Vitamin D Recommendations for Canadian Elderly:

An evaluation of adequacy of current clinical practices for nutrition and skeletal health

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ABSTRACT

Vitamin D is a key nutrient in bone health. Seniors living in long-term care (LTC) facilities in Canada face a higher risk of deficiency as menus are often deficient in vitamin D and sunlight exposure is limited. Vitamin D status can be measured by serum 25-hydroxyvitamin D concentration (25(OH)D). Many seniors in Canada have low vitamin D status.

The global objectives of this thesis were to: (1) assess vitamin D intake and association with biomarkers of bone health in older men in a LTC facility of the Montréal region (latitude: 46°N); (2) assess the impact of an 8-wk supplementation regimen of 2000 IU/d of vitamin D_3 on 25(OH)D and markers of bone metabolism; (3) determine how much vitamin D_3 is needed to sustain vitamin D status using vitamin D_3 fortified foods.

Study 1 tested the first objective. Food intake and sunlight exposure of 30 men were measured for 1 year. Food intake was measured with 3-d weighed food records. Biomarkers of bone metabolism (serum 25(OH)D, parathyroid hormone (PTH), calcium, phosphate, osteocalcin (OC) and C-terminal telopeptide of collagen type 1 (CTX)) were tested. Descriptive statistics and change over time were analyzed.

A mean of 280±120 IU/d of vitamin D was consumed. In the winter months, over 30% of participants were below the 50 nmol/L of serum 25(OH)D concentrations suggested by the Institute of Medicine. Serum 25(OH)D rose by summer and declined in the fall. PTH was lower in the spring. CTX and OC were unchanged.

Study 2 tested the second and third objectives with a 2 phase trial. First, a Loading Phase used 2000 IU/d vitamin D_3 tablet supplementation (October to December; 8 weeks; RCT –8). A randomized controlled trial (RCT) followed (January to July; 24 weeks; RCT0–RCT24).

Participants were randomized to 3 groups: Placebo group (0 IU/d), 500 IU/d vitamin D_3 group or 1000 IU/d vitamin D_3. Vitamin D_3 was given as bite size fortified foods. The Placebo group received identical unfortified foods. Biomarkers of bone metabolism (serum 25(OH)D, PTH, calcium, phosphate, OC, CTX, osteoprotegerin and RANKL) were measured at RCT –8, RCT0 and RCT24. At RCT0 and RCT24, peripheral dual-energy X-ray absorptiometry, peripheral quantitative computed tomography (pQCT) and handgrip strength were measured on the non-dominant forearm. Differences were tested using mixed model ANOVA for fixed effects of time and group and interactions of time by group.

At RCT –8, mean vitamin D intakes and mean 25(OH)D concentrations were 494±380 IU/d and 56.9±13.3 nmol/L for the Placebo group, 769±526 IU/d and 54.6±15.0 nmol/L for the 500 IU/d group and 562±383 IU/d and 56.9±9.7 nmol/L for the 1000 IU/d group. The 2000 IU/d of vitamin D_3 raised serum 25(OH)D to a mean value of 65 nmol/L in all groups. During the RCT, the 500 IU/d and 1000 IU/d groups maintained serum 25(OH)D above 65 nmol/L. In 8 weeks, the 25(OH)D concentration values of the Placebo group reverted back to the values of RCT –8. Biomarkers of bone metabolism were stable. The pQCT values declined over time in trabecular volumetric bone mineral density (vBMD) for the 500 IU/d group and in vBMD at the diaphysis for the 1000 IU/d group. Total bone area in the diaphysis improved and areal bone mineral density improved in the 1000 IU/d group. For all groups, muscle area declined over time and muscle density did not change. Handgrip strength was stable in the 1000 IU/d group.

In conclusion, these studies suggest that vitamin D supplementation is needed to achieve and maintain healthy vitamin D status. The group getting 1000 IU/d of vitamin D_3 improved cross-sectional bone area at 66% of the forearm diaphysis suggesting remodeling with incomplete mineralization. As handgrip strength was improved in the 1000 IU/d group, vitamin

D_3 could positively influence muscle strength and, possibly reduce the risk of falls and fractures. More research is needed to understand the relationships among vitamin D and musculoskeletal health in older men living in LTC.

RÉSUMÉ

La vitamine D est vitale à la santé des os. La population âgée vivant en centres de soins de longue durée (CHSLD) au Canada a un risque élevé de carence car les menus manquent de vitamine D et l'exposition au soleil est limitée. Le taux sérique de 25-hydroxyvitamine D (25(OH)D) mesure la vitamine D. Plusieurs personnes âgées ont de faibles taux de 25(OH)D.

Les objectifs de cette thèse étaient: (1) évaluer les apports en vitamine D et liens avec différents biomarqueurs osseux chez des hommes âgés en CHSLD (région de Montréal; 46°N); (2) évaluer l'impact d'un ajout de 2000 UI/j de vitamine D_3 pour 8 semaines sur 25(OH)D et marqueurs du métabolisme osseux; (3) quantifier la dose de vitamine D_3 requise pour maintenir le taux sérique de 25(OH)D, via des aliments fortifiés en vitamine D_3.

L'étude 1 a testé le premier objectif. Durant 1 an, l'alimentation et l'exposition au soleil ont été suivies chez 30 hommes âgés. Les taux de 25(OH)D, hormone parathyroïdienne (PTH), calcium, phosphate, ostéocalcine (OC) et télopeptides c-terminal du collagène de type 1 (CTX) sériques ont été mesurés. Les données descriptives et les changements ont été analysés.

Les participants prenaient en moyenne 280±120 UI/j de vitamine D. En hiver, plus de 30% des participants avaient un taux sérique de moins de 50 nmol/L, valeur suggérée par l'Institute of Medicine. En été, les taux sériques de 25(OH)D ont augmenté et rebaissé à l'automne. PTH fut plus basse au printemps; CTX et OC inchangés.

L'étude 2 a testé les deuxième et troisième objectifs. 1) Étude pré/post : comprimés de 2000 UI/j de vitamine D_3 donnés à 60 participants (Période de charge; octobre-décembre; 8 semaines; ERC -8); 2) Essai contrôlé randomisé (janvier-juillet; 24 semaines; ERC0-ERC24) de 3 groupes : Placebo, 500 UI/j ou 1000 UI/j de vitamine D_3 via des aliments fortifiés. Le groupe

Placebo recevait les mêmes aliments non fortifiés. Les taux sériques de 25(OH)D, PTH, calcium, phosphate, OC, CTX, ostéoprotégérine (OPG) et ligand RANKL furent testés. À ECR0 et ECR24, l'absorptiométrie périphérique à rayons X biénergique (pDXA), tomographie périphérique assistée par ordinateur (pQCT) et force de préhension ont été mesurées sur le bras non-dominant. Les différences ont été testées par un modèle mixte ANOVA répétées à effets fixes du temps, des groupes et de leurs interactions.

À ERC –8, la vitamine D et le taux sérique de 25(OH)D étaient de 494±380 UI/j et 56.9±13.3 nmol/L pour le groupe Placebo, 769±526 UI/j et 54.6±15.0 nmol/L pour le groupe 500 UI/j et de 562±383 UI/j et 56.9±9.7 nmol/L pour le groupe 1000 UI/j. Les comprimés de 2000 UI/j de vitamine D_3 ont élevé le taux moyen de 25(OH)D à 65 nmol/L. Durant l'ERC, les groupes 500 UI/j et 1000 UI/j avaient des taux de 25(OH)D au-dessus de 65 nmol/L. A la 8^{e} semaine de l'ERC, les taux de 25(OH)D Placebo ont baissés au niveau des taux mesurés à ERC –8. Les biomarqueurs osseux furent stables. La densité minérale osseuse volumétrique trabéculaire du groupe 500 UI/j et la densité minérale osseuse volumétrique de la diaphyse du groupe 1000 UI/j ont baissé. L'aire totale de l'os de la diaphyse et la densité minérale osseuse mesurée par pDXA ont haussé pour le groupe 1000 UI/j. L'aire du muscle a diminué et la densité musculaire a été maintenue pour tous. La force de préhension fut stable pour le groupe 1000 UI/j.

En conclusion, ces études suggèrent qu'un supplément de vitamine D est requis pour atteindre et maintenir un taux de vitamine D adéquat. L'aire osseuse à 66% de la diaphyse du bras du groupe 1000 UI/j de vitamine D_3 a augmenté suggérant un début de remodelage osseux et une minéralisation incomplète. La force de préhension fut stable dans le groupe 1000 UI/j suggérant que la vitamine D_3 influencerait positivement la force musculaire et, pourrait baisser le

risque de chutes et de fractures. D'autres études sont requises pour mieux cerner les liens entre la vitamine D et la santé musculo-squelettique chez les hommes âgés vivant en CHSLD.

