June - September		
	n	Mean (CI*)	n	Mean (CI*)	p value
Men					
68 - 72 years	35	68.5 (56.9 - 80.0)	35	74.6 (66.1 - 83.1)	0.390
73 - 77 years	34	55.2 (46.5 - 63.9)	35	80.2 (68.9 - 91.5)	< 0.001
78 – 82 years	34	65.3 (55.6 - 75.0)	35	76.3 (66.7 - 85.8)	0.107
		p = 0.146		p = 0.701	
All	103	63.0 (57.3 - 68.8)	105	77.0 (71.5 - 82.5)	< 0.001
Women					
68 - 72 years	35	67.4 (58.4 - 76.4)	35	84.3 (74.7 - 93.9)	0.111
73 – 77 years	22	72.2 (55.9 - 88.6)	35	88.1 (77.0 - 99.2)	0.093
78 - 82 years	35	73.0 (63.7 - 82.3)	35	81.4 (71.0 - 91.7)	0.224
		p = 0.701		p = 0.647	
All	92	70.7 (64.6 - 76.7)	105	84.6 (78.1 - 90.4)	< 0.001

* CI = Confidence Interval

Table 3. 3. Multiple Regression of Predictors of Means of Serum 25(OH)D Concentration

	β Coefficient	β error	t	p value
Intercept	68.83	25.27	2.72	0.007
Age, years	-0.34	0.33	-1.03	0.306
Sex, (1 = male, 2 = female)	2.85	2.96	0.96	0.337
Season, summer	13.78	2.82	4.89	< 0.001
Vitamin D supplement intake (Yes / No)	17.24	3.26	5.29	< 0.001

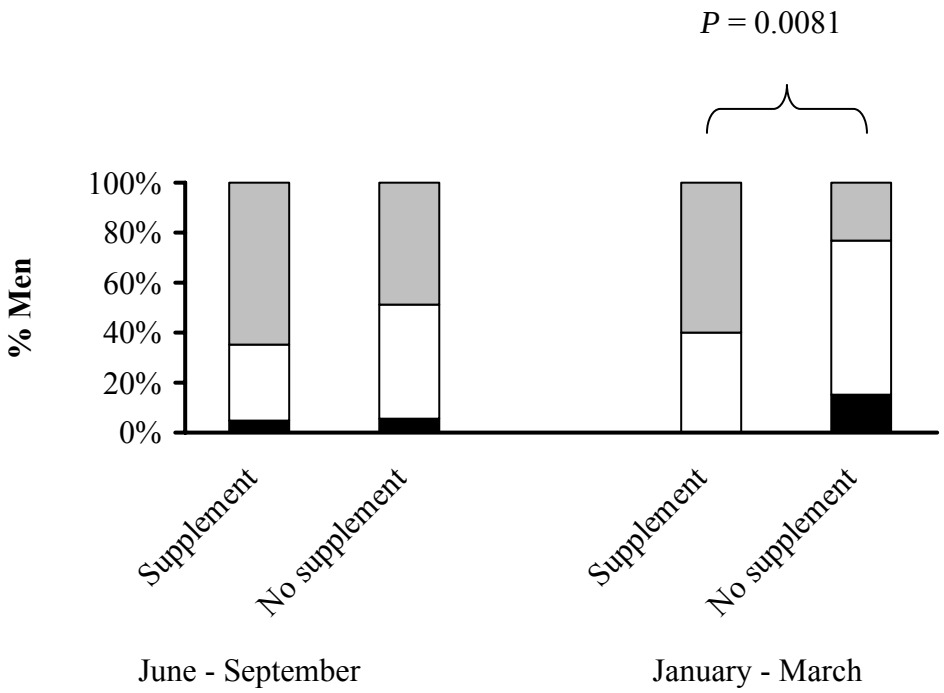

Figure 3.1. Percentage of Men with Optimal, Sub-optimal and Deficient Concentrations of 25(OH)D by Season and Supplement Use

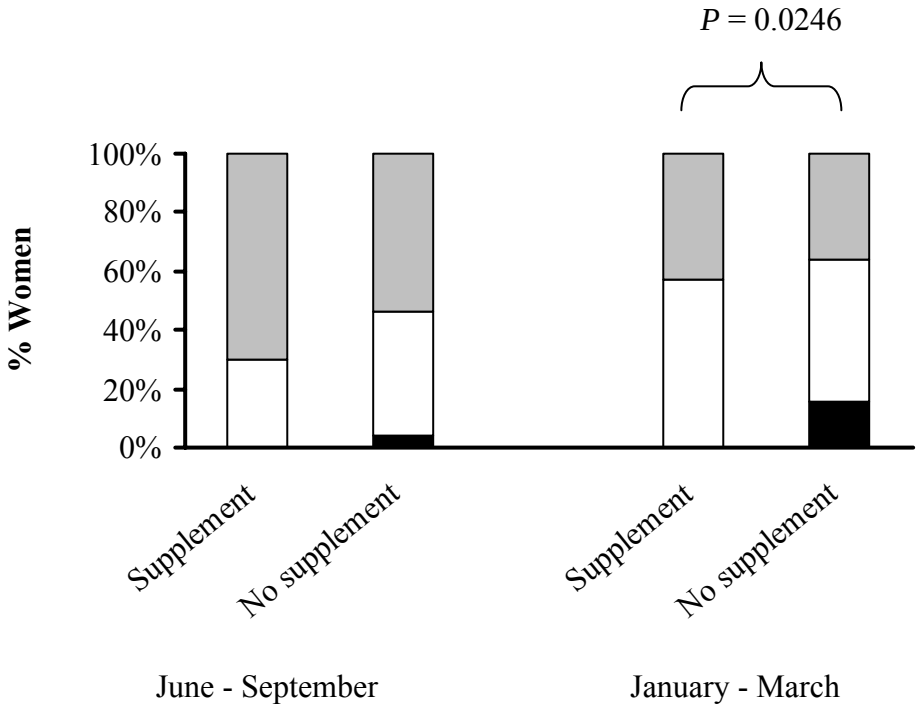

Figure 3.2. Percentage of Women with Optimal, Sub-optimal and Deficient Concentrations of 25(OH)D by Season and Supplement Use

Bridge 2

Compared to the high prevalence of vitamin D deficiency observed in hospitalized and older people with poor health conditions; it is apparent from our first manuscript that the healthy free-living population have a low prevalence of vitamin D deficiency as shown in Chapter 3. Only 7% of this population were vitamin D deficient, whereas, 48% had suboptimal status and 45% had optimal status. Intrigued by this result, we wanted to explore further the contribution of diet and or other factors that may have contributed to this result across the 3 groups of vitamin D status in our population to see what was required to reach optimal status. Additionally, we wanted to identify food groups that contribute most to vitamin D intake and examine predictors of optimal vitamin D status. We were also interested in ascertaining the level of vitamin D intake among subjects with optimal vitamin D status.

CHAPTER 4. MANUSCRIPT 2

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Vitamin D Supplement Consumption is Required to Achieve a Minimal Target 25 – Hydroxy Vitamin D Concentrations of ≥ 75 nmol/L in the Older People[1, 2]

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4.1 Abstract

Population level data on how older individuals living at high latitudes achieve optimal vitamin D are not fully explored. Our objective was to examine the intake of vitamin D among healthy older individuals with 25-hydroxy vitamin D [25(OH)D] concentrations ≥ 75 nmol/L and to describe current sources of dietary vitamin D. We conducted a population-based, cross-sectional study of 404 healthy men and women from (69 – 83 y) randomly selected from the NuAge longitudinal study, Québec, Canada. Dietary intakes were assessed by six 24-h recalls. We examined the contribution of foods and vitamin/mineral supplements to vitamin D intake. Serum 25(OH)D was assessed by radioimmunoassay (RIA). We assessed smoking status, season of 25(OH)D measurement, physical activity, and anthropometric and sociodemographic variables. Vitamin D status was distributed as: 7% (< 37.5 nmol/L), 48% (37.5 - 74.9 nmol/L) and 45% (≥ 75 nmol/L). Vitamin D intake from supplements varied across the 3 vitamin D status groups respectively; 0.5 µg, 4.1 µg and 8.9 µg ($P < 0.0001$). Adding food sources these total intakes were 4.6 µg, 8.7 µg, and 14.1 µg respectively. In multivariate analysis vitamin D from foods, supplements and by season were associated with vitamin D status. These healthy community-dwelling older men and women with 25(OH)D concentrations >75 nmol/L had average intakes of 14.1 µg/d from food and supplements. Supplement use is a significant contributor to achieve a minimal target of 25(OH)D concentration ≥ 75 nmol/L.

Key words: Dietary vitamin D intake, Supplements, Healthy elderly, Food groups, Minimal target 25(OH)D concentration

4.2 Introduction

Vitamin D deficiency and inadequacy has become a major public health concern in elderly populations. Severe vitamin D deficiency is known to cause osteomalacia and secondary hyperparathyrodism, high bone turnover, increased risk of fractures and osteoporosis may occur with sub-optimal vitamin D status (Lips, 2001). Additionally, recent epidemiological studies provide some support for an association of low circulating serum 25(OH)D concentration and some types of cancer (Giovannucci et al., 2006; Chen & Holick, 2003), diabetes (Hypponen et al., 2001), multiple sclerosis and other autoimmune diseases (Munger et al., 2004; Merlino et al., 2004).

The Dietary Reference Intake for vitamin D has been set at 10 μg/ d for ages 50 – 70 years using bone loss as an indicator of adequacy and 15 μg/ d for people > 70 y (Institute of Medicine, 1997). Although vitamin D is mainly acquired through dermal synthesis, this route is not available at certain latitudes in winter and may be affected by sun screen use, protective clothing and cloud cover (Lips, 2006). Dietary vitamin D is available through food sources including fortified milk and margarine in some settings, fatty fish, egg yolk, organ meats and mushrooms (Holick, 2004) as well as vitamin supplements. Given the high estimated needs for vitamin D in those over 70 y, older people face a challenge acquiring vitamin D. Reduced dermal synthesis is an independent risk factor in the elderly (MacLaughlin & Holick, 1985), in addition to concern about sun exposure including the use of sunscreen (Holick, 1994) and concealing clothing (Matsuoka et al., 1992). Reduced renal hydroxylation of 25(OH)D, decreased intestinal responsiveness to 1, 25 dihydroxy vitamin D [1,25$(OH)_2$D] (Gennari et al., 1990) and heavy use of

medication may further contribute to reduced vitamin D status (Walker–Bone et al., 2004; Di Munno et al., 2004).

There is no consensus on optimal 25(OH)D for the elderly. The suggestion of a minimum serum concentration of 75 nmol/L is based on bone mineral density, reduced fracture risk and lower extremity muscle strength (Bischoff-Ferrari, 2007). Although vitamin D experts favor a minimum serum 25(OH)D concentration between 70 and 80 nmol/L based on bone health; optimal concentrations may be much higher if we consider end- points other than skeletal health (Dawson – Hughes et al., 2005). Hence, in this manuscript we denote 25(OH)D concentrations of > 75 nmol/L as a "minimal target" vitamin D status required to achieve an acceptable vitamin D status. Dietary requirements needed to achieve serum thresholds of ≥ 75 nmol/L depend on baseline 25(OH)D concentrations, summer vitamin D tissue storage and variation of 25(OH)D concentrations across populations (Bischoff – Ferrari, 2007; McKenna, 1992). Several vitamin D supplementation randomized clinical trials suggest vitamin D intakes of 15 – 25 μg to reach serum 25(OH)D values of 75 –112 nmol/L (Vieth et al., 2004; Chapuy et al., 1992; Dawson – Hughes, 2008). In a recent 22-wk vitamin D supplementation trial, 225 elderly healthy subjects aged ≥ 64 y were randomized to 0, 5, 10 and 15 μg vitamin D/ d (Cashman et al., 2009). The author reported that a daily intake of 8.6 μg/d vitamin D maintained serum 25(OH)D above 25 nmol/L in 97.5% of the study sample and extrapolated to estimate that 42.8 μg/ d of vitamin D was required to ensure a level of serum 25(OH)D above 80 nmol/L for most (97.5%) of the population. However, DRI values were set to maintain status and not to remediate deficiency. Thus values to sustain 75 – 80 nmol/L may be less.

The current vitamin D set by the DRI committee on the dietary vitamin D intake recommended for healthy elderly over 70 y of age at 15 μg is lower than that recommended by some researchers. Thus, in this study we aimed to examine the intake of vitamin D among healthy elderly individuals with 25(OH)D concentrations > 75 nmol/L and to determine the best available vitamin D dietary sources needed to reach this level in a healthy aging population in Québec, Canada (Latitude = 45.7°).

4.3 Subjects and Method

4.3.1 Study setting and subjects

Data for this study were obtained from the NuAge cohort study of 1793 independently-living predominantly Caucasian men and women aged 68 to 82 y. The NuAge participants were recruited from the Québec health insurance registry (RAMQ) and were stratified by sex and three age groups 70 ± 2, 75 ± 2 and 80 ± 2 y with equivalent numbers in each stratum. Community-dwelling individuals were invited by mail to assess their eligibility and willingness to participate, after which, they were assessed by both telephone screening survey and clinical examination. Only participants who were free of disability in activities of daily living, were able to walk one block or to climb one flight of stairs without rest, had no cognitive impairment, spoke English or French and were able to commit to a 5 y follow-up period were recruited. The research protocol was approved by the ethics committees of the Institut universitaire de gériatrie de Montréal, University Institute of Geriatrics of Sherbrooke and McGill University. All participants provided written informed consent.

The sample of 404 subjects for the vitamin D study was selected from a random sample of all interviews conducted at baseline in the NuAge cohort between January and March 2005 (period of no dermal synthesis of vitamin D) and between June and September 2006, inclusively after stratification by age, sex and season. These sampling time-frames were selected to represent extremes for least likelihood (winter: January through March) of and greatest likelihood (summer: June through September) of endogenous synthesis of vitamin D. Fasting blood samples collected at the first annual follow-up were used for vitamin D analyses so as to have dietary data prior to measuring serum. Six 24-h dietary recalls were collected 3 at a time, 6 mo apart.