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ADVANCE OF SCHOLARLY KNOWLEDGE

I. Original contribution to knowledge

This doctoral dissertation provides a comprehensive analysis of vitamin D consumption in older men living in a long-term care (LTC) facility of the Montréal region (46°N). Canada's Food Guide for Healthy Eating has been recommending vitamin D supplementation of adults over the age of 50 y since 2007. In 2011, the Institute of Medicine (IOM) published new Dietary Reference Intakes for vitamin D which replaced the Adequate Intake (AI) of 600 IU/d with an Estimated Average Requirements (EAR: 400 IU/d) and a Recommended Dietary Allowance (RDA: 800 IU/d) for the older population of more than 70 y to maintain a 25(OH)D concentration of 50 nmol/L and protect bone health. The Endocrine Society had proposed a higher target for 25(OH)D concentration (> 75 nmol/L). The Canadian Community Health Survey and the NuAge study in Québec reported on vitamin D intakes of the senior population but only those living in the community. The first study reveals the low vitamin D intakes and low vitamin D status of older men living in LTC. It confirms that there is a need for adequate vitamin D supplementation since reaching the AI or the new RDA with food alone is nearly impossible for this frail senior population. Study 2 was designed to first promote vitamin D repletion for 8 weeks and subsequently assess the adequate maintenance dose (RCT of 24 weeks) with 3 groups: 0 IU/d Placebo group, 500 IU/d group and 1000 IU/d group using fortified foods to reduce polypharmacy and help to preserve quality of life. This protocol demonstrated the a loading dose of 2000 IU/d for 8 weeks could raise the mean 25(OH)D concentration over 65 nmol/L, but did not reach the 75 nmol/L as suggested by the Endocrine Society. The RCT segment demonstrated that a supplement between 500 to 1000 IU/d was sufficient to maintain the 25(OH)D status

which concurs with the IOM recommendations. A selection of vitamin D_3 fortified foods was well appreciated by the older men included in this study. This work could help to guide future research and help to bridge the gaps in bone health research for older men in LTC.

II. Research publications

- Germain I, Agellon S, Weiler H (2013) Insufficient Vitamin D Intake and Low Vitamin D Status in Men Over 80 Y of Age: Interventions Required To Meet Dietary Targets in Long-Term Care Facilities. Vitam Miner 2:113. doi:10.4172/vms.1000113

III. Abstracts and presentations

- Germain I and Weiler H. Impacts of vitamin D status on health of Canadian veterans living in long-term care. Research Day – Ste-Anne's Hospital (2008) (Oral presentation)
- Germain I and Weiler H. Vitamine D: Besoins, apports et impacts sur la santé de la personne âgée au Canada. Institut universitaire de gériatrie de Montréal (2008) (Oral presentation)
- Germain I and Weiler H. Impacts du statut en vitamine D sur la santé de vétérans Canadiens, Féd. Producteurs d'œufs de consommation du Québec, Ass. Générale spéciale (2008) (Oral presentation)
- Germain I and Weiler H. Vitamin D Intake and Status in Veterans Living in Long-Term Care Facility. 9th Interdisciplinary Graduate Student Research Symposium (IGSRS) (2009) (Oral presentation)

- Germain I and Weiler H. Impacts of vitamin D status on health of Canadian veterans living in long-term care facility. Dietitians of Canada, Annual Conference (2009) (Oral presentation)
- Germain I and Weiler H. Vitamin D intake and status in veterans living in long-term care facility. ASBMR (2011) (Poster presentation)
- Germain I and Weiler H. Impact of vitamin D_3 fortified foods on vitamin D status and radial bone mineral density in elderly men during the winter and spring seasons: A randomized controlled trial. CIHR (2013) (Poster presentation)
- Germain I, Vanstone C, Hazell T, Bianchini C, Agellon S, Weiler H. Impact of vitamin D_3 fortified foods on vitamin D status and radial bone mineral density in elderly men during the winter and spring seasons: A randomized controlled trial. Clinical Musculoskeletal Research (2014) (Poster presentation)
- Germain I, Vanstone C, Hazell T, Lee M, Bianchini C, Agellon S and Weiler H. Foods specially fortified with vitamin D preserve winter-time status and BMD in elderly men. MUHC Bone Rounds (2014) (Oral presentation)

LIST OF ABBREVIATIONS

$1,25(OH)_2D$:	1,25-dihydroxyvitamin D or calcitriol (active state)
$24,25(OH)_2D$:	24,25-dihydroxyvitamin D
25(OH)D:	25-hydroxyvitamin D or calcidiol
aBMD:	Areal bone mineral density
AI:	Adequate Intake
ANOVA:	Analysis of variance
AU:	Australia
BMC:	Bone mineral content
BMD:	Bone mineral density
BMI:	Body mass index (kg/m2)
BTM:	Bone turnover marker
CaMos:	Canadian Multicentre Osteoporosis Study
CAN:	Canada
CCHS:	Canadian Community Health Survey
CLIA:	Chemiluminescent immunoassay
CONSORT:	Consolidated Standards of Reporting Trials
CNF:	Canadian Nutrient File
CTX:	C-terminal telopeptides of Type 1 collagen
d:	Day
DBP:	Vitamin D binding protein
DEQAS:	Vitamin D External Quality Assessment Scheme
DIN:	Drug Identification Number

DRI:	Dietary Reference Intakes
DXA:	Dual-energy X-ray absorptiometry
EAR:	Estimated Average Requirement
ELISA:	Enzyme-linked immunosorbent assay
ES:	Spain
EU:	European Union
FEFA:	Frail Elderly Functional Assessment
FI:	Finland
FR:	France
FRAX:	Fracture risk assessment tool
GB:	Great Britain
HPLC:	High performance liquid chromatography
IM inj.:	Intramuscular injection
IOM:	Institute of Medicine (US)
IR:	Ireland
IU:	International Unit [1 IU vitamin D = 0.025mg]
LC-MS/MS:	Liquid chromatography tandem mass spectrometry
min:	Minutes
MMSE:	Mini-mental state examination
mo:	Month
MSC:	Mesenchymal stem cell
N/A:	Not available
NHANES:	National Health and Nutrition Examination Survey (US)

NIH:	National Institutes of Health (US)
NL:	The Netherlands
NO:	Norway
NZ:	New Zealand
OPG:	Osteoprotegerin
pDXA:	Peripheral dual-energy X-ray absorptiometry
pQCT:	Peripheral quantitative computed tomography
QCT:	Quantitative computed tomography
RANKL:	Receptor activator of nuclear factor-Kß ligand
PTH:	Parathyroid hormone
RCT:	Randomized controlled trial
RDA:	Recommended Dietary Allowance
RIA:	Radioimmunoassay
RO:	Romania
RXR:	Retinoid X receptors
SAS:	Statistical Analysis System
SZ:	Switzerland
UK:	United Kingdom
UL:	Tolerable Upper Intake Level
US:	United States of America
UVB:	Ultraviolet beta
vBMD:	Volumetric bone mineral density
Vitamin D_2:	Ergocalciferol

Vitamin D_3:	Cholecalciferol
VDR:	Vitamin D receptor
VDRE:	Vitamin D receptor elements
wk:	Week
WHO:	World Health Organization
y:	Year

CONTRIBUTION OF AUTHORS

Manuscripts 1, 2 and 3: The authors' contributions were as follows: Isabelle Germain and Dr. Weiler conceived the studies and obtained funding, coordinated and led the data collection, oversaw laboratory analyses, conducted the data analysis, provided technical oversight and input into all aspects of the study, drafted the research manuscripts and had primary responsibility for the final content.

Manuscript 1: Sherry Agellon and Christina Bianchini conducted laboratory analyses. Isabelle Germain and Sonia Jean-Philippe organized and recorded the weighed food intakes and completed the analysis. All authors contributed to the review and revision of the manuscript and read and approved the final manuscript. All other authors declared no conflicts of interest related to this study.

Manuscripts 2 and 3: Isabelle Germain prepared the fortified foods, organized and collected the weighed food intakes and audited the analysis. Michelle Lee, Christina Bianchini and Sonia Jean-Philippe recorded food intakes. Monica Bashaw completed the nutrient analysis and prepared the final report. Michelle Lee designed and validated the vitamin D food appreciation questionnaire, led interviews and wrote final report. Isabelle Germain, Tom Hazell and Catherine Vanstone performed pDXA and pQCT scans and data audits; Sherry Agellon and Christina Bianchini conducted laboratory analyses; and all authors contributed to the review and revision of the manuscript and read and approved the final manuscripts. All authors declared no conflicts of interest related to this study

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"When we try to pick out anything by itself, we find it hitched to

everything else in the Universe"

John Muir

This research work began as a simple idea and morphed into something much greater than I could have ever imagined. I soon realized that everything is attached. A number of important individuals made this possible.

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CHAPTER 1 – INTRODUCTION

1.1 Background and Rationale

Vitamin D is extensively studied as a key factor in achievement of bone health [1-19] and in many chronic diseases including cancer (colon, pancreatic), diabetes, autoimmune diseases, depression, neurocognitive decline and functional capacities [6, 20-38] as well as overall mortality [39-42]. With mounting proof of the involvement of vitamin D metabolites in several physiological pathways, vitamin D is known to benefit skeletal health [43] and believed to benefit general health. However, given the conflicting results of recent meta-analyses and the complexity of the action of vitamin D and its pathophysiological pathways, more research is required to bridge knowledge gaps that exist for advanced aging [17, 19, 44-46].

By 2025, 20 % of Canadians will be ≥ 65 y [47]. The 2011 Canadian Census reported that nearly 30 % of seniors 85 y and over will be living in long-term care (LCT) facilities [48]. Thus optimizing care in LTC facilities for frail seniors will require effective novel treatments for the treatment of osteoporosis, osteomalacia and bone health. Vitamin D intake as a means to improve vitamin D status is highly regarded in the treatment of osteoporosis [49]. Abundant work has focused on bone health in postmenopausal women. However, gender is now recognized as a modulator of bone health outcomes in aging [50]. Furthermore, natural food sources and solar exposure are not sufficient to maintain vitamin D status. Further investigation is needed to fill the knowledge gaps regarding vitamin D and health of elderly male populations living in LTC facilities.

Vitamin D can be obtained in 2 ways: 1) endogenous synthesis, through solar irradiation (wavelengths of 290-315 nm, Ultraviolet beta (UVB)), where 7-dehydrocholesterol in the epidermis is converted to pre-vitamin D_3 or, D_2) exogenously, through foods or supplements containing ergocalciferol (vitamin D_2) or preferentially cholecalciferol (vitamin D_3). Skin pigmentation is known to affect efficiency of synthesis [51]. In the older population, endogenous synthesis remains active [19] however, some studies showed a decreased conversion of 7-dehydrocholesterol to pre-vitamin D [52, 53]. Recommendations from the Canadian Cancer Society [54] encourage use of sunscreen in the prevention of skin cancer at all ages, but these block the transformation of 7-dehydrocholesterol in the skin to previtamin D_3. For those in institutions the issues related to endogenous capacity are of a lesser concern because UVB exposure is limited in general and dermal synthesis of vitamin D is limited to the months of April through October in Canada due to limited solar UVB radiation [55]. This leads to the conclusion that in LTC, the primary sources of vitamin D for Canadians are foods or supplements. In fact, the dietary recommendations for vitamin D in North America assume no UVB exposure [21].

The most recent recommendations of the Institute of Medicine (IOM) on Dietary Reference Intakes for Calcium and Vitamin D [21] were updated in 2011. The Adequate Intake (AI) values were updated to Estimated Average Requirements (EAR) and Recommended Dietary Allowances (RDA). For adults of >70 y, the daily AI of 600 IU (15 μg) was changed to a daily EAR of 400 IU (10 μg) and RDA of 800 IU (20 μg). The Upper Tolerable Intake Level (UL) was doubled to 4000 IU/d. Fracture risk was stated as the most important indicator of status due to the associated risks of morbidity and mortality. In the closing remarks, the IOM committee highlighted the lack of long term vitamin D dose-response studies in the older population and the need for more information regarding specific effects of vitamin D on health outcomes other than

bone health. The underrepresentation of men in recently published meta-analyses [1, 3, 5, 6, 10, 11, 16] on vitamin D supplementation and bone health (averages of 85% female cohorts) is an important knowledge gap that needs to be closed.

Serum calcidiol (25(OH)D) is the best indicator of vitamin D status reflecting both endogenous synthesis and dietary sources. The IOM recommends a serum 25(OH)D concentration of at least 50 nmol/L to achieve bone health [17]. Historically, the healthy target for vitamin D status was based on a non-linear inverse relationship between parathyroid hormone (PTH) and serum 25(OH)D concentrations [56]. Higher PTH stimulates bone resorption and increases risk of fractures [57]. Overall, a plateau in PTH (36 pg/mL) is observed at 78 nmol/L of 25(OH)D [56] giving rise to the suggested target of > 75 nmol/L of 25(OH)D by the US Endocrine Society [58]. Although, this relationship has been confirmed in an elderly cohort [59], higher values of 25(OH)D may be needed for the plateau of PTH to be reached in advanced aging [60]. However, institutionalized older Canadians [61, 62] often have low serum concentrations of 25(OH)D (means of 40 to 45 nmol/L) and insufficient vitamin D intake [61, 63-68] despite health care supervision. More RCT, especially dose-response designs, are required to provide evidence to support the RDA of 800 IU (or otherwise) and its target serum 25(OH)D values of 50 nmol/L and to establish the benefits of vitamin D on bone health in this important life stage group [21, 69].

Vitamin D available from food sources is limited. Foods naturally providing vitamin D are fatty fish such as salmon, mackerel, tuna, plus smaller amounts in beef, eggs and irradiated mushrooms. Vitamin D content varies according to species, provenance and cooking method [70-73], season of consumption [74] or industry fortification methods [75-78]. Recently, the content of 25(OH)D has also been investigated and suggested as a possible factor influencing the

discrepancy vitamin D intake and serum concentration of 25(OH)D [79]. Canadian legislation mandates fortification of milk and margarine with vitamin D. Other foods with vitamin D such as meal supplements, apple or orange juice and yogurts are available and provide small amounts of vitamin D per serving [80, 81]. In the United States, vitamin D fortification of margarine is mandatory and for milk and standardized cereal flours and other standardized bakery products, the vitamin D fortification is optional [82]. In the Canadian Community Health Survey (Cycle 2.2, Nutrition, 2004) dietary intake was on average 252±183 IU/d in older men (>70 y). The main sources of vitamin D were milk products (49% of dietary vitamin D), followed by meat and meat-alternatives (31.1%) [83].

Vitamin D status in older men is underrepresented in meta-analyses (published to date) looking at vitamin D and bone health [1-3, 5, 6, 19, 84]. In recent decades, large prospective cohort studies have been designed to document bone health of men that live in the community. The MrOs study [85], the InChianti study [86] and the CHAMP [87] study are good examples of these efforts. However, very old men living in LTC facilities remain an under studied population and sound research is needed. Based on LTC settings in Canada, residents have low vitamin D intake ranging from 100 to 295 IU/d [61, 63, 64, 88]. Meta-analyses demonstrate vitamin D fortified foods and supplements improve vitamin D status [18, 19, 21, 89]. High-dose bolus and daily dosing regimens of vitamin D supplementation can successfully increase 25(OH)D [90-93], with vitamin D_3 being more efficient than vitamin D_2 [94, 95]. Recent work shows that bioavailability of vitamin D_2 and D_3 can be affected by the supplemental forms [96, 97]. High-dose bolus may increase 24-hydroxylase, causing increased catabolism of 25(OH)D and $1,25(OH)_2D$ [98]. Dose-response studies, using daily regimens, are needed to establish the total

daily vitamin D intake required to maintain healthy vitamin D status and musculoskeletal health which is the premise of this thesis research.

1.2 Statement of Purpose and Objectives

Evidence-based recommendations for vitamin D intake and status in association with bone health in older men are fundamental to clinical practice of physicians, nutritionists, and other allied health professionals. To date, no dose-response study has looked at the impact of optimizing vitamin D status on bone metabolism using fortified foods in very old men living in LTC facilities. At the time of designing this research, a needs assessment was required to guide the intervention. Thus, the objectives of this thesis research in men > 70 y living in LTC were conducted in two studies as outlined below.

1) Prospective cohort study (manuscript 1):

   Primary objectives: to evaluate dietary consumption of vitamin D and its impact on vitamin D status, PTH, OC and (CTX) in older men living in a LTC facility across all seasons.

   Secondary objectives, to evaluate functional and cognitive state with the Frail Elderly Functional Assessment (FEFA), handgrip strength, and the Mini-mental state examination (MMSE) tools in relation to 25(OH)D in older men living in a LTC facility.

   Hypotheses:

   It was hypothesized that during the prospective cohort study residents would not meet the RDA requirements for vitamin D despite clinical supervision of intakes by dietitians;

seasonal variation would be apparent; biomarkers of bone metabolism would be influenced by serum 25(OH)D concentration.

2) Randomized control trial (manuscripts 2 and 3):

RCT - Phase I (Before After Study – Run In of 8 weeks): to evaluate the impact of 2000 IU/day of vitamin D_3 supplementation on serum concentrations of 25(OH)D, PTH, calcium and phosphate.

RCT - Phase II (RCT of 24 wk; winter to spring): after Phase I, to test the amount of vitamin D needed to maintain 25(OH)D status with a RCT of vitamin D fortified foods (0, 500 or 1000 IU/d for 24 wk) on the basis of change over 24 wk in:

a) vitamin D status using multiple metabolites including serum 25(OH)D concentration and biomarkers of bone and mineral metabolism (OC, CTX, RANKL and OPG); (primary outcome);

b) areal bone mineral density (aBMD) of the forearm using peripheral dual-energy X-ray absorptiometry (pDXA);

c) volumetric bone mineral density (vBMD) of radius plus forearm muscle cross-sectional area using peripheral quantitative computed tomography (pQCT);

d) handgrip strength.

Hypotheses:

It was hypothesized that, prior to RCT study entrance, all participants screened for study entry would have a serum 25(OH)D < 75 nmol/L; a supplement of 2000 IU/day for 8 weeks would increase 25(OH)D concentration to 75 nmol/L or more in 100% of participants, but not exceed 125 nmol/L; only the food-based delivery systems (fortified foods) containing 1000 IU/d of vitamin D would maintain serum 25(OH)D ≥ 75 nmol/L and thereby prevent values from reverting to baseline values or lower; aBMD and vBMD would be maintained and biomarkers of bone metabolism would be influenced by vitamin D intake; handgrip strength would increase with increased vitamin D status.

CHAPTER 2 – REVIEW OF THE LITERATURE

2.1 Present State of Knowledge

2.1.1 History of Vitamin D

In 1912, the biochemist Casimir Funk was the first to express the concept of 'vital amines' which would encompass essential micronutrients with specific actions present in foods and required for health and survival. He conceptualized that simple amines could cure specific diseases. Although thiamine was an amine that cured beriberi, other vitamins were not amines *per se*. Therefore, the word vitamin is now spelt without the 'e' to acknowledge the molecular diversity. Professor Funk developed *Oscodal* lozenges which were the first vitamin preparation accepted by the American Medical Association. Funk's theory led to the discovery of a several components complementary to the 3 main macronutrients, protein, carbohydrate and fat, which were then believed to be the basis of an adequate diet [99, 100].