4.3.2 Data collection and measurements

4.3.2.1 Biochemistry

Blood samples were collected after a 12-h fast, centrifuged and stored in aliquots at - 80º C (2005 – 2006). Serum 25(OH)D was assessed by radioimmunoassay (RIA) using a commercial kit (DiaSorin, REF: 68100E, Stillwater, MN, USA) at McGill University (2007 – 2008), with an inter-assay CV < 8%. The assay measures both $25(OH)D_2$ and $25(OH)D_3$. All samples were analyzed in duplicate; data were expressed in nmol/L with values for "deficiency status" set at < 37.5 nmol/L (Institute of Medicine, 1997), "insufficiency status" at 37.5 to 74.9 nmol/L and "minimal target" at ≥75 nmol/L (Dawson – Hughes et al., 2005). In terms of accuracy, the kit's low control on average was 52.9 nmol/L and was within the manufacturer specification 23.3 – 57.5 nmol/L, and the high control on average was 119.5 nmol/L and within manufacturer specification 85.0 – 202.0 nmol/L.

4.3.2.2 Diet

Six 24-h dietary recalls were collected; three non-consecutive 24-h dietary recalls including one weekend day (1 face-to-face and 2 telephone interviews) and another three non-consecutive 24-h dietary recalls were done by telephone. These two sets of three 24-h recalls were gathered 6 mo apart. A 24-h recall consisted of a detailed description of all foods and beverages consumed during the previous day, including cooking methods and brand names. Repeated interviews provided precise estimates of usual energy and nutrient intakes (Payette & Gray – Donald, 1991). Portion size models aided in the estimation of quantities consumed. The recalls were done by registered dietitians who followed a rigorous 10 d training period. An average intake from the 6 24-h recalls was used to measure dietary intake of vitamin D from both sources ergocalciferol D_2 and cholecalciferol D_3. These 6 d of recall reliably measured usual intake of most nutrients including protein, calcium, vitamin D, phosphorus and magnesium given the somewhat reduced day-to-day variability observed in elderly populations (Payette & Gray – Donald, 1991). Dietary analysis was done using the CANDAT program (Godin and Assoc, London ON) which uses the 2007b Canadian Nutrient File, Health Canada, and was augmented by a database of >1200 additional foods that were developed on site (Johnson – Down et al., 2006). The program allowed for the development of tailor-made food groupings, which was used to examine the extent to which each vitamin D containing food component, or food group, contributed to nutrient intake. Supplement use was recorded at each recall and entered based on Drug Identification Numbers (DIN). Where the exact composition of the supplement was not known a default value of the most common supplement of that type (eg 500 mg for a calcium supplement) was used (Troppmann et al., 2002). To measure the contribution of particular foods to nutrient

intake we created initially 10 food groupings based on their contribution to vitamin D intake, eventually, food groups were merged to 6 food groups [dairy products, (butter/ margarine), fish, (meat/ organ meat), eggs, others], and one group for vitamin/mineral supplements, in order to examine the extent to which the intake of different foods (e.g. dairy products, butter/ margarine, fish) contribute to vitamin D intake. The data output from the CANDAT program consisted of the intake of each food grouping in grams and the nutrients in their habitual units of measure provided by each food for each participant. Foods grouped together were similar in density to allow for meaningful interpretation of quantities of the food by weight

4.3.2.3 Potential confounding variables

Self-reported variables including Physical Activity Scale for the Elderly questionnaire (PASE) (Friedenrieich et al., 1998), and socioeconomic and demographic information that included age, sex, smoking status, education, medical information and perception of income were collected at baseline through a structured-computer assisted interview. Standing height and weight were measured with participants dressed in light indoor clothing without shoes, using a stadiometer and a beam balance. Body mass index (BMI) was calculated. Waist circumference was measured.

4.3.3 Statistical analysis

The approach of examining the predictors of serum 25(OH)D > 75 nmol/L was used given the potential for misclassification in the reported dietary/supplement intake at the individual level. Difference in participants' baseline characteristics across the different 25(OH)D cut-offs were expressed in terms of means ± SD for continuous variables using

one-way analysis of variance ANOVA, and in terms of frequencies and percentages for categorical variables using chi–square tests. Means of dietary vitamin D intake from supplement intake, total food intake or intake from separate food groupings were analyzed using one-way ANOVA. Multiple linear regression models were used to examine different food groups and other independent correlates of serum 25(OH)D concentrations.

Statistical inferences were made based on a two-sided significance level of $P < 0.05$. All statistical analyses were performed using SAS version 9.1 (SAS institute Inc, Cary, NC, USA).

4.4 Results

Data for 404 healthy elderly community men and women participating in the NuAge study were analyzed **Table 4.1**. The characteristics of 207 men and 197 women aged between 69-83 y were stratified by serum 25(OH)D concentration cut-offs (Table 4.1). Serum 25(OH)D < 37.5 nmol/L which represents deficiency status was observed in 7% of the study sample, whereas 48% subjects had 25(OH)D concentrations between 37.5 – 74.9 nmol/L, and 45% had concentrations above 75 nmol/L.

The over all mean serum 25(OH)D was 74.0 nmol/L with means of 30.9, 55.3 and 100.6 nmol/L in deficient, insufficient and minimal target groups, respectively. Means of 25(OH)D were lower in January through March 66.7 nmol/L compared to June through September 80.8 nmol/L ($P < 0.0001$). Ranges of serum 25(OH)D were between 17.0 – 175.0 and 26.0 – 185.0 nmol/L for men and women respectively. Vitamin D supplements were consumed by 45% of women and 17% of men. Vitamin D supplements came in two

levels of vitamin D tablets (10 μg and 25 μg) from Centrum Select (multivitamin with 10 μg of vitamin D), Carbol (calcium with 10 μg vitamin D), Calcium 500/ 600/ 650 mg with 10 μg of vitamin D) and cod liver oil, with 10 μg being the most common form of vitamin D supplement consumed (65%).

Data for total energy and micronutrient intake of the NuAge sample were stratified by the three vitamin D status categories **Table 4.2** Macronutrient intakes (protein, carbohydrates and fat) were not significantly different by vitamin D status category. As expected, women's energy intake was less than that for men however, dietary intakes of calcium and vitamin D were higher in women in both adjusted or unadjusted for energy intake. ($P < 0.0001$). Means ± SD of total dietary vitamin D consumed from food and supplements were 8.2 ± 6.6 μg and 13.6 ± 11.4 μg for men and women respectively ($P < 0.0001$). For men 5.3 ± 3.4 μg and 2.9 ± 5.6 μg came from food and supplements, where as 4.3 ± 2.3 μg and 9.3 ± 11.2 μg came from food and supplements for women, following the pattern of absolute intake. The overall means ±SD of dietary vitamin D adjusted per 4,185 kJ (1000kcal) were 1.5, 4.8 and 8.3 μg for deficient, insufficient and minimal target vitamin D status ($P < 0.001$). Vitamin D consumed from food alone did not differ across the 3 vitamin D status categories (Table 4.2). Dietary vitamin D from supplement consumption showed important differences across the three different vitamin D status categories ($P < 0.0001$).

In order to examine whether there were particular quantities of particular foods consumed by those of differing vitamin D status levels, we divided the foods into groups of milk,

margarine/ butter, fish, meat, and eggs. No differences in food consumption were detected across the 3 categories with the exception of butter/ margarine food group ($P = 0.0053$) (data not shown). The contribution of different food categories and vitamin D supplement category was examined **Figure 4.1**. Daily mean vitamin D intake from food and supplements was 10.8 μg. Vitamin D supplementation accounted for 56% of total vitamin D intake. When excluding the contribution of supplements to total vitamin D intake, dairy products (mainly fortified milk) were the main food source comprising 45% of vitamin D intake from food.

In order to verify that vitamin D from food and supplement has an independent effect over that of season and other potential confounders, we conducted a multivariable analysis. Age, sex, season, BMI, smoking, PASE, education and perception of income were not associated with 25(OH)D when investigated in a multivariable analysis. In the final multivariable model summer season, intake of vitamin D containing supplements (vitamin D, vitamin D with calcium, cod liver oil and multivitamins) and vitamin D from food, were strong independent correlates of 25(OH)D concentration **Table 4.3**. Those tested in summer or early fall had 25(OH)D concentration 12.3 nmol/L higher than those tested in winter. Consumption of an extra one μg of vitamin D from food predicted an increase of 3.1 nmol/L of 25(OH)D, whereas, those consuming supplements containing vitamin D had an increase of 1.4 nmol/L for each one μg of supplements, or 14 nmol/L for the most common used supplement of 10 μg. The seemingly higher changes in 25(OH)D concentrations from food sources are overridden in a practical sense, because of the greater difficulty increasing vitamin D intake through food sources alone. In

examining the association of 25(OH)D in a regression with multiple food categories there was an effect for butter/margarine ($P < 0.0001$) and a weak effect of milk ($P = 0.045$) (data not shown).

4.5 Discussion

Based on this study dietary vitamin D from food sources alone appears to be inadequate to achieve the minimal target for vitamin D status in a healthy elderly population in Québec. The contribution of vitamin D from food is enhanced importantly by vitamin supplementation and sunlight exposure in summer/spring seasons. The mean intake of vitamin D from food and supplements among those with 25(OH)D concentrations > 75 nmol/L was 14.1 µg (95 % CI: 12.36 – 15.75). The dietary recommendation for vitamin D by the DRI panel currently set at 15 µg can be seasonally achieved when vitamin D supplements are consumed. Vitamin D fortified foods (milk, margarine and butter) were the main contributors to habitual vitamin D intake from food in this population In our study, the over all mean serum 25(OH)D of 74.0 nmol/L was remarkably close to the very recently published results of the Canadian Health Measures Survey. They estimated mean 25(OH)D concentrations of 73.5 for Canadian men and women aged 60-79 y (Statistics Canada, 2009).

Several studies have investigated the inadequacy of dietary vitamin D consumption (Carriere et al., 2007; Rodriguez et al., 2008; Sharkey et al., 2002; Lengyel et al., 2008; Lee et al., 2008) to better understand why levels of 25(OH)D are not optimal, however, only a few attempted to investigate the relationship between dietary intake of vitamin D and 25(OH) concentrations. Similar to our study, researchers in Denver investigated

dietary vitamin D intake and its association with vitamin D status in 80 ambulatory men and women with a mean age of 77.8 y (Linnebur et al., 2007). Dietary intake as assessed by food frequency questionnaire indicated 5.3, 7.0 and 6.4 µg /d from food in deficient, insufficient and sufficient groups as defined by 25(OH)D concentrations. While there were no differences in intake from food, supplement levels among the 3 vitamin D status categories however, were very different across the 3 vitamin D status categories.1.6, 11.0 and 15.5 µg/ d. In a cross-sectional study investigating prevalence of vitamin D deficiency in 95 healthy community-dwelling Irish women aged 51-75 y late in winter, researchers reported a mean vitamin D intake of only 2.9 µg from food and supplement assessed by 14 d diet history. This group's mean serum 25(OH)D concentrations of 57.2 nmol/L assessed by enzyme – linked immunosorbent assay (ELISA) for women < 70 y and high performance liquid chromatography (HPLC) for those ≥ 70 y, was lower than observed in our study (25(OH)D = 66.7 nmol/L) (Hill et al., 2006). In unhealthy populations the problem of vitamin D adequacy is more pronounced. A study in Baltimore of 64 nursing home and 52 home-care older residents aged ≥ 60 y, using a 3-d food record, found vitamin D intakes of 7 and 3 µg/d for the 2 groups respectively were associated with very low mean concentration levels of 25(OH)D at 36.2 and 26.0 nmol/L (Gloth et al., 1995). Despite the variation in population groups, and data collection methods (diet history, food recall, food frequency questionnaires and 24-h recall), it is clear that vitamin D intake from food alone is not sufficient to insure the minimal desired vitamin D status in older individuals. Our study clearly shows that on average consuming 15 µg/ d of vitamin D intake from food and supplements is associated with vitamin D

values > 75 nmol/L, and anything short of that intake may put older individuals at risk of vitamin D deficiency or insufficiency.

To our knowledge, the only dose-response trial to quantify vitamin D requirement in older individuals was a 22 wk clinical trial that randomized 216 elderly subjects (mean age 70.7 ± 5.4 y) from two Irish cities to 0, 5, 10 and 15 μg/d vitamin D (Cashman et al., 2009). The investigators reported a dose- related increase in 25(OH)D concentrations up to 10 μg. Based on a hypothetical mathematical model extrapolating to higher levels of intake, the investigators estimated a daily intake of 38.7 μg are required to achieve 25(OH) concentrations of > 80 nmol/L in 97.5% of the population. Given the very high variability in 25(OH)D concentrations in individuals with the same dietary intakes of vitamin D, leads to high estimates of intake required to meet the needs of 97.5 % of the population.

Although natural food sources of vitamin D are limited, vitamin D fortification policies in North America serve to increase the amount of vitamin D in the diet. In industrialized countries there are different fortification policies and differing proportions of people using supplements. Data from the Third National Health and Nutrition Examination Survey (NHANES III) indicate that fortified milk and cereals contribute 63% and 42% in men and women respectively to total vitamin D intake as compared to a 30% and 40% consumption from supplements in young Caucasian adults (Park et al., 2001). In the United Kingdom, where all margarine and some cereal products are fortified, vitamin D fortified foods contribute by 34 % and 29% to total vitamin D intake in men and women 50 - 64 y old compared to 21 and 24% from supplement intake (Henderson et al., 2003). In Norway, 27% and 23% of vitamin D came from margarine, the only fortified food

source (Jorde et al., 2000). In contrast, supplements on the other hand contributed by 42 % and 49 % in Norwegian men and women. In Japan where food fortification policies are not mandatory, fish contributed 91% of dietary vitamin D intake in women (Nakamura et al., 2000). These studies are not limited to older people but give an idea of the relative contribution of foods and supplements to the total vitamin D consumption. In our study 27 % of total vitamin D intake came from fortified food (milk and margarine), whereas 56.0 % came from supplements. These findings suggest that different food fortification policies contribute substantially to vitamin D intake and while supplement intake is recommended in North America, and recommended by the revised Canada's Food Guide (10 µg) for men and women > 50 y (Health Canada, 2009), it is not the major source of vitamin D in most reports. Enhanced food fortification with vitamin D, in addition to recommendations for supplement consumption may help the majority of the population achieve optimal vitamin D status.