Inspired by the work done by McCollum in xerophtalmia [101] using vitamin A containing foods such as butterfat and cod liver oil, Sir Edward Mellanby thought a dietary compound could be responsible for the onset of rickets in children [102]. After providing dogs with the typical diet of Scottish people, he noticed that they developed rickets which could then be cured by giving cod liver oil. He concluded that the lack of vitamin A was responsible for rickets. Professor McCollum, further testing the hypothesis put forward by Sir Mellanby, decided to destroy vitamin A in cod liver oil by oxidation and proved that cod liver oil could still cure rickets, but not xerophthalmia. Not long afterwards, Professor Elmer McCollum and his team correctly identified the presence of a fourth vitamin compound, alphabetically labelled vitamin D, in 1922 [103]. This vitamin is currently known more specifically as vitamin D_2 and D_3.

Health Canada has recommended cod liver oil in order to prevent rickets in children since 1928 [104].

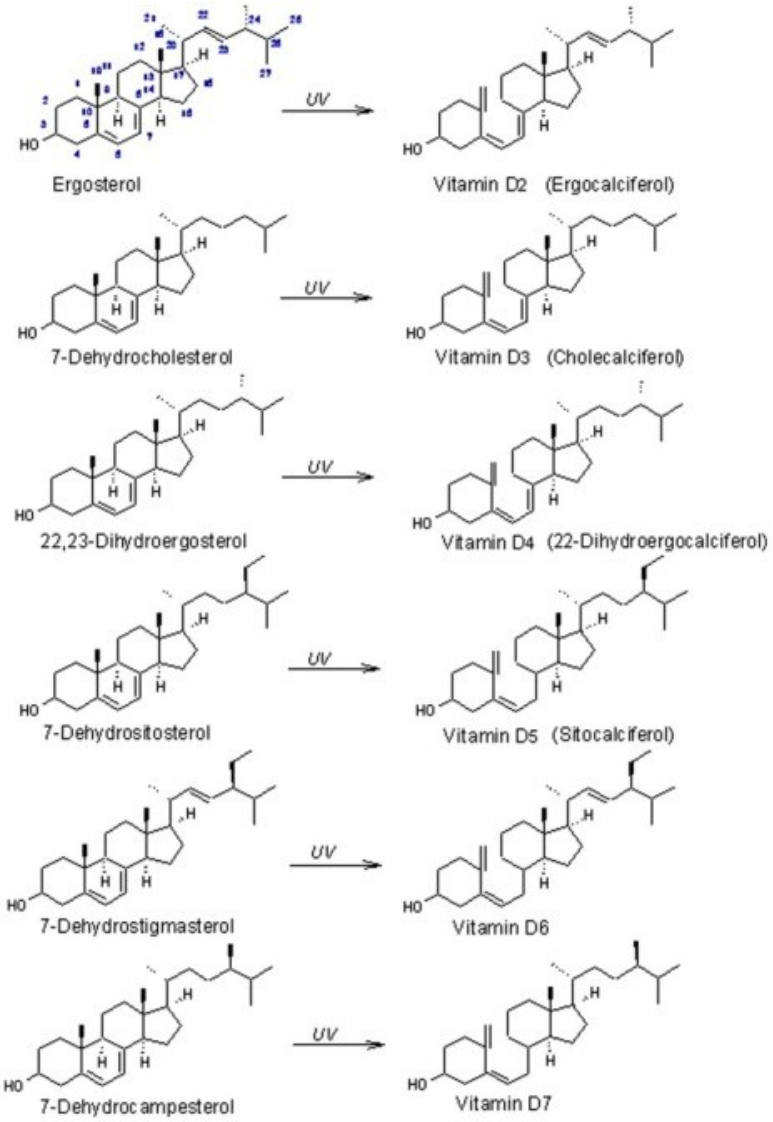
International research teams in Austria [105] and the United States [106] worked on finding more vitamins during that period. However, it was an English team led by Harry Steenbock in 1925 that found that the absence of sunlight was connected to negative calcium balance [107]. They discovered that irradiation of food and animals with ultraviolet (UV) light could cure rickets. They discovered that a lipid present in food and in the skin could be converted by UV light into an active molecule to prevent and cure rickets [108-110]. Just recently, with the advances in chromatography, research has led to the identification of a new vitamin D compound in mushrooms which is now labelled vitamin D_4 (22-dihydroergocalciferol) [111]. This finding continues to unveil the complexity of vitamin D and its isomers (Figure 2.1).

2.1.2 Sources of Vitamin D

2.1.2.1 Endogenous Production

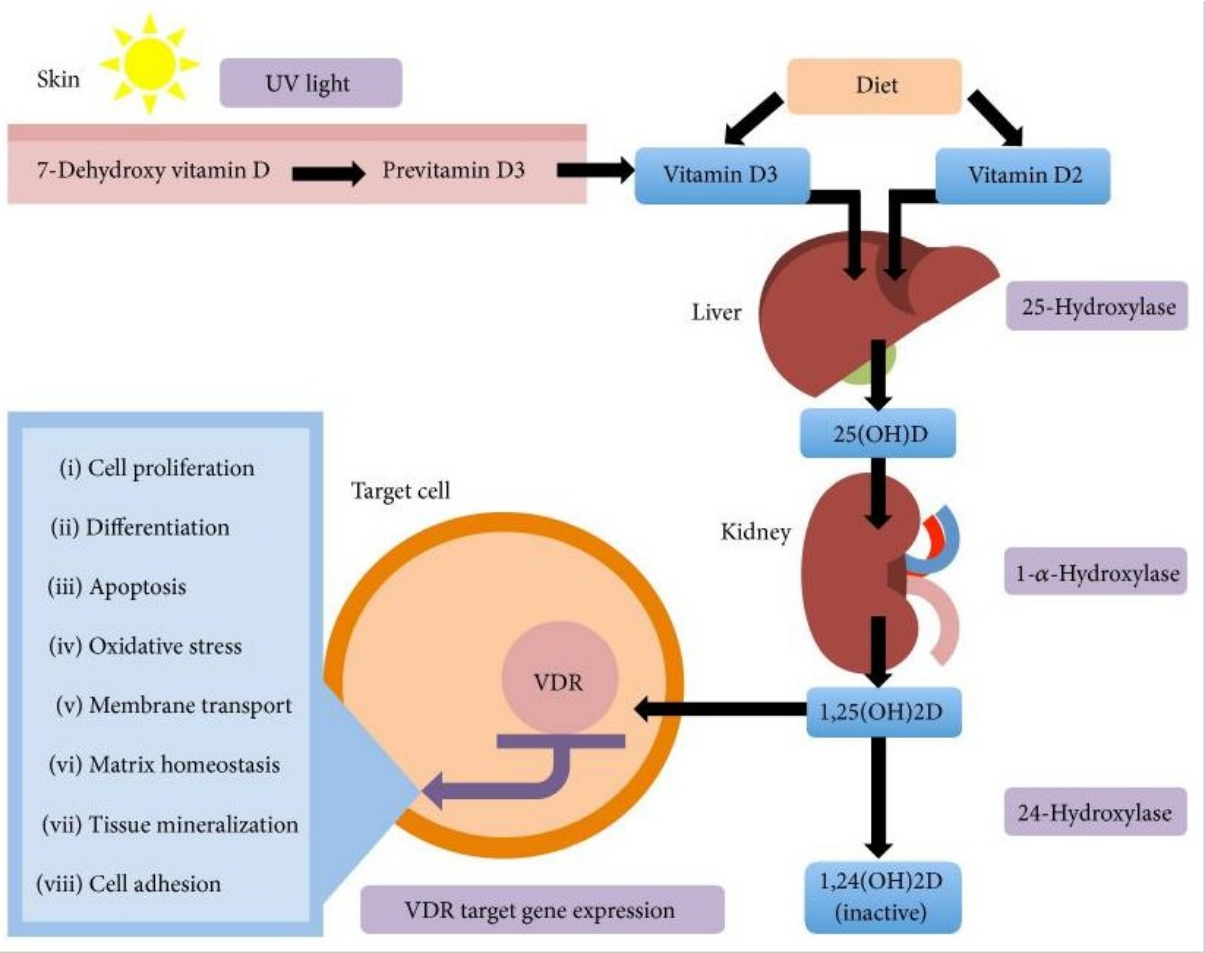
Although Steenbock concluded that skin exposure to UV light produced vitamin D in humans in 1925 [112], the photosynthesis process was only proven more than 50 years later. In 1977, Holick and his team demonstrated that previtamin D_3 was formed by exposure of 7-dehydrocholesterol of the skin to UV light levels of 282-310 nm (Figure 2.2). This precursor of previtamin D_3 is found in the epidermis and the dermis. In 1981, Holick also demonstrated that pigmentation of the skin affected the UV penetration. Populations with fair skin color pigmentation have a deeper penetration of the UV rays (epidermis and dermis) which can reach the stratum basale, the lowest segment of the dermis. Although the largest quantity of 7-dehydrocholesterol per unit of skin is in the stratum spinosum, it is in the stratum basale layer of

Figure 2.1: Molecular structures of vitamin D compounds



Source: Phillips KM, Horst RL, Koszewski NJ, Simon RR. Vitamin D_4 in Mushrooms. Lobaccaro J-MA, ed. *PLoS ONE*. 2012;7(8):e40702.