The strength of our study lies in its ability to assess usual vitamin D intake reliably with six 24-h recalls. Our study population was composed of community dwelling older individuals who make up the vast majority of older individuals in industrialized countries.

In conclusion, healthy community-dwelling elderly men and women living in Québec, Canada did not meet the recommended adequate intake (AI) set by the DRI committee by diet alone. Supplement intake is required to meet the DRI. The mean intake of vitamin D from food and supplements for those with vitamin D status > 75 nmol/L was 14.1 µg. Due to concerns for adequate intakes of vitamin D and limited availability of dietary sources, it is apparent that the need for additional fortification of a wider range of food

products and emphasis on recommending vitamin D supplements is necessary for maintaining appropriate levels of vitamin in elderly free living Quebecers, especially in winter.

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RB and KGD were involved in the study concept and design, data and statistical analysis and interpretation of data, and writing the manuscript. HW and RB analyzed the 25(OH)D concentrations, HW was involved in the study design and the preparation of the manuscript. HP is the PI (principal investigator) of the NuAge study. HP and HW helped with the study design, data interpretation and the preparation of the manuscript. All authors read and approved the final manuscript.

4.7 Caption to figure

Figure 4.1. Contribution of different food categories and vitamin D supplements in μg to mean daily vitamin D intake in 404 healthy elderly community–dwelling Quebecers from the NuAge study.

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Table 4.1. Baseline Characteristics For 404 Healthy Elderly Men and Women From The Nuage Study Stratified by Different Serum 25(OH)D Cut-offs

	Serum 25 (OH)D in *nmol/L*						
	< 37.5		37.5 – 74.9		≥ 75		p value
n	29		192		183		
Body mass index, kg/m^2	28.68	± 3.77	27.89	± 4.9	27.31	± 4.15	0.2149
Education, *y*	11.34	± 3.86	11.61	± 4.4	11.18	± 4.4	0.6280
PASE*	74.22	± 37.49	90.1	± 50.18	86.97	± 46.28	0.2426
Sex, male	19	(66)	104	(54)	84	(46)	0.0776
Season							< 0.0001
January - March	21	(11)	109	(56)	64	(33)	
June - September	8	(4)	83	(39)	119	(67)	

Table 4.1. Continued

	Serum 25 (OH)D in *nmol/L*						
	< 37.5		37.5 – 74.9		≥ 75		p value
Age, *y*							0.7502
69 – 73	6	(5)	60	(51)	52	(44)	
74 – 78	9	(7)	59	(49)	54	(44)	
79 – 83	14	(9)	73	(34)	77	(47)	
Smoking (smokers)	5	(18)	6	(3)	11	(6)	0.0007
Supplement intake							< 0.0001
Consumers	1	(1)	50	(40)	72	(59)	

Data are presented in means ± SD or numbers (%)* PASE "Physical Activity Scale for the Elderly" questionnaire, score range (0 – 277), higher scores indicate higher activity level

Table 4.2. Energy and Dietary Micronutrient Intakes of 404 Healthy Elderly Men and Women From The Nuage Study Stratified by Different Serum 25(OH)D Cut-offs

	Serum 25 (OH)D in *nmol/L*						
	< 37.5		37.5 – 74.9		≥ 75		p value
n	29		192		183		
Energy, *kJ/d*	8499	± 1968	8009	± 2098	7641	± 1825	0.0417
Men	9348	± 2098	8838	± 1955	8487	± 1859	0.3632
Women	8788	± 1763	7038	± 1838	6921	± 1465	0.0036
Total dietary vitamin D, *μg/d*	4.6	± 3.5	8.7	± 6.8	14.1	± 11.6	< 0.0001
Vitamin D from foods	4.1	± 2.7	4.6	± 2.4	5.1	± 3.4	0.1185
Vitamin D from supplements	0.5	± 1.5	4.1	± 6.5	8.9	± 11.5	< 0.0001
Dietary calcium, *mg/d*	755	± 315	988	± 444	1224	± 661	< 0.0001
Dietary magnesium, *mg/d*	311	± 98	332	± 124	339	± 107	0.4549
Dietary potassium, *mg/d*	1190	± 303	1212	± 336	1226	± 342	0.8315

Data are presented in means ± SD, $p < 0.05$

Table 4.3. Energy–adjusted Vitamin D Intake From Foods and Supplements as a Predictor of 25(OH)D Concentrations

	β coefficient	Standard error	p value
Age, *y*	- 0.40	0.32	0.2193
Sex, male = 1; female = 2	1.28	2.99	0.6689
Season, summer	12.26	2.79	< 0.0001
Vitamin D from food, μg	3.08	0.96	0.0014
Vitamin D from supplements, μg	1.45	0.26	< 0.0001

Multivariate analysis controlling for smoking, energy intake, and waist circumference, $p < 0.05$

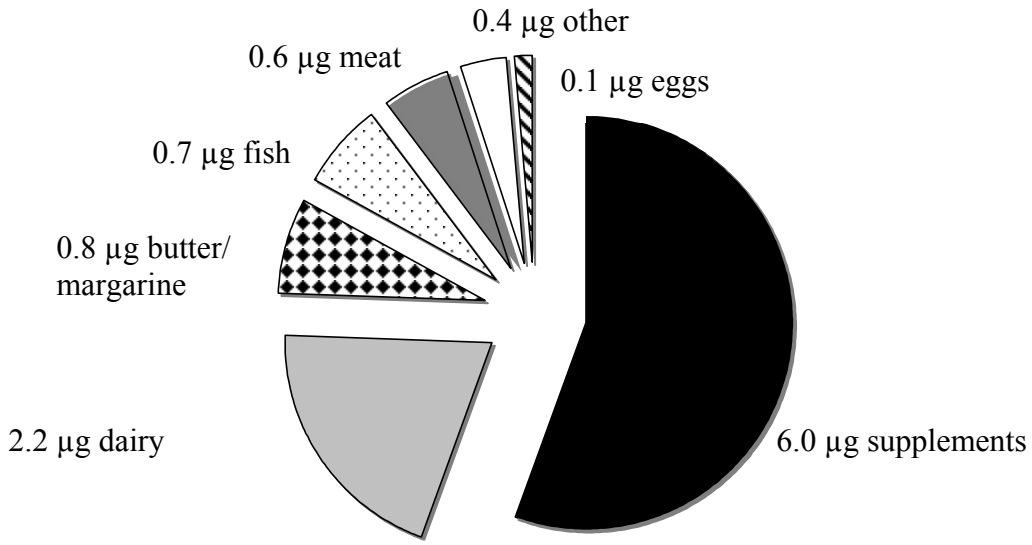

Figure 4.1. Contribution of Different Food Categories and Vitamin D Supplements in µg to Mean Daily Vitamin D Intake in 404 Healthy Elderly Community–dwelling Quebecers from the NuAge Study

Bridge 3

The literature has demonstrated an association between very low 25(OH)D concentrations and decline in physical functional performance in older people. This association, however, is not very clear in healthy older populations. Studies so far report conflicting findings. This cohort of healthy people, with 2 year follow up measurements of a number of muscle function, and strength, provided an excellent avenue to follow the different levels of vitamin D found in a healthy free living population. This study helped elucidate whether different vitamin D levels had an association with physical functional decline after controlling for a host of confounders.

CHAPTER 5. MANUSCRIPT 3

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Optimal Vitamin D Status Does Not Protect Against Functional Performance and Muscle Strength in a Healthy Elderly Population

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Running head:

Vitamin D and functional decline in the elderly

5.1 Abstract

Background: The impact of vitamin D status on physical functional decline is not fully understood. This study aims to examine whether optimal vitamin D status can prevent decline in physical functional status and muscle strength in a healthy older population.

Method: This prospective cohort study examined 357 older men and women aged 68 – 82 years from the NuAge longitudinal study, Québec. Serum 25(OH)D was assessed by radioimmunoassay (RIA), and other potential confounders were measured at baseline. Functional performance tests (walking speed, chair stand test, Timed "Up and Go", one leg stand balance, total functional score, muscle strength tests (elbow flexor, maximum isometric strength of knee extensors, maximal voluntary handgrip strength) were assessed at baseline, and after one and two year follow up.

Results: Serum concentrations of 25(OH)D were distributed as follows: 6% < 37.5 nmol/L, 48% between 37.5 and 74.9 nmol/L and 46% ≥ 75 nmol/L. Mean scores of all functional performance and muscle strength tests with the exception of the walking speed test, declined significantly from baseline over 1 ($p = < 0.001$) and 2 years ($p = < 0.001$). Baseline vitamin D status was not associated with a decline in percent change in any of the functional performance or muscle strength measures over 1 and 2 years as assessed by multiple regression analysis.

Conclusion: Vitamin D status was not associated with declines in physical functional status or muscle strength in a healthy elderly Québec population.

Key words: vitamin D status, physical functional performance, muscle strength, decline, healthy elderly population, Canada

5.2 Introduction

Low concentrations of vitamin D are recognized as a global problem, particularly in people residing in latitudes above 40° (Institute of Medicine, 1997). In addition, dermal synthesis of vitamin D is reduced with aging, potentially enhancing the risk for this group (MacLaughlin & Holick, 1985). Several studies have shown a high prevalence of vitamin D deficiency in hospitalized and home-bound older people (Gloth et al., 1995; Goldray et al., 1989; Thomas et al., 1998), whereas for community-dwelling older people few individuals appear to be deficient with values below 37.5 nmol/L (Melin et al., 2001; Van dam et al., 2007).

Although it has long been established that vitamin D plays a pivotal role in bone health, it has more recently been proposed that optimal vitamin D status may protect against impaired muscular function and the risk of falls and fractures (Janssen et al., 2002). There is a reasonable body of evidence associating 25(OH)D < 30 nmol/L with severe proximal myopathy (Ceglia, 2008; Prabhala et al., 2000). Vitamin D receptors exist in several tissues and organs including muscle and nerve tissues, implying a possible role of vitamin D in muscle strength and function (Campbell & Allain, 2006). Cross-sectional studies have shown an association between 25(OH)D concentrations and physical functional status; an association was reported between 25(OH)D concentrations < 37.5 nmol/L and lower muscle strength in women, but not in men (Zamboni et al., 2002), and lower-extremity strength in ambulatory men and women aged 60 years and older (Bischoff-Ferrari et al., 2004; Houston et al., 2006). Houston et al.(2006) have also reported an association between very low serum 25(OH)D concentrations (< 25 nmol/L) and physical performance measures in community-dwelling elderly men and women. In contrast,

cohort studies exploring the relationship of decline in muscle function and strength and vitamin D status provide conflicting results. Three studies report no association between 25(OH)D concentrations and functional decline. Two other cohort studies have shown an association between decline in physical functional tests and 25(OH)D concentrations, however, the strength of this association was attenuated as 25(OH)D concentrations approached optimal levels (Visser et al., 2003; Wicherts et al., 2007). Clinical trials of vitamin D supplementation indicate improvements in musculoskeletal functional tests of 3.7% to 11.1% in two studies of hospitalized older individuals (Bischoff et al., 2003; Corless et al., 1985), however no such effect was evident among healthy elderly community-dwelling participants (Kenny et al., 2003).

Given the uncertainty of surrounding the impact of vitamin D status on physical functional decline, our objective was to explore the relationship between vitamin D status and decline in physical functional status and muscle strength in a cohort of healthy older Quebecers after one and two year follow-up periods.

5.3 Methods

5.3.1 Subjects

Data for this study were obtained from the NuAge cohort study of 1793 independently-living predominantly Caucasian men and women aged 68 to 82 years. The NuAge participants were recruited from the Québec health insurance registry (RAMQ) and were stratified by sex and three age groups 68 - 72, 73 -77 and 78 - 82 years with equivalent numbers in each stratum. Community-dwelling individuals were invited by mail to assess their eligibility and willingness to participate, after which, they were assessed by both

telephone screening survey and clinical examination. Only participants who were free of disability in activities of daily living, had no cognitive impairment, were able to walk one block or to climb one flight of stairs without rest, spoke English or French and were able to commit to a 5 year follow-up period were recruited. The research protocol was approved by the ethics committees of the Institut universitaire de gériatrie de Montréal, University Institute of Geriatrics of Sherbrooke and McGill University. All participants provided written informed consent.

The sample (n = 405) for the vitamin D study was selected from a random sample of all interviews conducted at baseline in the NuAge cohort between January and March 2005 (period of no dermal synthesis of vitamin D) and between June and September 2006, inclusively after stratification by age, sex and season. Fasting blood samples and physical performance measures were collected at baseline, and physical performance measures were collected after one and two year follow up. Vitamin D assessment was measured prior to all measures of change in function so as to have clear temporal precedence in the study. (It is possible that decreased physical performance could reduce food intake or sunlight exposure). After one-year follow-up, 25 subjects were lost for different reasons (**Figure 5.1)**. An additional 24 participants were lost by the second year follow-up, leaving 357 subjects for the analysis.

5.3.2 Data collection and measurements

5.3.2.1 Biochemistry

Fasting blood samples (12 h fast) were collected, centrifuged and stored in aliquots at -80º C (2005 – 2006). Serum 25(OH)D was assessed by radioimmunoassay (RIA) using a commercial kit (DiaSorin, REF: 68100E, Stillwater, MN, USA) at McGill University (2007 – 2008), with an inter-assay CV < 8%. The assay measures both $25(OH)D_2$ and $25(OH)D_3$. All samples were analyzed in duplicate; data were expressed in nmol/L with values for "deficiency" set at < 37.5 nmol/L (Institute of Medicine, 1997), "suboptimal" at 37.5 to 74.9 nmol/L and "optimal" at ≥75 nmol/L (Dawson-Hughes et al., 2005). In terms of accuracy, the kit's low control on average was 52.9 nmol/L and was within the manufacturer specification 23.3 – 57.5 nmol/L, and the high control on average was 119.5 nmol/L and within manufacturer specification 85.0 – 202.0 nmol/L.