Figure 2.2: Summary diagram of vitamin D metabolism and function



Source: Lugg ST, Howells PA, Thickett DR. Optimal Vitamin D Supplementation Levels for Cardiovascular Disease Protection. *Disease Markers*. 2015;2015:864370.

the skin that the highest concentration of 7–dehydrocholesterol (per mg of lipid) is found [113]. In the plasma membrane, vitamin D_3 is then transformed via isomerization into vitamin D_3, reflecting a temperature-dependent balance [114]. Vitamin D_3 is translocated in the extracellular space and diffused into the capillaries of the dermis and bound to vitamin D binding protein (DBP). Intoxication by sunlight over exposure of the skin will not result in excessive production of vitamin D_3 as previtamin D_3 will be photoisomerized to lumisterol and tachysterol, which are inert compounds. These 2 molecules have no binding affinity to DBP and would be sloughed off by natural exfoliation of the skin. Furthermore, lumisterol and tachysterol could revert back to previtamin D_3 if exposed to UV radiation [113]. Therefore, endogenous vitamin D synthesis is affected by UV light exposure, photochemical regulation and skin pigmentation.

2.1.2.1.1 Seasonal Variation

The fluctuation of previtamin D_3 due to season and latitude was well demonstrated by the work of Webb and colleagues in 1988 [115]. This now classic work shows an important increase in previtamin D_3 formation for the populations of the northern hemisphere, between the months of May and October followed by a rapid decline in the winter months. Due to its association to UV light, specifically UV beta radiation (UVB; wavelengths of 290-315 nm), dermal synthesis of vitamin D_3 from sunlight is only possible beginning in the month of April through to the end of October in Canada. Northern locations above the 49^{th} parallel are affected by changes in wavelengths of the solar rays [55] and photometabolism is halted in the late fall and over the entire winter.

In fact, this periodicity has been documented in subsequent studies looking at vitamin D status in various populations and countries below the 49°N. In 2002, Looker and colleagues used

a logistic contingency of the NHANES data collection approach to compare the season-latitude impact on vitamin D status in adults and adolescents. The data collection for the northern U.S. population (Median latitude: 39°N – range: 35°N to 47°N) was done in the summer whereas it was done in the winter for the southern populations (Median latitude: 32°N – range: 25°N to 41°N) which provided maximized seasonal values for the northern group. They found little evidence of vitamin D deficiency in both groups. Interestingly however, insufficiency, defined as less than <37.5 nmol/L at the time, was common in adults and adolescents of the winter-southern population.

A decade later, a 4-year, population-based, randomized, placebo-controlled double-blinded vitamin D clinical trial including 1063 non-Hispanic white postmenopausal women (n=1063; 67.6±7.3 y) from rural Nebraska (at latitude of 41.4°N) looked at factors affecting the response of vitamin D_3 supplementation of 1100 IU/d [116]. After observing an important variance in the response to vitamin D supplementation, the authors identified the independent contribution of the season of blood collection as a factor predicting the variability in the response to vitamin D. The baseline serum 25(OH)D concentrations were significantly higher in subjects enrolled in the summer with an average of 75.0±19.9 nmol/L (±SD) when compared to the winter recruitment group at 66.6±21.0 nmol/L ($p< 0.001$). After 12 months of supplementation, the group selected in the summer had an increase of 5.1±19.3 nmol/L and the winter group had an increase of 13.4±20.3 nmol/L ($p< 0.001$). The authors suggest that a protective endogenous mechanism could be at play to protect against vitamin D intoxication during the summer months. This concurs with the thermal sensitivity of photoisomerization published by Holick [113] in 1981.

In a secondary analysis of a large, multicenter, randomized, placebo-controlled colorectal adenoma chemoprevention trial, a recent publication by Rees and al. [117] presented results looking at factors associated with serum 25(OH)D concentrations in adult participants, living in the United States and Puerto Rico, involving vitamin D_3 supplementation (n=699; age: 58.2±6.9 y; 64% male). All of the participants resided in study centers at latitude below the 45°S parallel. After the vitamin D supplementation, half of the variability in the change in serum 25(OH)D was associated with being female, lower baseline 25(OH)D values, adherence to supplementation intake, wearing long pants and sleeves while being exposed to sunlight, moderate activity level, additional vitamin D supplement over the trial supplementation treatment, and season of blood draw. Internationally, researchers have observed similar effects. In Mongolia [118] (46°N), summer mean serum 25(OH)D concentration was 56.3 nmol/L (95% CI: 36.3 nmol/L, 81.3 nmol/L) and winter mean value was 19.3 nmol/L (95% CI: 11.5 nmol/L, 27.0 nmol/L). In a very large sample of the Korean population analyzed by Yu and al. [119] (n=95;137 participants; 47.8% female), concentrations of 25(OH)D were more than 12 nmol/L higher in the Fall period when compared to the Spring season for the men ≥ 70 y and, 15 nmol/L higher for the women ≥ 70 y when looking at the same seasons.

These studies collectively demonstrate that, even in populations living in latitudes below the 49°N parallel, two important factors affect endogenous transformation of 7-dehydrocholesterol of the skin by UV light: clothing and season. Seasonal variability is observed in healthy men and women as well as in all age groups. However, the impact of advanced aging (80+) and residing in long-term care (LTC) remains clearly understudied.

In recent years, strong recommendations coming from the dermatology associations [120] and Canadian and America Cancer Societies as well as the European Code against Skin Cancer

[121-123] encourage wearing of hats and long sleeve clothing as well as use of sunscreen in prevention of skin cancer all year long to protect the skin of UV light exposure. Therefore, these approaches could result in inhibiting the natural transformation of 7-dehydrocholesterol from the skin to previtamin D_3 even during the summer months and further support that foods containing vitamin D and supplements are essential all year long to meet vitamin D requirements. For seniors, and especially if living in LTC facilities, long sleeves clothing, hats and long pants, are often the attire of choice. The UV light exposure recommendations, coupled with the evidence of season variation to date leads to the conclusion that, in winter, the primary sources of vitamin D for populations become tissue vitamin D stores or exogenous sources in foods or supplements. Furthermore, for those in LTC the loss of physical mobility results in extended time spent indoors, even during the summer season. This adds to their dependency on vitamin D supplementation to achieve adequate vitamin D status.

2.1.2.2 Exogenous Sources

2.1.2.2.1 Natural Vitamin D Food Source in Canada

Natural vitamin D available from food sources is limited to a few foods (Table 2.1). Foods providing vitamin D_3 include animal-based foods such as 1) organs (kidney and liver) of fish, beef, pork or chicken, 2) flesh of fish such as salmon, mackerel, tuna, and 3) meat from beef, pork, lamb as well as chicken and turkey. Egg yolk also provides vitamin D [124]. The highest vitamin D_3 values in foods are found in fish liver with content ranging from 4440 IU per 100 g in anglerfish liver to more than 130,000 IU per 100 g in tuna liver. These exceptionally high values would help explain the success of cod liver oil (9280 IU per 100 g [125]) at curing rickets in the early 1800s [126]. Fish fillet also provides vitamin D_3 and reported values range

from 11 IU per 100 g in perch [127] to more than 5440 IU per 100 g in Japanese pilchard [128]. Tilapia, a common fish eaten in Canada, provides 1800 IU per 100 g [129]. Organs from meat would provide vitamin D_3 in lesser amounts with the highest values reported at 5600 IU per 100 g for beef liver [130]. Regular egg yolks (approximately 5 eggs) would provide up to 232 IU per 100 g [128]. Vitamin D_2 and vitamin D_4 are present in wild or irradiated mushrooms [73, 78, 111, 131-138].

It is important to note that seasonal and local variations of vitamin D content as well as 25(OH)D concentrations are also present in various foods leading to the understanding that a fluctuation of natural vitamin D available from the food supply throughout the year is also a possibility [124]. Recently, questions regarding the quantification of 25(OH)D content in foods have been raised [74, 79, 139, 140] as these metabolites are present in the foods we eat and could increase the true vitamin D intake of populations. Recent work in pigs [141] and in humans [142] has demonstrated that supplementing with 25(OH)D_3 would be more efficient in raising 25(OH)D concentrations, therefore further explaining the variable effect of foods on vitamin D status. However, challenges remain in accurate and reproducible measurement of 25(OH)D in food [143]. The Canadian Nutrient File does not include values of 25(OH)D [144], nevertheless it provides data on 5690 foods and 152 food components, including vitamin D (D_2+D_3). This database also contains nutrient values derived from the United States Department of Agriculture whenever the foods are eligible for the Canadian market [144]. Table 2.1 presents vitamin D values of staple foods.