5.3.2.2 Physical performance

Physical performance was assessed using four functional performance tests at baseline and then 1 and 2 years later: Walking speed, chair-stand, Timed "Up and Go" and One leg standing balance, and a Total functional score which is the sum of each participant's quartile ranking in each of the four functional performance tests (maximum score 16).

Walking speed: Participants were asked to walk at their usual speed over three lines marked on the floor (two red lines at 0 and 4 meters and one white line at 1 meter). Timing started when the participant crossed the white line and finished 3 meters later at the red line. The best time of two trials was recorded and expressed as meters/second. Use a cane or a walker was allowed, but not the help of another person (Avila-Funes et al., 2008). The test-retest reliability over a 2 week interval is very good [Interclass

Correlation Coefficient (ICC) = 0.79] (Jette et al., 1999) and inter-rater reliability is excellent [ICC = 0.93] (Nevitt et al., 1989).

Chair-stand test: This test assesses lower extremity function, balance and coordination. Participants were asked to stand up and sit down from a standard chair, with arms folded across the chest, as fast as possible. Timing started from initial sitting position and ended at the final standing position at the end of the fifth stand (Avila-Funes et al., 2008; Guralnik et al., 1995). The test - retest reliability is good (ICC range = 0.67 – 0.73) (Seeman et al., 1994).

Timed "Up and Go": This test evaluates mobility and balance. Participants were asked to sit with their back rested on the back of the chair and arms rested on armrests. Participants were asked to rise from the chair, walk 3 meters at a comfortable pace, return to the chair and sit down. Timing started when the participant was asked to start and ended when the participant was reseated. The test-retest (ICC = 0.99) and inter-rater (ICC= 0.99) reliability is excellent (Payette et al., 1998).

One Leg Standing Balance: This is a valid and reliable test (Winograd et al., 1994) measuring maximum time subjects can stand on one foot with hands placed on their waist. The participant, not wearing shoes, was positioned approximately 1 meter from a wall and was instructed to stand on one foot lifting the dominant leg to calf level for as long as possible. The test was repeated for the other leg. Timing started when the participants took their leg off the ground and stopped when the foot touched the ground, the arm position was modified or 60 seconds elapsed. The best time for either leg was used (Avila –Funes et al., 2008).

Total functional score: Five levels of physical functional performance were created for each of the four tests. Scores from 1 (worst) to 4 (best) were assigned according to the

quartile of time needed to carry out the test. A score of 0 was assigned for those who could not do or did not complete the test because they felt unable to do so. The possible score range was 0 to 16. The validity of this global measure has been previously reported in the NuAge population (Avila – Funes et al., 2006).

Change in total functional score: This measure was calculated by subtracting total functional score measurements at baseline from those measured after one and two years follow – up.

There were also three muscle strength tests that assessed handgrip strength, biceps and quadriceps strengths.

Maximal voluntary handgrip strength: This was measured with a Martin Vigorimeter and expressed in kiloPascals (kPa). The participant was seated on a standard straight back chair without arm rests. Hips and knees were at right angles. Upper extremity position standardized (American Society of Hand Therapists) to enable good test-retest reliability (Payette et al., 1998). The participants were instructed to squeeze the bulb as hard as they could upon the examiner's signal. Verbal encouragement was provided. Effort did not exceed 10 seconds. Three measurements were taken for both the right and left hands. Recommended rest intervals to allow muscular recovery between replicate trials were provided. The highest score for either hand was used.

Maximum isometric strength of knee extensors (quadriceps): This was measured using a hand held dynamometer Microfet2 and expressed in terms of Newtons according to a standardized protocol (Bohannon, 1986; Payette et al., 1998; Reed et al., 1993). Muscle testing, using the belt-resisted, 'make' test, was performed bilaterally for each muscle group. The subject was seated in a straight back arm chair; knee extension was

measured with the subject seated with the knee at 60° from full extension and foot resting on the floor. Three maximal contractions were recorded on each side. All contractions were of 4 seconds duration and completed about 30 seconds apart. The highest score was used (Payette et al., 1998).

Elbow flexors (Biceps): This was measured using a hand held dynamometer Microfet2 and expressed in Newtons according to standardized protocol (Desrosiers et al., 1995). The elbow flexion was measured with the participant's arm resting on the arm of the chair, with the limb segment and dynamometer positioned as described by Bohannon (Bohannon, 1986). The belt was secured to the arm of the chair, tightly enough to stabilize the forearm but not so tight as to create pressure. The test was repeated 3 times bilaterally and the best reading was used.

5.3.2.2 Potential confounding variables

Demographic and socioeconomic information included age, sex, smoking status, perception of revenue adequacy, education, medical information, medication and supplement use, and the Physical Activity Scale for the Elderly questionnaire (PASE) and Geriatric Depression Scale (GDS). These variables were collected through a structured computer-assisted interviewer carried out the same day as the blood draw at baseline. Baseline standing height and weight were measured with subjects dressed in light indoor clothing without shoes, using a stadiometer and a beam balance. Body mass index (BMI) was calculated and waist circumference was measured. Season of the blood draw was classified as summer or winter as indicated in the section on sample selection.

5.3.3 Sample size and statistical analysis

Our sample of 357 healthy older men and women had a relatively large number of individuals with 25(OH)D concentration ≥ 75 nmol/L. The changes in strength and functional status over time that could be expected to be picked up (80% power) comparing the deficient group with optimal group are as follows; the strength measures vary from 9-14% change; the overall functional performance score, 12% change; The TUG score 11%, and chair stands 21%. The balance measure is highly variable and for this reason was only reported as part of the overall score. Differences in subject characteristics across different 25(OH)D concentration cut-off levels were expressed in terms of means ± standard deviation using one-way ANOVA, or as frequencies and percentages using chi-square tests. Declines over time in each functional measure were examined using paired t-tests. Multiple linear regression models were used to assess vitamin D status category (3 levels of 25(OH)D concentrations) as an independent predictor of percent change in each functional performance score and each muscle strength test. All variables listed above were examined as potential confounders. Statistical inferences were made based on a two-sided significance level of $p < 0.05$. All statistical analyses were performed using SAS version 9.1 (SAS institute Inc, Cary, NC, USA).

5.4 Results

Complete data were available for 357 participants from the NuAge study (179 men and 178 women). Characteristics of this sample stratified by different serum 25(OH)D concentration levels are presented in **Table 5.1**. Serum 25(OH)D < 37.5 nmol/L

representing deficiency status was observed in 6%, serum 25(OH)D between 37.5 – 74.9 nmol/L (suboptimal status) was observed in 48%, and serum 25(OH)D concentrations ≥ 75 nmol/L (optimal status) was observed in 46% of participants.

The overall mean (SD) serum 25(OH)D was 75.3 (30.7) nmol/L with means of 30.1 (5.2), 55.3 (9.6) and 101.8 (23.6) in deficient, suboptimal and optimal groups, respectively. Only 2 participants had vitamin D concentrations below 27 nmol/L. Means of 25(OH)D were lower between January through March 67.9 (30.0) nmol/L compared to June through September 82.1 (29.8) nmol/L ($p < 0$ .001). Ranges of serum 25(OH)D were 17 – 174 and 26 – 185 nmol/L for men and women respectively.

One and two year declines in muscle strength and function test scores are shown in **Table 5.2**. The baseline and absolute decline values are reported**.** All muscle strength tests significantly declined (4 % decline over one year and 8 % decline over two years for each test). Muscle function scores also significantly declined by one and two years with the exception of the "Timed Up and Go" with 0 % decline after one year of follow up and 4 % decline over 2 years, and walking speed test which did not decline over one and two years. Two year declines were larger than one year declines for most variables indicating the consistent declines in this study group ranging from 4 % to 14%.

An investigation of the relationship of categories of serum 25(OH)D with percent change in total functional score, walking speed, chair stands and TUG over one and two years in a multiple regression model was undertaken (**Table 5.3).** Potential confounders including number of medications consumed, education, perception of revenue, PASE score for

physical activity, and GDS score were tested in different regression models, but were omitted in the final model as they did not improve the model significantly. The final regression model was adjusted for age and sex as well as season, smoking status and waist circumference as these were related to vitamin D status. There were no differences in the percentage decline for any of the tests related to either deficient or suboptimal 25(OH)D concentrations.

Changes in percent change in muscle strength tests in relation to vitamin D status are shown in **Table 5.4**. Vitamin D deficient participants with serum 25(OH)D < 37.5 nmol/L did not show greater declines in strength than those with optimal levels. The only statistically significant finding was a greater improvement in handgrip strength in the vitamin D deficient and suboptimal status groups when compared to the reference group of optimal status when followed up for a one or two year period. The reason for this is unclear.

In addition to a lack of association of suboptimal and deficient vitamin D levels with functional declines over 1 and 2 years, a cross-sectional analysis of serum 25(OH)D concentration categories at baseline indicated no association with any of the muscle function tests and muscle strength tests at recruitment into this study.

When examining the relation between percent changes in muscle strength and muscle function tests' scores with vitamin D concentrations using vitamin D as a continuous variable in a regression model, similar results were observed (data not shown). There was

no association of lower levels of 25(OH)D being associated with greater declines in functional status.

5.5 Discussion

The findings of this study indicate that there is no association of vitamin D status with declines over a one or two year period in functional status or muscle strength in a healthy older population aged 68 to 82 years. Although there is a clear decline over one and two years in all measures except the walking speed, vitamin D status was not associated with this decline.

The gradual declines in strength and functional status with aging are well established. A decline over 3.7 years in hand grip strength, usual–pace walking speed, chair–stand and tandem walk test results was also reported in 6357 elderly community–dwelling women over 65 years old participating in the Study of Osteoporotic Fractures (SOF) who were taking vitamin D supplements (Faulkner et al., 2006). In addition, the Longitudinal Aging Study Amsterdam (LASA) reported a 13.2% decline in hand grip strength among men and women aged 55 – 85 years over 3 years (Visser et al., 2003). Findings from our study were consistent with these studies.

There has been considerable interest in knowing whether or not vitamin D might be associated with these declines, with the hypothesis that one might be able to decrease the declines seen in some people if vitamin D status is optimized. There have been clinical trials indicating the effectiveness of supplementation. One study reported that one dose of 600 000 IU of intramuscular ergocaliferol injection, administered to patients from fall

clinics with 25(OH)D levels < 30 nmol/L led to improved muscle function determined by an aggregate functional performance time, but not quadriceps strength when compared to a placebo group (Dhesi et al., 2004). In addition institutionalized women with a mean age of 85 years and 25(OH)D < 30 nmol/L reported in over 50 % of the study population showed improvement in functional scores, when randomized to 1200 mg calcium plus 800 IU vitamin D vs. 1200 mg calcium alone for 3 months (Bischoff et al., 2003). The previous studies suggest that vitamin D supplementation may be effective only when offered to very vitamin D depleted older people.

While these studies of vitamin D depleted older subjects indicate some improvements in muscle function and strength, studies of healthy aging individuals with relatively higher vitamin D concentrations do not show these effects. The SOF study, where prevalence of vitamin D deficiency was only 4.6% among the participating women, reported no significant association of 25(OH)D concentrations with any of the neuromuscular function tests (chair–stand time, hand grip strength, walking speed and balance–walk time) or with change of these measures over a 3.7 year follow up of older women consuming vitamin D supplements (Faulkner et al., 2006). Similarly, the Women's Health and Aging Study (WHAS) reported no association between 25(OH)D concentrations (mean = 53 nmol/L) and change in physical performance measures (hip flexor, knee flexor, hand grip strength, walking speed or repeated chair stand) in moderately to severely disabled community – dwelling women aged > 65 years, after a 3 year follow – up (Verreault et al., 2002). Likewise, findings reported from a Japanese study failed to find an association between vitamin D status and gravity center sway or hand grip strength in community dwelling older women with a mean 25(OH)D concentration of 60 nmol/L (Nakamura,

2006). In addition, in a trial of healthy community-dwelling men, randomized to 1000 IU vitamin D plus 500 mg of calcium/day *vs*. only 500 mg Ca/day, no significant improvements in either muscle function measures or muscle strength tests were observed after a 6 month follow-up (Kenny et al., 2003).

In contrast, investigators reported an association between 25(OH)D concentration and decline in physical performance after a 3 year follow up of healthy men and women recruited from the LASA study (Wicherts et al., 2007). A plausible explanation may be that 25(OH)D concentration were very low in this study population wherein 9.6% had 25(OH)D concentrations < 25 nmol/L and 1.3% less than 12.5 nmol/L. Vitamin D fortification does not exist in the Netherlands which may explain the low vitamin D status reported. In our study only 0.5% subjects had 25(OH)D concentrations less than 25 nmol/L. In our study there was a statistically significant association of low vitamin D concentrations and increased handgrip strength over time. Our results are similar to findings reported from the SOF study in which hand grip measurements improved significantly over time in women with low 25(OH)D concentrations. This was not observed for other strength or muscle function tests such as quadriceps, chair-stand or balance tests (Faulkner et al., 2006). Conversely, a significant association of low vitamin D concentrations and declines in hand grip strength was reported in the LASA study (Visser et al., 2003) wherein some individuals had very low levels of 25(OH)D.

Several factors, in addition to aging and declining activity levels, such as drug interactions (Dukas et al., 2002), age related decreased activity of renal 1-α-hydroxylase (Francis et al., 1984), decreasing cofactors for activation of sex hormones (Castillo et al.,

1977), and decreased number of vitamin D receptors and the genotypic variations (Geusens et al., 1997) may cause signs of vitamin D deficiency despite the presence of 25(OH)D concentration ≥ 70 nmol/L. Further study is necessary to ascertain which factors may explain the similar rate of decline in muscle strength and function that was observed across the three 25(OH)D groups in our study.

The strength of our cohort study is the relatively large sample size, with equal number of men and women, the comprehensive assessment of muscle function and muscle strength measures, the reasonably long follow–up period, in addition to controlling for a number of potential confounders such as physical activity and depressive symptoms. In combination with data from the trial of supplementation among healthy elderly and most other cohort studies it would appear that supplementation may only be beneficial in those with very low levels of vitamin D. In our study the group of participants with deficient levels was limited making it difficult to detect small declines over one or two years, however a significant improvement in muscle strength in the deficient group was detected. Although one cannot rule out the fact that there may be a few individuals among a healthy cohort who could benefit from higher levels of vitamin D, in general, supplementation will not help reduce the natural decline in functional status observed with aging.

5.6 Conclusion

In conclusion, vitamin D status in healthy aging Canadians is not related to physical functional declines or declines in muscle strength. Although supplementation with vitamin D is recommended to attain optimal levels for a number of health benefits, it does

not appear that vitamin D status will help to avoid functional declines with aging in healthy older populations with low rates of true deficiency.

5.7 Acknowledgements

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5.8 Caption to figure

Figure 5.1. Study sample from the NuAge Cohort Study and reason for attrition and exclusion.

5.9 References

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Table 5.1. Baseline Characteristics of 357 Healthy Elderly Men and Women Recruited from the NuAge Study Stratified by Different Serum 25(OH)D Cut-offs

	Serum 25(OH)D concentrations in nmol/L			
	< 37.5	37.5 – 74.9	≥ 75	p value
n	21	171	165	
Age, years	76.6 ± 4.3	75.2 ± 4.3	75.8 ± 4.4	.362
Sex (% men)	12 (57)	93 (54)	74 (45)	.174
Weight, kg	74.7 ± 11.9	73.8 ± 15.5	70.3 ± 13.3	.059
BMI, kg/m^2	28.5 ± 3.1	27.9 ± 5.0	27.3 ± 4.1	.345
Waist circumference, cm	98.5 ± 10.3	96.3 ± 13.5	93.5 ± 11.9	.059
GDS[1]	4 ± 2	5 ± 4	5 ± 4	.465
PASE[2]	77 ± 32	91 ± 49	87 ± 46	.471
Season of blood collection, n (%)				
January – March	16 (76)	93 (54)	74 (45)	< .001
June – September	5 (24)	78 (46)	91 (55)	
Number of medications used	4.6 ± 3.7	4.9 ± 3.1	5.0 ± 3.2	.776
Smoking status, n (%)	3 (14)	4 (2)	16 (6)	.030
Education, years	10.9 ± 3.6	11.6 ± 4.5	11.1 ± 4.5	.539
Perception of Income, n (%)				
Very adequate	14 (67)	69 (40)	78 (47)	.145
Adequate	7 (33)	95 (56)	84 (51)	
Not adequate	0 (0)	7 (4)	3 (2)	

Values are means ± standard deviations or number (percentage within columns).

[1]GDS "Geriatric Depression Scale" questionnaire, score range (0 – 23), scores ≥ 11 and ≤ 20 indicate mild depression.

[2] PASE "Physical Activity Scale for the Elderly" questionnaire, score range (0 – 277), higher scores indicate higher activity level.

Table 5. 2. Absolute Change in Means of Muscle Strength, Muscle Function, and Total Functional Score Tests Over One and Two Years for a Sample of Healthy Elderly Men and Women from the NuAge Study

		Change over one year				Change over two years			
	Baseline means	n	Mean	SE*	p value	n	Mean	SE*	p value
Muscle strength tests									
Elbow flexor test, Newton	42.2	345	- 1.7	0.4	< .001	344	- 3.5	0.4	< .001
Maximum isometric strength of knee extensor test, Newton	56.2	342	- 2.4	0.7	< .001	334	- 4.3	0.7	< .001
Maximal voluntary handgrip strength, kPa †	65.5	354	- 1.8	0.5	< .001	352	- 5.0	0.5	<.001
Muscle function tests									
Timed "Up and Go", seconds	10.9	352	0.0	0.1	.674	346	0.4	0.1	< .001
Chair stand test, seconds	12.1	339	0.8	0.2	< .001	332	0.5	0.2	< .001
One leg standing balance test, seconds	12.7	330	1.8	0.7	.010	328	1.5	0.7	.024
Walking speed test, m/s	1.1	353	0.0	0.0	.823	347	0.0	0.0	.524
Total functional score	9.8	316	- 0.4	0.1	< .001	306	- 0.4	0.1	< .001

* SE: Standard error
† kPa: kilopascal

Table 5.3. Serum 25(OH)D as a Predictor of Percent Change in Total Functional Score and Separate Functional Performance Tests in Healthy Men and Women From the NuAge Study

	Percent Change Over One Year											
	Total functional score			Walking speed			Chair stand			Timed "Up and Go"		
25 (OH)D nmol/L	n	ß	95% CI[1]	n	ß	95% CI[1]	n	ß	95% CI[1]	n	ß	95% CI[1]
< 37.5	17	- 2	(-11; 7)	21	4	(-3; 10)	21	5	(-6; 17)	21	4	(-2; 10)
37.5 - 74.9	149	2	(-2; 7)	167	-1	(-5; 2)	158	-5	(-10; 1)	167	0	(-3; 3)
≥ 75	150		(reference)	165		(reference)	160		(reference)	164		(reference)
total	316			353			339			352		
	Percent Change Over Two Years											
	Total functional score			Walking speed			Chair stand			Timed "Up and Go"		
25 (OH)D nmol/L	n	ß	95% CI[1]	n	ß	95% CI[1]	n	ß	95% CI[1]	n	ß	95% CI[1]
< 37.5	15	-7	(-16; 3)	20	-3	(-10; 4)	19	-1	(-12; 10)	20	0	(-7; 7)
37.5 - 74.9	145	-5	(-15; 4)	165	-1	(-5; 2)	156	2	(-3; 7)	165	0	(-3; 4)
≥ 75	146		(reference)	162		(reference)	157		(reference)	161		(reference)
total	306			347			332			346		

[1] CI: Confidence Interval

Adjusted for age, sex, season, waist circumference and smoking using multiple regression.

Table 5.4. Serum 25(OH)D as a predictor of Percent Change in Muscle Strength Tests in Healthy Elderly Men and Women from the NuAge Study

Percent Change Over One Year									
		Biceps[1]			Quadriceps[2]			Handgrip strength[3]	
25(OH)D nmol/L	n	β	95 % CI[4]	n	β	95 % CI[4]	n	β	95 % CI[4]
< 37.5	20	-3	(-12; 6)	20	-5	(-16; 6)	21	8	(1; 15)
37.5 – 74.9	167	-3	(-7; 2)	166	- 5	(-10; 0)	170	2	(-1; 6)
≥ 75	158		(reference)	156		(reference)	163		(reference)
total	345			342			354		
Percent Change Over Two Years									
		Biceps[1]			Quadriceps[2]			Handgrip strength[3]	
25(OH)D nmol/L	n	β	95 % CI[4]	n	β	95 % CI[4]	n	β	95 % CI[4]
< 37.5	20	- 3	(-12; 7)	17	- 8	(-19; 3)	21	9	(1; 16)
37.5 – 74.9	166	- 3	(-7; 2)	165	- 4	(-8; 1)	170	0	(-3; 4)
≥ 75	158		(reference)	152		(reference)	161		(reference)
total	344			334			352		

[1]Biceps strength tested by "Elbow flexor test"; [2]Quadriceps strength tested by "Maximum isometric strength of knee extensor test"; [3]Hand grip strength tested by "Maximal voluntary Hand Strength test"; [4] CI: Confidence interval;

Adjusted for age, sex, season, waist circumference and smoking using multiple regression.

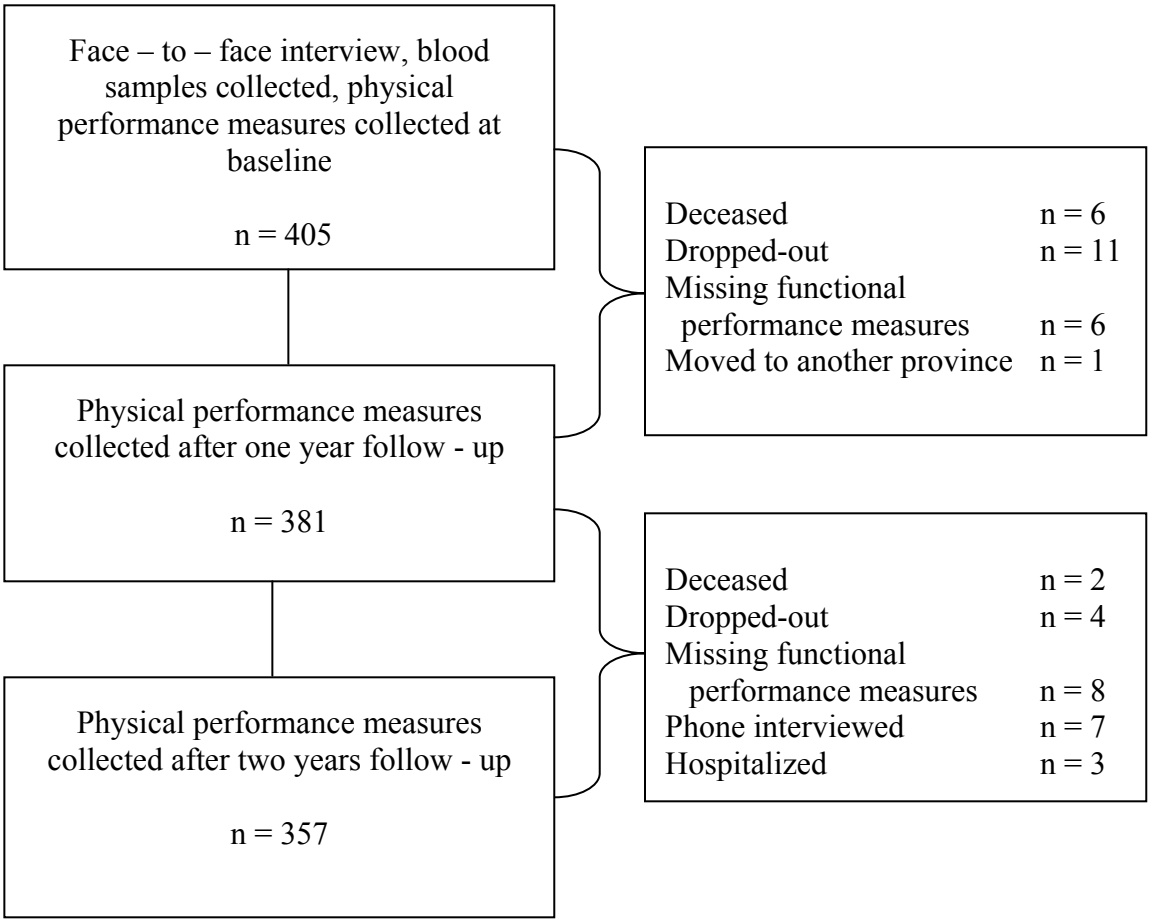

Figure 5.1. Study Sample from the NuAge Cohort Study and Reason for Attrition and Exclusion

CHAPTER 6. OVERALL SUMMARY AND CONCLUSIONS

Population aging, characterized by decline in the proportion of children and young people and increase in the proportion of older people, is set to increase worldwide. Although, this phenomenon varies widely among countries, ranging from 4% in Cambodia to nearly 14% in Japan (World Health Organization Regional Office for the Western Pacific Region), older people > 60 years are expected to reach the 1.2 billion mark by 2025 (World Health Organization). In 2030 in the US alone there are expected to be 71.5 million older people with 9.6 million in the category "oldest, old ≥ 85 years" (Administration on Aging 2003). Unfortunately, the longevity and continued "graying" of the world is usually accompanied with a rise in prevalence of chronic diseases which leads to compromised quality of life and increased financial burden (Brown & Josse, 2002). Hence, it is necessary to examine possible factors that may affect older people to help this growing sector of society to age gracefully with minimum negative health outcomes.

Despite the general impression that vitamin D is readily available due to the ability of humans to dermally synthesize it, this process is impeded by several factors including use of sunscreen, concealing clothing, cultural dress codes, skin color, body fat, weather conditions and geographical location (latitude) in general. Reduced dermal vitamin D synthesis that accompanies the aging process along with heavy consumption of medication that interferes with vitamin D's absorption or metabolism in older people put aging populations at additional risk for synthesizing vitamin D (Holick, 2006). Moreover,

scarcity of dietary resources, in adherence to supplement consumption and lack of mandatory vitamin D fortification policies are additional risks hindering the acquisition of this important vitamin (Buhr & Bales, 2009; Calvo & Whiting, 2005; Lips, 2006).

Emerging evidence suggests that nutrition and lifestyle may be key determinants of healthy aging (Payette, 2005). Vitamin D stands out as one nutrient that is directly associated with numerous health outcomes in an aging population. The best substantiated effect is on bone health. Recently, amounting body of epidemiological evidence has associated vitamin D deficiency or insufficiency with several chronic diseases such as cardiovascular disease, diabetes, immune system disorders and some types of cancer (Holick, 2004). Additionally, cross-sectional studies, longitudinal cohort studies, and a few clinical trials have suggested an association between vitamin D status and muscle strength and function, however, reports from these studies lack consistency.

To date, no study in Canada has examined vitamin D status and its association with functional performance in free living healthy men and women. An excellent opportunity to develop and design a study addressing vitamin D status determinants and the possible consequences of low circulating serum 25(OH)D concentrations in healthy older people living in Québec was possible due to data available from the NuAge Longitudinal Study. This study of 1793 healthy free-living older people living in Québec, stratified by sex, season and 3 age groups of approximately equal numbers, provided precise and comprehensive biological, dietary, anthropometric, socio-demographic and functional performance measurements. Data were collected at baseline and annually for a 5 year period by well trained staff.