Table 2.1: Vitamin D content of selected foods from the Canadian Nutrient File[1]

Vitamin D Content of Selected Foods	Portion size	Vitamin D (IU)	Vitamin D (IU/100g)
Dairy Products			
Milk (skim, 1%, 2% or 3.25% M.G.)	250 ml	103	40
Yogourt, plain, Mediterranean (6-9% M.G.)	175 g	4	2
Yogourt with added vitamin D	175 g	42 - 80	24 - 46
Dessert, frozen, ice cream, vanilla, 11% M.F.	125 ml	6	9
Cream, table (coffee), 15% M.F.	30 ml	2	13
Brie	50 g	10	20
Camembert	50 g	8	16
Cheddar (18% M.G.)	50 g	6	12
Processed cheese, cheddar, slices	30 g	1	3
Butter, regular	10 g	2	20
Breads and Breakfast Cereals			
* Bread, multigrain, commercial	1 slice	5	14
* Bread, white, commercial	1 slice	5	14
* Bread, sprouted wheat with added calcium and vitamin D, commercial	1 slice	25	81
Kellogg's, Cereal, ready to eat (Corn Pops, Froot Loops, Rice Krispies, Special K, Frosted Flakes)	30 g	56 - 65	187 - 217
Eggs			
Egg, chicken, whole, cooked, poached	2 large	58	58
Egg, chicken, whole, cooked, fried	2 large	89	89
Pancake, plain, homemade	1	10	27
Margarine			
Margarine, tub, hydrogenated, canola oil	10 g	66	660
Margarine, tub, non-hydrogenated, canola oil (includes some sunflower oil)	10 g	66	660
Margarine, non-hydrogenated, canola+fish oil	10 g	72	720
Margarine, non-hydrogenated, canola+olive oil	10 g	72	720
Animal Fats			
Animal fat, beef tallow	10 g	3	30
Animal fat, lard (pork)	10 g	10	100
Animal fat, chicken	10 g	17	170
Animal fat, turkey	10 g	19	190
Mushrooms			
* Mushroom, white, raw	125 ml	4	8
* Mushroom, shiitake, raw	4 pieces	14	18
* Mushroom, Chanterelle, raw	125 ml	60	210
* Mushroom, morel, raw	125 ml	72	206
* Mushroom, Maitake, raw	125 ml	409	1122
Fortified Foods			
* Plant-based beverage, soy, enriched, all flavours, reduced fat	250 ml	54 - 87	21 - 34
Juice, frozen concentrate, diluted, OR canned with added calcium and Vitamin D (Orange or Apple)	125 ml	9 - 25	7 - 19

Table 2.1: Vitamin D content of selected foods from the Canadian Nutrient File[1]-(cont'd)

Vitamin D Content of Selected Foods	Portion size	Vitamin D (IU)	Vitamin D (IU/100g)
Beef			
Beef, loin, strip loin (New York) steak, boneless, lean and fat, 3mm (1/8") trim, cooked, broiled	75 g	4	5
Beef, chuck, blade roast, boneless, lean, cooked, braised	75 g	7	9
Beef, chuck, blade roast, boneless, lean and fat, 3mm (1/8") trim, cooked, braised	75 g	12	16
Chicken			
Chicken, stewing, dark meat only, stewed	75 g	8	11
Chicken, stewing, light meat only, stewed	75 g	8	11
Game			
Game meat, elk, loin, separable lean only, cooked, broiled	75 g	6	8
Lamb			
Lamb, New Zealand, shoulder, whole, lean braised	75 g	2	3
Fish			
Fish, haddock, baked or broiled	75 g	9	13
Fish, cod (gray cod), pacific, baked or broiled	75 g	18	24
Fish, smelt, rainbow (American, capelin), raw	75 g	28	37
Fish, tuna, light, canned in oil, drained, unsalted	75 g	36	48
Fish, catfish (wolfish), Atlantic, baked or broiled	75 g	50	67
Fish, tuna, white, canned with oil, drained, unsalted	75 g	60	80
Fish, mackerel, Spanish (Atlantic), baked or broiled	75 g	81	108
Fish, halibut, Atlantic or Pacific, baked or broiled	75 g	144	192
Fish, trout, rainbow, farmed, baked or broiled	75 g	192	256
Fish, salmon, Atlantic, wild, baked or broiled	75 g	245	327
Fish, salmon, chinook (spring), smoked, lox	75 g	315	420
Fish, salmon, chinook (spring), baked or broiled	75 g	387	516
Fish, salmon, pink (humpback), canned, solids with bone and liquid, salted	75 g	411	548
Fish, eel, mixed species, baked or broiled	75 g	699	932
Fish, mackerel, salted	75 g	754	1005
Fish, carp, baked or broiled	75 g	950	1319
Pork			
Pork, loin, centre cut (centre steak), boneless, lean and fat, broiled	75 g	8	11
Pork, loin, centre cut (centre chop), bone-in, lean and fat, roasted	75 g	19	25
Pork, shoulder, whole, lean and fat, roasted	75 g	46	61

Table 2.1: Vitamin D content of selected foods from the Canadian Nutrient File[1]-(cont'd)

Vitamin D Content of Selected Foods	Portion size	Vitamin D (IU)	Vitamin D (IU/100g)
Turkey			
Turkey, all classes, drumstick, meat and skin, roasted	75 g	10	13
Turkey, all classes, back, meat and skin, roasted	75 g	10	13
Veal			
Veal, ground, pan-fried	75 g	41	55
Veal, foreshank, osso buco, lean and fat, braised	75 g	32	42
Organs			
Beef, liver, raw	75 g	37	49
Beef, liver, braised	75 g	37	49
Turkey, all classes, liver, raw	90 g	45	50
Pâté, liver, unspecified meat, canned	55 g	17	31
Pâté de foie gras, (goose liver pate), smoked, canned	55 g	26	47
Beef, kidney, simmered	55 g	34	62
Fish oil, cod liver	10 ml	855	9283
Fish, burbot (loche), native, liver, raw	90 g	11 444	12715
Native Canadian Foods			
Game meat, native, sea lion, stellar, meat, raw	90 g	1	1
Animal fat, native, beluga oil	10 g	23	230
Game meat, native, caribou (reindeer), liver, raw	90 g	50	56
Game meat, native, bearded seal meat, boiled	75 g	54	72
Game meat, native, ringed seal, brain, raw	90 g	54	60
Game meat, native, ringed seal, eyes, raw	90 g	94	104
Game meat, native, ringed seal, blubber, raw	125 g	80	64
Game meat, native, muskox, fat, raw	125 g	160	128
Game meat, native, ringed seal, liver, raw	90 g	385	428

[1]Data source: *Canadian Nutrient File* [125]. **Ergocalciferol (vitamin D_2)*

2.1.3 Absorption, Metabolism and Excretion

Vitamin D can also be obtained by consuming foods that provide either ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3). These are both referred to as vitamin D to ease the description and both of these liposoluble seco-steroid vitamins are absorbed from the intestinal micelles carrying fat. Bile salts facilitate the passive diffusion of vitamin D into the intestinal cell. The absorption occurs rapidly in the duodenum; however vitamin D can be absorbed throughout the small intestine with the largest amount being absorbed in the ileum. In the intestinal cell, vitamin D is incorporated into chylomicrons which then enter the lymphatic system. Some vitamin D from the chylomicron can be transferred to the DBP for delivery to extrahepatic tissues.

Circulation of vitamin D in the blood stream is mainly complexed to DBP [145, 146]. DBP is also referred to as group specific component (Gc). It is a multi-functional plasma glycol-protein synthesized in the liver [147]. More specifically, 88% of 25(OH)D is bound to DBP, 10% is bound to albumin and 0.03% travels in a free form [146]. It is also DBP that transports 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) [148]. It is highly polymorphic serum protein and several variants have been identified and influenced by ancestry (D432E (rs7041), T436K (rs4588, A246del (rs.529043158), P253S and C311F (rs.780473721)). These variants may affect vitamin D metabolism as they have been correlated with total circulating 25(OH)D and/or $1,25(OH)_2D$ concentrations in European, East Asian, Hispanic and African American populations [149-153]. This polymorphism may affect the protein's function and its ability to bind to vitamin D ligands, its bioavailability and could differ throughout racial groups [154-156]. It is also believed that DBP supports the preservation of stable stores of vitamin D [154, 157].

Although inconsistent in the literature, comparisons of certain DBP phenotypes have been associated with increased rick of bone fracture in perimenopausal white women and low radial bone mineral density (BMD) in Japanese women [158, 159]. In men, a positive correlation was observed between DBP and lumbar and femoral neck BMD [160] and in BMD and vertebral fractures in men (Controls: n= 21; Median age: 65 y; Range: 40-77 y versus Experimental group: n= 26; Median age: 64 y; Range: 27-72 y) [161]. However, no impact on BMD was seen in non-black older women (age: 65-90 y) [162]. Similarly to vitamin D status, Larcombe et al. [163] demonstrated a seasonal variation for DBP with lower concentrations in summer 204.5±78.8 μg/mL when compared to winter 364.3±124.6 μg/mL ($p \leq 0.005$), which was seen as beneficial to overcome the lack of UV exposure during the winter months in the Dené population (northern Canada). Since its discovery, DBP has been linked to several more important functions such as actin scavenging, fatty acid transport, macrophage activation and chemotaxis [147].

The vitamin D present within the chylomicron remnants continues its course to the liver, arriving by the portal vein. The apolipoprotein E on the surface of the chylomicron remnants binds with receptors on the hepatocyte plasma membrane [164] to activate internalization. The lipid portion is hydrolyzed and released into the cell. Resynthesis of the lipid compounds occurs in the hepatocyte [165]. Vitamin D, in itself is not biologically active. In the microsomes of the liver cells, vitamin D is hydroxylated at C-25 and converted to 25(OH)D. This later form of vitamin D is the most prevalent and stable circulating form of vitamin D in the body and is thus used as a biomarker of vitamin D status [166, 167]. Several cytochromes of the P450 family of enzymes, including CYP2R1, CYP27A1 and CYP2D25 are possible enzymes responsible for the conversion of vitamin D to 25(OH)D [168]. However, CYP2R1 appears to be a key hydroxylase for the transformation of vitamin D as it has been linked with rickets in patients presenting a

mutation preventing them to produce it [169-171]. Studies in CYP2R1 null mutant mice provided evidence that this enzyme is a significant compound for the 25-hydroxylation of vitamin D as concentrations of $25(OH)D_3$ are drastically reduced. However, some synthesis remains and leads scientists to believe that other vitamin D 25-hydroxylases could be present contributing, with a lower capacity, to the production of $25(OH)D_3$ [172]. In 2015, Thacher and colleagues demonstrated that CYP2R1 was the principal 25-hydroxylase in Nigerian children with familial rickets. Vitamin D supplementation was ineffective to increase 25(OH)D levels in individuals with homozygous mutation whereas, individuals presenting with an heterozygous mutation presented a rise in serum 25(OH)D [173].

From the liver, 25(OH)D is carried in circulation by DBP to other tissues where it may be further metabolized. It is in the kidney that 25(OH)D is fully activated to $1,25(OH)_2D$. The uptake of 25(OH)D is facilitated by megalin and cubilin which are 2 transmembrane lipoprotein receptors present on the proximal tubular epithelial cells of the kidney. Their binding with DBP results in endocytic internalization of 25(OH)D [174-176]. A second hydroxylation at C-1 then occurs in the proximal tubules of the kidney, via the CYP27B1 enzyme, generating the active hormonal form $1,25(OH)_2D$. Binding to DBP, it is released in the blood stream where it begins its endocrine activity. In the intestine, $1,25(OH)_2D$ interacts with vitamin D receptor (VDR) and increases the expression of calcium channel producing an increased transport of calcium.

Initially believed to be only expressed by the kidney and the placenta, CYP27B1 is now known to be present in several extraskeletal tissues. Both VDR and CYP27B1 enzyme have been detected as active in many tissues such as macrophages, epithelial of epidermis, prostate and colon, liver, pancreatic islets and osteoblasts [177-179]. The purpose of the active form $1,25(OH)_2D$ in such numerous cell types remains to be clarified, but it is theorized that calcium

homeostasis, at the cellular level, might be a key objective [180] along with gene transcription for over 35 proteins. To either promote or inhibit gene transcription, $1,25(OH)_2D$ binds to the VDR that, when heterodimerized with RXR, in return will then bind to the vitamin D response element (VDRE) [22]. The DBP can bind all metabolites in the vitamin D family, but has much less affinity for $1,25(OH)_2D_3$ than 25(OH)D.

In the kidney, hydroxylation at C-24 of $25(OH)D_3$ and $1,25(OH)_2D_3$ by CYP24A1 - another cytochrome P-450 enzyme – will result in auto regulation of the circulating concentration of the metabolites. The resulting compounds, $24,25(OH)_2D_3$ and $1,24,25(OH)_3D_3$ are destined for degradation pathways and modulates the cellular responses [181]. Studies of mutation of CYP24A1 in children and adults have demonstrated that they present with hypercalcemia, hypercalciuria and recurrent nephrolithiasis [182, 183]. Both CYP27B1 and CYP24A1 are tightly controlled. Hypocalcemia will induce the production of PTH. This will result in an increase of $1,25(OH)_2D_3$ synthesis. The elevation of $1,25(OH)_2D_3$ concentration will then suppress the PTH production. $1,25(OH)_2D_3$ auto-regulates its own synthesis by inhibiting CYP27B1 [184]. The phosphaturic factor fibroblast growth factor 23 (FGF23) and αklotho transmembrane protein suppress CYP27B1 and subsequent conversion of 25(OH)D to $1,25(OH)_2D$ and activates CYP24A1 which will increase catabolism of $1,25(OH)_2D_3$ [185]; both leading to reduced circulating $1,25(OH)_2D$.

2.1.4 Bone Cells and Remodeling

Bone is a complex tissue. Unlike any other tissue human tissue, bone is a solid mineralized connective tissue which provides support for the body. It also serves several other purposes such as mobility, protection of organs, storage for minerals and synthesis of growth

factors [186, 187]. After skeletal maturity is reached in the second and third decade of life, the maintenance of bone will be achieved by a well-balanced replacement of damaged or old bone by newly formed bone. Bone can remodel throughout the life span [186, 188-192] and is highly regulated. It occurs within bone cavities named basic multicellular units in distinct phases: quiescence, activation, resorption, reversal, formation, mineralization and termination [188, 190, 193]. Bone cells are of variable types including osteoblasts, osteoclasts, osteocytes and bone-lining cells. They are responsible for the balance between formation and resorption of bone through complex interactions of hormones, growth factors and their receptors. Autocrine, paracrine and endocrine signaling systems regulate bone remodeling.

Osteoblasts are cuboidal shaped cells derived from mesenchymal stem cells (MSC) [191, 194]. They are involved in the synthesis of the bone matrix by setting up the organic matrix and its mineralization. The first step of bone matrix formation involves the secretion of collagen proteins and proteoglycans. Subsequently, mineralization occurs with the release of matrix vesicles carrying calcium. Due to their negative charge, the proteoglycans immobilize the calcium ions from the vesicles. Via calcium channels, called annexins, the calcium ions are released from the vesicles to the matrix membrane. Phosphate-containing compounds are degraded by the alkaline phosphatase (ALP) secreted by osteoblasts, releasing phosphate ions inside the vesicles. Calcium and phosphate form hydroxyapatite crystals. When supersaturation of calcium and phosphate ions occurs inside the matrix, the fibrillar phase takes place and mineralization is completed. Osteoblasts also release receptor activator of nuclear factor kappa-B ligand (RANKL) which will activate osteoclasts in binding to RANK receptor on the surface of their precursor [195]. The mature osteoblasts will morph into osteocytes, bone lining cells or undergo apoptosis [196, 197]. In senescence, MSC are believed to lose their efficiency to

differentiate into osteoblast. The evidence is controversial. However, it could help explain age-related bone loss [50].

Osteocytes are a sub-group of osteoblasts that become embedded in the bone matrix at the end of the formation process. Long believed to be passive structural cells, they are now known to act as mechanosensors. Osteocytes present cytoplasmic processes that connect to other osteocytes, osteoblasts and bone lining cells. Messengers such as ATP, nitric oxide, Ca^{2+} and prostaglandins are released upon mechanical stimulation and modify bone physiology [198]. A third cell derived from the MSC includes the bone lining cells; these are flat shaped osteoblasts that lye on the bone surface. They prevent direct contact between osteoclast and bone matrix. They produce osteoprotegerin (OPG) which can impede the activation of osteoclast by binding to RANKL [199] and affect osteoclast differentiation. In response to RANKL, osteoclasts originate from the hematopoietic stem cell lineage and are responsible for resorbing mineralized matrix. To do so, osteoclasts express an H^+-ATPase active on the ruffled border which acidifies the remodeled region and enables the degradation of the matrix.

2.1.5 Bone Turnover Markers

As remodeling of bone is a dynamic process, the bone turnover markers (BTMs) are generated during bone formation and bone resorption. Markers of bone formation are products of osteoblasts. They can be by-products of collagen synthesis such as propeptides of type 1 collagen (C-terminal-P1CP or N-terminal:P1NP), osteoblast enzymes (alkaline phosphatase ALP) or matrix proteins (osteocalcin) [200]. Markers of bone resorption are the result of osteoclasts activity. They can be products of collagen degradation such as telopeptides of type 1 collagen (C-terminal: CTX-1, CTX-matrix metalloproteinases: MPP or N-terminal: NTX-1),

hydroxyproline or pyridnium crosslinks (pyridinoline: PYD or deoxypyridinoline: DPD). They also generated by the degradation of noncollagenous proteins (bone sialprotein) or osteoclastic enzymes (cathepsin K or tartrate-resistant acid phosphatase). Lastly, they can be the result of osteocyte activity markers such as RANKL, OPG and sclerostin [200]. Genetic, epigenetic, and environmental factors may play a role in the activation of these various BTMs, affecting peak bone mass and rate of bone loss. Studies have shown ethnicity based variations [201, 202]. The BTMs have been associated with the risk of fractures in postmenopausal cohorts [203, 204]. Complementary to bone mineral density assessments, BTMs could indicate deterioration of the microarchitecture of bones. Serum or plasma analyses of BMTs currently present limitations such as analytical variability and ethnic variations. In 2011, the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) evaluated the potential of BTMs for prediction of fracture risk and for monitoring treatment [205]. They recommended serum P1NP as bone formation marker and serum CTX as bone resorption marker for future large observational and intervention studies to improve knowledge in regard to BTMs [205]. However, recent research trials have not been able to demonstrate that BTMs influence bone structural integrity in older adults and further investigation is required to understand their impact on bone health [206-208].