In our first study, we examined vitamin D status, 25(OH)D concentrations assessed by RIA, in 405 older men and women with no major health problems stratified by sex, age (3 age groups) and season of blood collection fall/winter – spring/summer). This subgroup of the NuAge cohort was selected randomly and there are no indications that the subsample is different than the entire cohort at baseline. In an examination comparing baseline characteristics of the 1793 NuAge participants with the studied sample, there were no obvious differences in terms of a number of variables examined, including dietary intake, muscle function and strength and such lifestyle factors as smoking.

We observed a low prevalence of vitamin D deficiency, approximately 7 %, in this healthy population. Vitamin D supplement consumption was a major determinant vitamin D status; 45 % of women consumed vitamin D compared to only 17% of men. No vitamin D deficiency was observed in women vitamin D consumers in either summer or winter. Season was another significant determinant of vitamin D status. Both men and women achieved higher 25(OH)D concentrations in summer. Sex, age and adiposity were not determinants of vitamin D status In a multiple regression model, 25(OH)D concentration was nearly 14 nmol/L higher for those tested in summer, and about 17 nmol/L higher for those using vitamin D supplements. We controlled for physical activity, smoking, and adiposity in addition to sex and age.

The majority of studies examining vitamin D status were not in agreement with our finding; they reported much higher prevalence of vitamin D deficiency. Most of these studies, however, were performed in hospitalized, institutionalized or in elderly people with clinical conditions related to bone health (Chapuy et al., 1996; Glowacki et al., 2003;

Ramel et al., 2009). Institutionalized older people may have less sun exposure and not have optimal dietary sources (Gloth et al., 1995; Liu et al., 1997). Most of the studies did not report vitamin D supplement consumption; however, one study reported that only 10 % of the women suffering from hip fractures were vitamin D supplement users (Bischoff-Ferrari et al., 2008). Even when comparing findings from this study with the few studies carried out in healthy people, still prevalence of vitamin D deficiency in this healthy population remained lower than what was reported in other studies examining vitamin D status in younger age groups (Rucker et al., 2002; Weiler et al., 2007). We have assessed adiposity by examining both BMI and waist circumference in our study. BMI may not be the best indicator to assess adiposity in older people due to physiological changes that may accompany aging such as sinle kyphosis, thining of weight-bearing cartilages and shortening of the spinal vertebrae (Cook et al., 2005). Because of the co-leaniarity between BMI and waist circumference, and due to our observation that larger mean waist circumference showed a trend with lower 25(OH)D concentration in our third manuscript; we chose to control for waist circumference in our regression models. Although adiposity has been identified as one of the determinants of vitamin D status in some studies (Bolland et al., 2006; Snijder et al., 2005), there was no effect in our study, despite a reasonably wide variation in adiposity among our group of subjects. Perhaps if measured more precisely by DXA instead of weight and waist circumference measurements, better detection of association between adiposity and vitamin D status would have been possible. One vitamin D supplementation study was in agreement with our findings (Canto – Costa et al., 2006). This study had measured body fatness by means of DXA and subjects were classified into 3 groups accordingly, response to vitamin D supplementation was similar across the 3 adipose tissue mass catigories, implying that

adiposity may not be necessarily an issue with vitamin D status. In the NuAge cohort there were no participants who were at all disabled and all could get outdoors on their own. It is not clear whether adiposity is a risk factor on its own or is the association confounded by heavier people covering up more, being less active and less outdoors or consuming a less healthy diet.

In the second study, investigating the same people from study 1 in a similar cross-sectional design, we assessed vitamin D intake by means of six 24 – hour recalls. We also examined contribution of foods and supplements to total vitamin D intake. The findings demonstrated that older people with optimal vitamin D status consumed on average 14 μg (560 IU) of vitamin D daily; however, this intake was not achieved from food sources alone. Food sources did not exceed the 5 μg (200 IU) mark at best, and had to be augmented with an additional mean intake of about 9 μg (360 IU) from vitamin D containing supplements. When looking further across the 3 vitamin D status groups, those in vitamin D deficient group consumed 0.5 μg (20 IU) vitamin D from supplements compared to 8.9 μg (356 IU) intake in the optimal group. Fortified milk intake was a significant contributor to vitamin D status as well as vitamin D supplement consumption. Reinforcing findings from our first study, season remained a strong determinanat of vitamin D status.

In agreement with other studies, dietary intakes of vitamin D were positively related to 25(OH)D concentrations (Cashman et al., 2009; Gloth et al., 1995; Linnebur et al., 2007). Quantifying vitamin D intake from food was not always accurately measured: in some studies it was estimated from food frequency questionnaires (Carriere et al., 2007;

Linnebur et al., 2007). When measured by three 24-hour recalls, intakes were still less than what was reported in our study, but vitamin D consumption from supplements was not always reported (Hill et al., 2006; Masse et al., 2004; Sharkey et al., 2002). Vitamin D fortified milk was the major food contributor to vitamin D intake in this population, which reinforces the importance of food fortification. Perhaps fortifying more food staples may help achieve adequate intakes of vitamin D, especially in those who do not adhere to supplement intake.

Findings from the third study, which was a longitudinal study examining the association of vitamin D and decline in functional performance in 357 older people over 1 and 2 years, clearly demonstrate the lack of such an association. Although some cross-sectional studies reported a relationship between vitamin D and muscle strength and function, cross – sectional designs cannot prove causality. We cannot tell whether low vitamin D status precedes poor muscle function or strength, or poor muscle function leads to fear of falls, more confinement to home and less exposure to sun, thus less vitamin D synthesis. Longitudinal cohort studies, on the other hand, provide conflicting results. Three studies found no association between 25(OH)D concentrations and muscle strength or function (Faulkner et al., 2006; Nakamura et al., 2007; Verreault et al., 2002); whereas, two studies demonstrated positive associations (Visser et al., 2003; Wicherts et al., 2007). However, those studies that reported positive associations either had not controlled for some important confounders such as smoking and no information about out door activity (Wicherts et al., 2007) or found an association only with those with very low serum 25(OH)D concentrations < 25 nmol/L (Visser et al., 2003). When reviewing supplementation clinical trials two studies demonstrated positive association and one

failed to do so. The positive associations were seen in studies performed on geriatric care facility residents (Bischoff et al., 2003) or older people recruited from falls clinics (Dhesi et al., 2004) with low baseline levels of 25(OH)D (< 30 nmol/L). The only supplementation study performed on healthy free- living older people did not show association of vitamin D with decline in physical performance (Kenny et al., 2003).

Our work for this dissertation does not come without limitations, concern is raised over not having serum 25(OH)D measurements for the same subjects in both seasons, which would have been able to provide a better representation of seasonal effects on vitamin D status. However, examining equal numbers of participants of both sexes and across the 3 age groups similar in both seasons provided an unbiased interpretation of the seasonality effect in this study. It may also come to mind that this study was underpowered to detect changes in muscle strength and function measurements; however, this study was sufficiently powered to detect an inverse association between vitamin D status and hand grip strength. Finally, 98 % of the participants in this study were Caucasian, though representative of older people of this age group in Canada; we cannot generalize findings of these studies to all the elderly.

Although, this study was not able to resolve the issue of the uncertainty regarding the association between vitamin D status and decline in physical performance; we confidently side with studies demonstrating a lack of presence of this association. Most studies measured some but not all measures (Faulkner et al., 2006; Verreault et al., 2002). This study had comprehensively measured all important physical performance tests. Four muscle function, 3 muscle strength and a balance test were measured in sets of 3.

Additionally a total functional score was created to account for muscle function tests. Our sample size was relatively large to detect difference in measurements over one and two years. Additionally, when we undertook a regression measure examining vitamin D as a continuous measure [25(OH)D concentration], no such predictive effect was observed.

Despite the few limitations of this study, the strengths listed below allow our findings to bridge the gaps in knowledge in this area. In addition to investigating a large sample size, with equal number of men and women, and equal number of older age groups, controlling for all potential confounders; obtaining comprehensive and precise measurements of outcomes of interest (vitamin D status, vitamin D dietary intake and physical performance) was the strongest point in this study. The cohort studied was very representative of the healthy elderly population with some health conditions such as diabetes, hypertension and arthritis, but they were functioning well on their own as most elderly Canadians. Two sets of three 24-hour recalls/ participant, were collected 6 months apart, with dietary analysis performed using the "CANDAT" program which uses the Canadian Nutrient File in addition to an institute file that periodically updates the database with additional foods (last update was performed in summer 2009). This enabled us to capture dietary vitamin D intake with a high degree of precision. We can state with certainty that vitamin D supplement consumption is necessary to achieve the adequate intakes currently recommended by the DRI committee.

Following our results, still there is more to be explored in the future. Studies of vitamin D prevalence in healthy older people with populations of multiethnic backgrounds are

needed to be able to generalize our findings. In addition, in groups of unwell subjects who appear to have much more serious vitamin deficiency levels, it will be important to understand the risks to low vitamin D status and how this can be corrected. It appears that adherence to supplementation is not appreciated in those with clinical problems related to vitamin D deficiency (Bischoff-Ferrari et al., 2008). A review of the vitamin D recommendations is underway and the optimal levels of vitamin D need to be defined (Millen & Bodnar, 2008; Yetley et al., 2009). Following agreement on the optimal level of 25(OH)D, vitamin D supplementation trials in older people are highly needed to quantify optimal vitamin D intakes needed to achieve optimal vitamin D status. Finally, more supplementation studies are needed to investigate the relationship between vitamin D intake and decline in physical function, if present, at different thresholds in different races and ethnicities; perhaps a genetic variation in response to vitamin D intake exists.

Vitamin D deficiency is uncommon in healthy elderly and it is clear that more widespread supplementation (or increased food fortification) is necessary to arrive at a high proportion of individuals meeting what is currently believed to be optimal levels. In the absence of important deficiency in vitamin D there does not appear to be any association of vitamin D level with functional decline. It is important to distinguish a healthy elderly cohort from the many repeated studies of chronically ill patients recruited in ambulatory care clinics or long-term care settings.

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APPENDICES

Appendix 1. Study setting and methodology

Appendix 2. General section questionnaire

Appendix 3. PASE: Physical Activity Scale for the Elderly questionnaire

Appendix 4. 24-hour recall form

Appendix 1. Study setting and methodology

Study setting

The data for this study have been obtained from the NuAge cohort, a 5 year longitudinal observational study of 1793 independently-living men and women aged 68 to 82 years at baseline recruited from Sherbrooke and Laval/ Montreal. The NuAge sample recruitment stratified participants into three age categories 70 ± 2 years, 75 ± 2 years and 80 ± 2 years with equivalent numbers of men and women in each group. The older age groups were intentionally over sampled so as to have good precision in all age groups. Names of those meeting desired age-sex criteria were obtained from a random sample within each age sex stratum from the Quebec health insurance registry (RAMQ) and potential subjects were sent an invitation letter and telephoned to assess eligibility and willingness to participate. Only community dwelling people were contacted. Inclusion and exclusion criteria were assessed in two phases; a telephone screening survey and a clinical examination.

Inclusion criteria

Community-dwelling men and women aged 68-82 years, French or English speaking, willing to commit for a 5 year-period, able to walk without help (cane acceptable), free of disabilities in activities of daily living, no cognitive impairment (3MS > 79), able to walk at least 100 meters (one block) or to climb one flight of stairs without rest and able and willing to sign informed consent.

Exclusion criteria

People suffering from class II heart failure, chronic obstructive pulmonary disease (COPD) requiring home oxygen therapy or oral steroids, inflammatory digestive diseases

or cancer treated by radiation therapy, chemotherapy or surgery in the 5 years prior to enrollment (with the exception of skin baso-cellular carcinoma). The exclusion criteria were assessed by a registered nurse.

Sample size

For our first manuscript (chapter 3) which examined vitamin D status in a healthy free living older people living in Québec, we sampled 405 subjects and analyzed their 25(OH)D concentration levels. Based on a mean 25(OH)D concentration of 74 nmol/L, with SD of 30 from our data, this sample provides 80% power to detect a difference in 25(OH)D concentration between men and women of 8.3 nmol/L. The detectable difference by age group is limited to differences of at least 10.5 nmol/L. We used the same set of data of theses 405 subjects to assess vitamin D intake from foods and supplements in our second manuscript (chapter 4). For our third question that investigated the relationship between vitamin D status and decline in physical performance measures, we initially started with data for the same 405 subjects and lost 48 subjects over 2 years of follow-up as discussed in manuscript 3 (chapter 5). We based our sample size calculations on changes in strength and functional status over time that could be expected to be picked up (80% power) comparing the vitamin D deficient group with the vitamin D optimal group are as follows; the strength measures vary from 9-14% change; the overall functional performance score, 12% change; The TUG score 11%, and chair stands 21%.

Samples

Four hundred and five subjects were chosen at random from those with complete data set required for this secondary study at year two from the NuAge study, which was the baseline in our study. The sample was stratified by sex and by season for Vitamin D work. The two seasons are January to March, inclusive, when subjects rely on dietary vitamin D intake for synthesis of 25(OH)D and June to September when the relationship of diet to vitamin D status is expected to be affected by sun exposure. The 405 stored samples were analyzed in duplicate. 25(OH)D serum levels were analyzed from frozen sera samples. Dietary measures of the relationship of foods and nutrients to vitamin D levels were examined from data provided for these 405 subjects. Research protocol has been approved by the ethics committees of the Institut universitaire de gériatrie de Montréal (IUGM), Sherbrooke (IUGS) and the McGill University (Faculty of Agricultural and Environmental Sciences). All participants have signed the informed consent form, after being fully informed of the study objectives and procedures and their right to withdraw from the study at any time. A significant person was designated before the start of the study in the event that the participant becomes unable to give informed consent over the course of the study.

Measurements

Demographic variables: Sex, age, education level, semi-rural or urban dwelling. Age and sex are obviously strongly related to health status and dietary intake and may act as confounders. The role of education level in this group born between 1921 and 1935 is not known, nor is the effect of living in a semi-rural or urban environment (in this case the

semi-rural environment is not isolated from major service delivery of any sort). The latter two variables were examined for their potential to confound the relationships examined.

Smoking status: (current, ever, never) has been measured (Levasseur, 1987). Smoking and dietary quality in the adult Canadian population are known to be related (Palaniappan et al., 2003).

Physical activity: Present physical activity was quantified using the Physical Activity Scale for the Elderly "PASE" questionnaire (Friedenreich et al., 1998). Individuals reported on leisure time activity, household activity and work-related activity in the past last week. Daily average of time spent doing each activity was added and weighted by intensity.

Anthropometric indices: Standing height and weight were measured with subjects dressed in light indoor clothing without shoes, using a stadiometer and a beam balance. BMI (wt/ ht^2). Waist circumference was measures using a measuring tape.

Diet: Three non-consecutive 24-hour dietary recalls (1 face-to-face and 2 telephone interviews) (Dubois & Boivin, 1990; Payette & Gray-Donald, 1991) including one weekend day have been gathered at baseline, and at 6 months (at the 6 month visit all three recalls were done by telephone). A 24-hour recall consists of a detailed description of all foods and beverages consumed during the previous day, including cooking methods and brand names. Repeat interviews provide precise estimates of usual energy and nutrient intakes (Payette & Gray-Donald, 1991). Portion models aid in the estimation of portion sizes. The recalls were done by registered dietitians who followed a rigorous

10 day training period for data collection procedures. An average intake from the first 6 24-hour recalls will be used to predict vitamin D status. Dietary analysis were done using the CANDAT program (Godin and Assoc, London ON) which uses the 2007 Canadian Nutrient File, Health Canada, and is augmented by a database of >1200 additional foods that we have developed on site (Johnson-Down et al., 2006). The program allows for the development of food groupings which can be used to examine the extent to which each food component, or food group, contributes to nutrient intake (Johnson-Down et al., 2006). Supplement use has been recorded and entered based on drug identification numbers (DIN). Where possible, exact composition of the supplement was used but in some cases a default value of the most common supplement of that type (eg 500 mg for a calcium supplement) was used (Troppmann, et al., 2002). Nutrients (vitamin D, calcium, phosphorus, magnesium) from food intakes alone, and food plus supplements, were compared to the dietary reference Intakes using the methodology outlined in the DRI report (Institute of Medicine, 2000).

Biochemical analysis of 25(OH)D in serum collected between 07:30 and 08:30 after 12 hour fast were analyzed by RIA using a commercial kit (DiaSorin, REF: 68100E, Stillwater, MN, USA);. The assay for 25(OH)D equally measures the D_2 and D_3 isoforms and has excellent agreement with HPLC methodology at IDS values = 0.94 x HPLC values + 2.8 nmol/L. Samples have been frozen in aliquots at -80°C. The date of the blood draw was used to control for seasonal variation in vitamin D level due to potential sunlight exposure. The values for 25(OH)D used as the biomarker for vitamin D status. Results were expressed in nmol/L and values for «deficiency» denoted by values < 37.5

nmol/L (Institute of medicine, 1997), «adequate» denoted by values within the range 37.5 to 74.9 nmol/L and «optimal» denoted by values 75 nmol/L and higher (Dawson-Hughes et al., 2005).

Maximal voluntary handgrip strength: This was measured with a Martin Vigorimeter and expressed in kiloPascals (kPa). The participant was seated on a standard straight back chair without arm rests. Hips and knees were at right angles. Upper extremity position standardized (American Society of hand Therapists, 1981) to enable good test-retest reliability (Payette et al., 1998). The participants were instructed to squeeze the bulb as hard as they could upon the examiner's signal. Verbal encouragement was provided. Effort did not exceed 10 seconds. Three measurements were taken for both the right and left hands. Recommended rest intervals to allow muscular recovery between replicate trials were provided. The highest score for either hand was used.

Maximum isometric strength of knee extensors (quadriceps): This was measured using a hand held dynamometer Microfet2 and expressed in terms of Newtons according to a standardized protocol (Bohannon, 1986; Payette et al., 1998; Reed et al., 1993). Muscle testing, using the belt-resisted, 'make' test, was performed bilaterally for each muscle group. The subject was seated in a straight back arm chair; knee extension was measured with the subject seated with the knee at 60° from full extension and foot resting on the floor. Three maximal contractions were recorded on each side. All contractions were of 4 seconds duration and completed about 30 seconds apart. The highest score was used (Payette et al., 1998).

Elbow flexors (Biceps): This was measured using a hand held dynamometer Microfet2 and expressed in Newtons according to standardized protocol (Desrosiers et al., 1995). The elbow flexion was measured with the participant's arm resting on the arm of the chair, with the limb segment and dynamometer positioned as described by Bohannon (Bohannon, 1986). The belt was secured to the arm of the chair, tightly enough to stabilize the forearm but not so tight as to create pressure. The test was repeated 3 times bilaterally and the best reading was used.

Walking speed: Participants were asked to walk at their usual speed over three lines marked on the floor (two red lines at 0 and 4 meters and one white line at 1 meter). Timing started when the participant crossed the white line and finished 3 meters later at the red line. The best time of two trials was recorded and expressed as meters/second. Use a cane or a walker was allowed, but not the help of another person (Avila-Funes et al., 2008). The test-retest reliability over a 2 week interval is very good [Interclass Correlation Coefficient (ICC) = 0.79] (Jette et al., 1999) and inter-rater reliability is excellent [ICC = 0.93] (Nevitt et al., 1989).

Chair-stand test: This test assesses lower extremity function, balance and coordination. Participants were asked to stand up and sit down from a standard chair, with arms folded across the chest, as fast as possible. Timing started from initial sitting position and ended at the final standing position at the end of the fifth stand (Avila-Funes et al., 2008; Guralnik et al., 1995). The test - retest reliability is good (ICC range = 0.67 – 0.73) (Seeman et al., 1994).

Timed "Up and Go": This test evaluates mobility and balance. Participants were asked to sit with their back rested on the back of the chair and arms rested on armrests. Participants were asked to rise from the chair, walk 3 meters at a comfortable pace, return to the chair and sit down. Timing started when the participant was asked to start and ended when the participant was reseated. The test-retest (ICC = 0.99) and inter-rater (ICC= 0.99) reliability is excellent (Payette et al., 1998).

One Leg Standing Balance: This is a valid and reliable test (Winograd et al., 1994) measuring maximum time subjects can stand on one foot with hands placed on their waist. The participant, not wearing shoes, was positioned approximately 1 meter from a wall and was instructed to stand on one foot lifting the dominant leg to calf level for as long as possible. The test was repeated for the other leg. Timing started when the participants took their leg off the ground and stopped when the foot touched the ground, the arm position was modified or 60 seconds elapsed. The best time for either leg was used (Avila-Funes et al., 2008).

Total functional score: Categories for performance were created for 3 muscle function measures (walking speed test, TUG and chair-stand test). Those who completed the tests were assigned a score from 1 to 4. These scores were corresponding to the time needed to complete the test. For each test, time needed to perform the task was stratified by sex and age group; after which, divided into 4 quintiles. Those who performed the task at the fastest time (1^{st} quintile were assigned a score of 4, 2^{nd} quintile a score of 2, 3^{rd} Quintile a score of 3 and 4^{th} quintile a score of 1. Those who did were not able to complete the task were assigned a score of zero. For the one leg balance test, a reversed scoring measure

was used, 4 was assigned for those who completed the 60 second stand or those who were classified into the quintile that stood longer. A sum of all for test was calculated to obtain a "Total functional score". Lowest possible score is 0 and the highest is 16 (Guralnik et al., 1994).

Statistical analysis:

For manuscript 1 (chapter 3), we examined the distribution of 25(OH)D concentrations presented as means and confidence intervals and examined differences by age-sex group using one-way ANOVA. The chi-square test was used to test differences in percentages; Fisher's exact test was applied when sample sizes were small. Correlations were estimated using Pearson's correlation. Independent correlates, of serum 25(OH)D were examined using multiple regression analysis. We undertook several regression models and the final model reported variables that contributed significantly to the model as well as some important confounders. We ran the analysis on both weighted and unweighted data.

For manuscript 2 (chapter 4), we presented baseline characteristics across the different 25(OH)D cut-offs expressed in terms of means ± standard deviation (SD) for continuous variables using one-way ANOVA, and in terms of frequencies and percentages for categorical variables using chi–square tests. Means of dietary vitamin D intake from supplement intake, total food intake or intake from separate food groupings were analyzed using one-way ANOVA. Both dietary vitamin D intakes adjusted per 1000 Kcal and as absolute intakes were assessed. Multiple linear regression models were used to

examine different food groups and other independent correlates of serum 25(OH)D concentrations.

For manuscript 3 (chapter 5), differences in subject characteristics across different 25(OH)D concentration cut-off levels were expressed in terms of means ± standard deviation using one-way ANOVA, or as frequencies and percentages using chi-square tests. Declines over time in each functional measure were examined using paired t-tests. Multiple linear regression models were used to assess vitamin D status category (3 levels of 25(OH)D concentrations) as an independent predictor of percent change in each functional performance score and each muscle strength test as well as in change in absolute measurement values. Also, 25(OH)D concentration was assessed as a predictor of functional decline, as a continuous variable in a multiple linear regression model. Other variables (indicated in the manuscript) were examined as potential confounders. All statistical inferences were made based on a two-sided significance level of $p < 0.05$. All statistical analyses were performed using SAS version 9.1 (SAS institute Inc, Cary, NC, USA).

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Appendix 2. General section questionnaire

Base en anglais section generate - T1

Numéro du sujet
Réponse:
Identification de I'évaluatrice Ré ponse:
SOCIO-DEMOGRAPHIC DATA
Réponse: ❑ Male
❑ Female
Country of birth
Réponse:
If born outside Canada, how many years have you lived abroad
Réponse:
Nationality (by right of birth) of your mother
Réponse:
Nationality (by right of birth) of your father
Réponse:
Race
Réponse: ❑ Caucasian
❑ Asian
❑ Black
❑ Hispanic
❑ Metis
What language do you speak most often at home?
Réponse: ❑ French
❑ English
❑ Other
Other language spoken at home, specify?
Réponse:
What is your current marital status?
Réponse: ❑ Single (never married)
❑ Widowed
❑ Married (including common law)
❑ Separated/divorced
How many years have you been a widow?
Réponse:
Do you have any children?
Réponse: ❑ Yes
❑ No
If yes, how many living children
Réponse:
Living arrangements (Who lives with you?) Answer YES or NO for each
Alone
Réponse: ❑ Yes

❑ No

Spouse

Réponse: ❑ Yes

❑ No

Child(ren)

Réponse: ❑ Yes

❑ No

Grand-child(ren)

Réponse: ❑ Yes

❑ No

Relatives, brothers or sii tens

Réponse: ❑ Yes

❑ No

Friends

Réponse: ❑ Yes

❑ No

Other seniors (not relatives)

Réponse: ❑ Yes

❑ No

Other

Réponse:

Do one or more of your children live close to your home?(Less than 30 minutes (one way) from your home)?

Réponse: ❑ Yes

❑ No

If yes, how many children live close to your home?

Réponse:

Are you……of your place of residence?

Réponse: ❑ Home owner

❑ Tenant

❑ Resident

❑ Resident at a relative or friend's home

Is your place of residence a...?

Réponse: ❑ Conventional individual residence (apartment or house)

❑ Individual or group housing with possibility of services (board and lodging, adapted dwelling, home for seniors)

❑ Religious congregation

How many years of education have you completed? R6ponse:

What kind of work have you done most of your life?

Réponse: ❑ Homemaker

❑ Farm worker

❑ Professional

❑ Administration

❑ Technician (skilled worker)

❑ Labourer (unskilled worker)

❑ Teaching

❑ Military

❑ Other

Occupational classification

Réponse: ❑ A. 0. Management
❑ B. 1. Business, finance and administration
❑ C. 2. Natural and applied sciences and related occupations
❑ D. 3. Health occupations
❑ E. 4. Social science, education govemement service and religion
❑ F. 5. Art, culture, recreation and sport
❑ G. 6. Sales and service occupations
❑ H. 7. Trades, transport and equipement operators
❑ I. 8. Occupations unique to primary industry
❑ J. 9. Processing, manufacturing and utilities

LIFE HABITS

Do you smoke (actually)

Réponse: ❑ No, never smoke
❑ Yes , occasionnally
❑ Yes , regularly
❑ No, but I used to smoke

How many cigarettes, cigars, pipes or tabacco, do you smoke each day?

Réponse:

How many years have you smoked?

Réponse:

How long ago did you stop smoking?

Réponse:

How many cigarettes, cigars, pipe, tabacco, did smoked each day?

Réponse:

Have you had any beer or ale in the past year?

Réponse: ❑ Yes
❑ No
❑ Not answered

Have you had any beer of ale in the past month?

Réponse: ❑ Yes
❑ No
❑ Not answered

Over the past month how often have you had beer or ale?

Réponse: ❑ 01-29 i
❑ 30-30+
❑ Not answered

When you had beer or ale(in the last month), how many cans or bottles did you usually have at one time?

Réponse: ❑ 1 or less
❑ 2
❑ 3-3+
❑ Not answered

Have you had any wine during the past year?

Réponse: ❑ Yes
❑ No

❑ Not answered

Have you had any wine in the past month?

❑ Yes

Réponse: ❑ No

❑ Not answered

Over the past month, how often have you had wine?

Réponse: ❑ 01-29

❑ 30-30+

❑ Not answered

When you had wine (in the last month), how many glasses did you usually have at one time?

Réponse: ❑ 1 or less

❑ 2

❑ 3-3+

❑ Not answered

Have you had any liquor in the past year, that is things like whiskey, vodka, gin, brandy, or liqueurs?

Réponse: ❑ Yes

❑ No

❑ Not answered

Have you had any liquor in the past month?

Réponse: ❑ Yes

❑ No

❑ Not answered

Over the past month, how often have you had liquor?

Réponse: ❑ 01-29

❑ 30-30+

❑ Not answered

When you had it (in the past month), how many drinks did you usually have at one time?

Réponse: ❑ 1 or less

❑ 2

❑ 3-3+

❑ Not answered

Has there ever been a time when you drank quite a bit more than you drink now?

Réponse: ❑ Yes

❑ No

❑ Not answered

SOCIAL NETWORK

How many people do you know well enough to visit in their home

Réponse: ❑ None

❑ One or Mix

❑ Three or four

❑ Five or more

❑ Not answered

About how many times did you talk to someone - friends, relatives, or others on the telephone in the past week (either you called them or they called you)?

Réponse: ❑ Not at all

❑ Once
❑ Twice
❑ Three or four times
❑ Once a day or more
❑ Not answered

How many times during the past week did you spend some time with someone who does not live with you, that is you went to see them or they came to visit you, or you went out to do things together?

Réponse: ❑ Not at all
❑ Once
❑ Twice
❑ Three or four times
❑ Once a day or more
❑ Not answered

Do you have someone you can trust and confide in?

Réponse: ❑ No
❑ Yes
❑ Not answered

Do you find yourself feeling lonely quite often, sometimes, or almost never?

Réponse: ❑ Almost never
❑ Sometimes
❑ Quite often
❑ Not answered

Do you see your relatives and friends as often as you want to or are you somewhat unhappy about how little you see them?

Réponse: ❑ As often as wants to
❑ Somewhat unhappy about how little
❑ Not answered

Is there someone who would give you any help at all if you were sick or disabled, for example your husband/wife, a member of your family, or a friend?

Réponse: ❑ No
❑ Yes
❑ Not answered

Is there someone who would take care of you as long as needed, or only for a time, or only someone who would help you now and then (for example, taking you to the doctor, or fixing lunch occasionally, etc.)?

a) Someone who would take care of you indefinitely (as long as needed)

Réponse: ❑ No
❑ Yes
❑ Not answered

b) Someone who would take care of you for a short time (a few weeks to 6 months)?

Réponse: ❑ No
❑ Yes
❑ Not answered

c) Someone who would help you now and then (taking you to the doctor or fixing lunch, etc.)?

Réponse: ❑ No

❑ Yes
❑ Not answered

HEALTH STATUS

Do you have any of the following illnesses at the present time? (check YES or NO for each of the following)

and IF YES

How much does it interfere with your daily activities?

Arthritis or rheu

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Oedema

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Asthma

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Emphysema, chronic bronchitis

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

High blood pressure

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Heart trouble

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Circulation trouble in arms or legs

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Diabetes

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Ulcers (of the digestive system)
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Other digestive problems (vomiting, constipation, diverticulosis)
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Liver or gallbladder disease
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Urinary problems (prostate)
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Osteoporosis
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Anemia
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Thrombosis, cerebral hemorrhage, CVA
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Parkinson's disease
Reponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Muscular dystrophy
Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal
Thyroid and gland problems
Réponse: ❑ No

❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Skin disorders

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Other diseases

Réponse: ❑ No
❑ Yes, Not at all
❑ Yes, A little
❑ Yes, A great deal

Specify

Résponse

Have you had a cancer or ever been treated for cancer throughout your life?

Réponse: ❑ No
❑ Yes

Type of cancer

Réponse: ❑ ovaries, uterus
❑ prostate
❑ lungs
❑ breast
❑ colorectal
❑ skin
❑ others

Year of diagnosis

Réponse:

Treatment

Réponse: ❑ Radiotherapy
❑ Chemotherapy
❑ Radio + chemo
❑ Hormonotherapy
❑ Curietherapy or Brachytherapy
❑ Surgery
❑ Other
❑ surgery and th4rapies

Throughout your life, did you undergo major surgery(ies)?

Réponse: ❑ No
❑ Yes

Type of surgery

Réponse: ❑ Digestive system
❑ Respiratory system
❑ Urinary system
❑ Genital organ
❑ Skeleton
❑ Muscles

❑ Nervous system
❑ Circulatory system
❑ Sense organs
❑ Teething
❑ Other

Type of surgery (2)
Réponse: ❑ Digestive system
❑ Respiratory system
❑ Urinary system
❑ Genital organ
❑ Skeleton
❑ Muscles
❑ Nervous system
❑ Circulatory system
❑ Sense organs
❑ Teething
❑ Other

Type of surgery (3)
Réponse: ❑ Digestive system
❑ Respiratory system
❑ Urinary system
❑ Genital organ
❑ Skeleton
❑ Muscles
❑ Nervous system
❑ Circulatory system
❑ Sense organs
❑ Teething
❑ Other

Throughout your life, have you suffered from any physical disabilities such as total or partial paralysis, missing or non-functional limbs, or broken bones?
Réponse: ❑ No
❑ Yes

Type of disability
Réponse: ❑ Total paralysis
❑ Partial paralysis
❑ Handicap (limbs)
❑ Broken bones (lower limbs)
❑ Broken bones (upper extremities)
❑ broken bones (>1)
❑ other

Have you ever been treated for or told you have rheumatoid arthritis?
Réponse: ❑ No
❑ Yes

Since the age of 45, have you experienced a fracture (broken bone) at any of the following sites (hip, rib, wrist)?
Réponse: ❑ No

❑ Yes

Hip fracture

Réponse: ❑ No

❑ Yes

Rib fracture

Réponse: ❑ No

❑ Yes

Wrist fracture

Réponse: ❑ No

❑ Yes

How old were you at menopause?

Réponse:

Do you currently take or have you ever taken estrogen?

(Examples include Premarin®, Estraderm®, Estring®, Estrace®, Ogen@)

Réponse: ❑ No

❑ Yes

INCOME

What are your main sources of income? First source of income

Réponse: ❑ None

❑ Guaranteed income supplement or Social assistance

❑ Federal pension/Old age security without supplement/Allowance for the survivor

❑ Rentes du Quebec (Quebec pension plan, surviving spouse's pension)

❑ Private pension plans (employer)

❑ Other personal savings and investments

❑ work

❑ not answered

❑ Other

Second source of income?

Réponse: ❑ None

❑ Guaranteed income supplement or Social assistance

❑ Federal pension/Old age security without supplement/Allowance for the survivor

❑ Rentes du Quebec (Quebec pension plan, surviving spouse's pension)

❑ Private pension plans (employer)

❑ Other personal savings and investments

❑ work

❑ not answered

❑ Other

Third source of income?

Réponse: ❑ None

❑ Guaranteed income supplement or Social assistance

❑ Federal pension/Old age security without supplement/Allowance for the survivor

❑ Rentes du Quebec (Quebec pension plan, surviving spouse's pension)

❑ Private pension plans (employer)

❑ Other personal savings and investments

❑ work
❑ not answered
❑ Other

Do you agree to give us an estimate of your annual family income?

Réponse: ❑ No
❑ Yes

Here is a list corresponding to different income levels. Which category best describes your annual family income, while taking into account all sources of income? (Montrez le carton de choix de reponses).

Réponse:

To what extent this income can satisfy your needs?

Réponse: ❑ Very well
❑ Decently
❑ Not so well
❑ Not at all

Appendix 3. PASE: Physical Activity Scale for the Elderly questionnaire

PASE anglais T3 - T3 anglais

Numéro du sujet
Réponse:
Identification de I'évaluatrice
Réponse:
Temps de mesure
Réponse: ❑ T1
❑ T2
❑ T3
❑ T4
❑ T5
Variable PASE1
LEISURE TIME ACTIVITY
Over the past 7 days, how often did you participate in sifting activities such as reading, watching TV or doing hancrafts
Réponse: ❑ never
❑ seldom (1-2 days)
❑ sometimes (3-4 days)
❑ often (5-7 days)
Variable PASE1B
On average, how many hours per day did you engage in these sitting activities?
Réponse: ❑ less than 1 hour
❑1 but less than 2 hours
❑2-4 hours
❑more than 4 hours
Variable PASE2 I
Over the past 7 days, how often did you take a walk outside your home or yard for any reason? For example, for fun or exercise, walking to work, walking the dog, etc.?
Réponse: ❑ never
❑ seldom (1-2 days)
❑ sometimes (3-4 days)
❑ often (5-7 days)
Variable PASE2A
On average, how many hours per day did you spend walking?
Réponse: ❑ less than 1 hour
❑1 but less than 2 hours
❑2-4 hours
❑ more than 4 hours
Variable PASE3
Over the past 7 days, how often did you engage in light sport or recreational activities such as bowling, golf with a cart, shuffleboard, fishing from a boat or pier or other similar activities?
Réponse: ❑ never

❑ seldom (1 -2 days)
❑ sometimes (3-4 days)
❑ often (5-7 days)

Variable PASE3B

On average, how many hours per day did you engage in these light sport or recreational activities?

Réponse: ❑ less than 1 hour
❑1 but less than 2 hours
❑ 2-4 hours
❑ more than 4 hours

Variable PASE4

Over the past 7 days, how often did you engage in moderate sport or recreational activities such as doubles tennis, ballroom dancing, hunting, ice skating, golf without a cart, softball or other similar activities?

R6ponse: ❑ never
❑ seldom (1 -2 days)
❑ sometimes (3-4 days)
❑ often (5-7 days)

Variable PASE4B

On average how many hours per day did you engage in these moderate sport and recreational activities?

Réponse: ❑ less than 1 hour
❑1 but less than, 2 hours
❑ 2-4 hours
❑ more than 4 hours

Variable PASE5

Over the past 7 days, how often did you engage in strenuous sport and recreational activities such as jogging, swimming, cycling, singles tennis, aerobic dance, skiing (downhill or cross-country) or other similar activities?

Réponse: ❑ never
❑ seldom (1 -2 days)
❑ sometimes (3-4 days)
❑ often (5-7 days)

Variable PASE5B

On average, how many hours per day did you engage in these strenuous sport and recreational activities?

Réponse: ❑ less than 1 hour
❑1 but less than 2 hours
❑ 2-4 hours
❑ more than 4 hours

Variable PASE6

Over the past 7 days, how often did you do any exercises specifically to increase muscle strength and endurance, such as lifting weights or pushups, etc.)?

Réponse: ❑ never
❑ seldom (1-2 days)
❑ sometimes (3-4 days)
❑ often (5-7 days)

Variable PASE6B
On average, how many hours per day did you engage in exercices to increase muscle strength and endurance?
Réponse: ❑ less than 1 hour
❑1 but less than 2 hours
❑ 2-4 hours
❑ more than 4 hours
Variable PASE7
HOUSEHOLD ACTIVITIES
During the past 7 days, have you done any light housework, such as dusting or washing dishes
Réponse: ❑ no
❑ yes
Variable PASE8
During the past 7 days, hale you done any heavy housework or chores, such as vacuuming, scrubbing floors, washing windows, or carrying wood?
Réponse: ❑ no
❑ yes
Variable PASE9A
During the past 7 days, did you engage in any of the following activities? Please answer YES or NO for each item
Home repairs like painting, wallpapering, electrical work, etc
Réponse: ❑ no
❑yes
Variable PASE9B
Lawn work or yard care, including snow or leaf removal, wood chopping, etc.
Réponse: ❑ no
❑ yes
Variable PASE9C
Outdoor gardening, sweeping the balcony or the stairs
Réponse: ❑ no
❑ yes
Variable PASE9D
Caring for another person, such as children, dependent spouse, or other adult
Réponse: ❑ no
❑ yes
Variable PASE10
WORK RELATED ACTIVITIES
During the past 7 days, did you work for pay or as a volunteer
Réponse: ❑ no
❑ yes
Variable PASE10A
How many hours per week did you work for pay and/or as a volunteer?
Réponse:
Variable PASE10B
Which of the following categories best describes the amount of physical activity required on your job and/or as a volunteer?

Réponse: ❑ Mainly sitting with slight arm movements (eg. office worker, bus driver)
❑ Sitting and standing with some walking (eg. cashier, light tool and machinery worker)
❑ Walking, with some handling of materials generally weighing less than 50 lbs (eg. mailman, waitress, construction worker)
❑ Walking and heavy manual work often requiring handling of materials weighing over 50 lbs (eg. lumberjack, stone mason, farm or general laborer)
valid questionnaire
Réponse: ❑ no
❑ yes

Appendix 4. 24-hour recall form

Rappel alimentaire de 24 heures pour le suivi 6ième mois Numéro du sujet :_____

ɘ Saisie dans CANDAT

Date du rappel:_____ Jour de la semaine:_____

Heure	Type de repas	Lieu	Quantité	Description de l'aliment

LÉGENDEType de repas : 1 : petit déjeuner 2 : collation AM 3 : dîner 4 : collation PM 5 : souper 6 : collation du soir 7 : autre repas
Lieu : M : maison R : restaurant C : cafétéria F : restaurant 'fast food' T : 'take out' V : visite

Rappel alimentaire de 24 heures pour le suivi 6ième mois Numéro du sujet :_____

ə Saisie dans CANDAT

Date du rappel:_____ Jour de la semaine:_____

Heure	Type de repas	Lieu	Quantité	Description de l'aliment

LÉGENDEType de repas : 1 : petit déjeuner 2 : collation AM 3 : dîner 4 : collation PM 5 : souper 6 : collation du soir 7 : autre repas
Lieu : M : maison R : restaurant C : cafétéria F : restaurant 'fast food' T : 'take out' V : visite

Rappel alimentaire de 24 heures pour le suivi 6ième mois Numéro du sujet :_____

ə Saisie dans CANDAT

Prenez-vous des suppléments de vitamines et minéraux? OUI = 1 NON = 0 ( )

DIN	Manufacturier	Description (inclure la composition)	Quantité

Est-ce que cette journée est représentative de votre alimentation habituelle? OUI = 1 NON = 0 | __ |

Si non, quelles sont les différences?

_____

_____

_____

Has your appetite changed over the last 6 months?

_____

ə No change ə Increased appetite ə Decreased appetite

Why?_____

Duration :_____

RECETTES

Détails à spécifier: ingrédients, mode de préparation et de cuisson ainsi que le rendement.

Nom de la recette	Nombre de portions	Ingrédients	Mode de préparation et de cuisson