2.1.6 Vitamin D Recommendations

Vitamin D is essential to bone health and calcium homeostasis. It can be synthesized by the skin and can be obtained naturally in a few foods. However, food sources are limited and sunlight exposure can be affected by skin color, latitude and season and limited in general for those in LTC. Traditionally, vitamin D and calcium have been recognized for their impact on skeletal health. From March 2009 to November 2010, the Institute of Medicine (IOM) conducted

a comprehensive review of the literature for the skeletal and extraskeletal impacts of vitamin D and calcium intakes. The IOM then published the Dietary Reference Intakes (DRI) for Calcium and Vitamin D [209]. The IOM replaced the Adequate Intakes (AI: 600 IU/d) which had been proposed in 1997 [210] with Estimated Average Requirements (EAR; 400 IU/d) and Recommended Dietary Allowance (RDA; 800 IU/d) values. It is important to remember that when providing recommendations to individuals, the RDA is used. However, when assessing and planning for general population recommendations, the EAR is emphasized. These recommendations are based on evidence of causal role of calcium and vitamin D on bone health in healthy general population of North America. They are gender and age specific and consider limited or no sunlight exposure. The recommendations apply for vitamin D_2 and D_3. The IOM reinforced that 25(OH)D continues to be the most useful marker of vitamin D status incorporating endogenous, dietary and supplement contributions to 25(OH)D concentration. They considered that a 40 nmol/L of 25(OH)D met the needs of 50% of the population (i.e., the EAR) whereas 50 nmol/L of 25(OH)D would meet the needs of 97.5% of the population (i.e. the RDA). A vitamin D concentration of 30 nmol/L or less is where the deficiency status has been set. However, similar to the Agency for Healthcare Research and Quality (AHRQ) concluded in 2007 [19], the reviewed evidence could not establish causality for the extraskeletal outcomes of vitamin D and hence the DRI values remain associated with skeletal health at this time.

In the context of osteoporosis prevention and treatment, several expert groups have put forward vitamin D recommendations taking into account the vitamin D status of the individual as well as their age and risk of deficiency. Risk is associated with prior fractures, bone loss or comorbid conditions that could lead to inadequate vitamin D absorption [49, 58, 211-216]. National and international recommendations are presented in Table 2.2a. For prevention, the

suggested daily vitamin D_2 or D_3 intakes range from 400 to 2000 IU. In the event of deficiency, which is defined differently by various expert groups (Table 2.2a), a loading dose of vitamin D of 7500 IU/d (300,000 IU for the total regimen that can last between 6 to 12 weeks) is proposed and should be followed by maintenance dose of 800 to 2000 IU/d. However, such high bolus dosages are now associated with increased risk of falls and need to be considered carefully [217]. For the Canadian National Osteoporosis Society in the United Kingdom [216], a sufficient vitamin D status is defined as 50 nmol/L of 25(OH)D whereas for the National Osteoporosis Foundation in the United States [211], the desirable status is > 75 nmol/L. Both groups suggest a loading corrective dose if deficiency is reported. For the US Endocrine Society [58] and Osteoporosis Canada [214], the suggested target 25(OH)D concentration is achieved when 25(OH)D is above 75 nmol/L.

Specific recommendations for LTC have recently been published for Canada and Australia [218-220] (Table 2.2b), whereas these are still awaited in the United States [221]. In general, LTC patients are older, frail and seldom exposed to sunlight. In Canada, a recommendation for year round daily vitamin D supplementation of 800 – 2000 IU for residents at high risk of fractures has been formulated and the expert group suggests the use of the same dosage for those not at risk of fractures. The recommendations are for vitamin D_3, as in Canada it is more affordable than vitamin D_2 [220].

Recently, several recent meta-analyses have obtained inconsistent results with regard to the impact of vitamin D intake, with or without calcium, in the prevention of fractures [1-3, 5, 6] (Figure 2.3). These conflicting outcomes might be due to analytic methods of 25(OH)D concentration [222] and lack of standardization of 25(OH)D measurement over the years [46] as

Table 2.2a: Recommendations and guidelines regarding vitamin D for older adults

Organizations	Target serum 25(OH)D concentrations	Groups	Intakes (IU/d)	Upper Level (UL)
Institute of Medicine (1997) [210]				
North America		Adequate Intake (AI)		
Normal healthy persons		51 - 70 y	400	2000
Minimal or no sun exposure		> 71 y	600	
Institute of Medicine (2011) [209]				
North America	40 nmol/L - Meet the needs of 50%	Estimated Average Requirement (EAR)		
Normal healthy persons	of the population	51 - 70 y	400	4000
Minimal or no sun exposure	50 nmol/L - Meet the needs of 97,5%	> 71 y	400	
	of the population	Recommended Dietary Allowance (RDA)		
		51 - 70 y	600	4000
	Deficiency: < 50 nmol/L	> 71 y	800	
	Inadequate for some: 30 - 50 nmol/L			
	Sufficient: > 50 nmol/L			

Table 2.2a: Recommendations and guidelines regarding vitamin D for older adults (cont'd)

Organizations	Target serum 25(OH)D concentrations	Groups	Intakes (IU/d)	Upper Level (UL)
Endocrine Society [58]				
North America	Deficiency: < 50 nmol/L	51 - 70 y	600	4000
At-risk individuals	Insufficiency: 52.5 - 72.5 nmol/L	> 71 y	800	
Some general population		Other recommendations:		
(Ex. dark skinned groups)		> 75 nmol/L	1500-2000	
		Corrective dose, if deficiency		
		- Loading dose for 8 weeks	6000	
		- followed by maintenance dose	1500-2000	10,000

Table 2.2a: Recommendations and guidelines regarding vitamin D for older adults (cont'd)

Organizations	Target serum 25(OH)D concentrations	Groups	Intakes (IU/d)	Upper Level (UL)
Osteoporosis Canada (2010) [49, 214]				
Canada	Deficiency: < 25 nmol/L	<u>Preventative supplementation:</u>		10,000
Prevention of osteoporosis	Insufficiency*: 25 - 75 nmol/L	Adults at *low* risk of deficiency	400-1000	
Adult > 50 y	**: Milder form of deficiency*	(< 50 y and without osteoporosis)		
	or Suboptimal	Adults at *moderate* risk of deficiency	800-1000	
	Desirable status: > 75 nmol/L	(> 50 y, with or without osteoporosis)		
	Potential adverse effect: > 250 nmol/L	Adults at *high* risk of deficiency	800-2000	
		(recurrent fractures, bone loss and/or comorbid conditions affecting absorption)		
		<u>Management of osteoporosis with pharmacologic therapy</u>		
		Adequate supplementation to replete 25(OH)D > 75 nmol/L	800-2000	
Canada Food Guide (2007) [223]				
Canada, General population		Supplement for > 50 y	400	

Table 2.2a: Recommendations and guidelines regarding vitamin D for older adults (cont'd)

Organizations	Target serum 25(OH)D concentrations	Groups	Intakes (IU/d)	Upper Level (UL)
National Osteoporosis Foundation (2014) [211]				
United States	Desirable status: > 75 nmol/L	> 50 y	800-1000	4000
		Corrective dose, if deficiency		
		- Loading dose for 8-12 weeks	7000	
		- Followed by maintenance dose	1500-2000	
National Osteoporosis Society (2014) [212, 216]				
United Kingdom	Deficiency: < 50 nmol/L Inadequate for some: 30 - 50 nmol/L Sufficient: > 50 nmol/L	Dose *for non urgent* correction of deficiency or use of antiresorptive therapy	800-2000	
		Dose for *rapid* correction of deficiency		
		- Loading dose for 6-10 weeks	7500	
		- followed by maintenance dose	800-2000	

Table 2.2a: Recommendations and guidelines regarding vitamin D for older adults (cont'd)

Organizations	Target serum 25(OH)D concentrations	Groups	Intakes (IU/d)	Upper Level (UL)
European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (2013) [215]				
Belgium	Deficiency: < 25 nmol/L	Elderly	800-1000	
France	Insufficiency: < 50 nmol/L	Postmenopausal women < 50 nmol/L	800-1000	
Germany	Sufficient[1]: 50 - 75 nmol/L	Osteoporotic patient < 50 nmol/L	800-1000	
Italy	Sufficient[2]: ≥ 75 nmol/L			
Switzerland	Potential adverse effect: > 125 nmol/L			
United Kingdom	[1] Neutral effect			
	[2] Desirable target for fragile elderly			
Australian and New Zealand Bone and Mineral Society (2012) [213]				
Australia and New Zealand	Severe deficiency: < 12.5 nmol/L	≤ 70 y	600	
	Moderate deficiency: 12.5 - 29 nmol/L	> 70 y	800	
	Mild deficiency: 30 - 49 nmol/L			
	Adequacy: ≥ 50 or 60 nmol/L			

Table 2.2b: Recommendations and guidelines regarding vitamin D for older adults in long-term care facilities

Organisations	Target serum 25(OH)D concentrations	Groups	Intakes (IU/d)	Upper Level (UL)
Osteoporosis Canada (2015) [220]				
Canada		<u>Recommendation for vitamin D_3:</u>	800-2000	
		Residents at *high* risk of fractures		
		<u>Suggestion:</u>		
		Resident *not at high* risk of fractures	800-2000	
Australian and New Zealand Bone and Mineral Society (2012) [219]				
Consensus Statement		All residents of residential aged care	1000	
Australia	Adequate: > 50 nmol/L	facilities		
	Optimal: > 75 nmol/L			

Figure 2.3: Forest plot on the effect of vitamin D supplementation on new fracture. Reproduced from [Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men (Review) Cochrane Database of Systematic Reviews 2014, Issue 4. Art. No.: CD000227] [5] with permission from John Wiley and Sons and Copyright Clearance Center

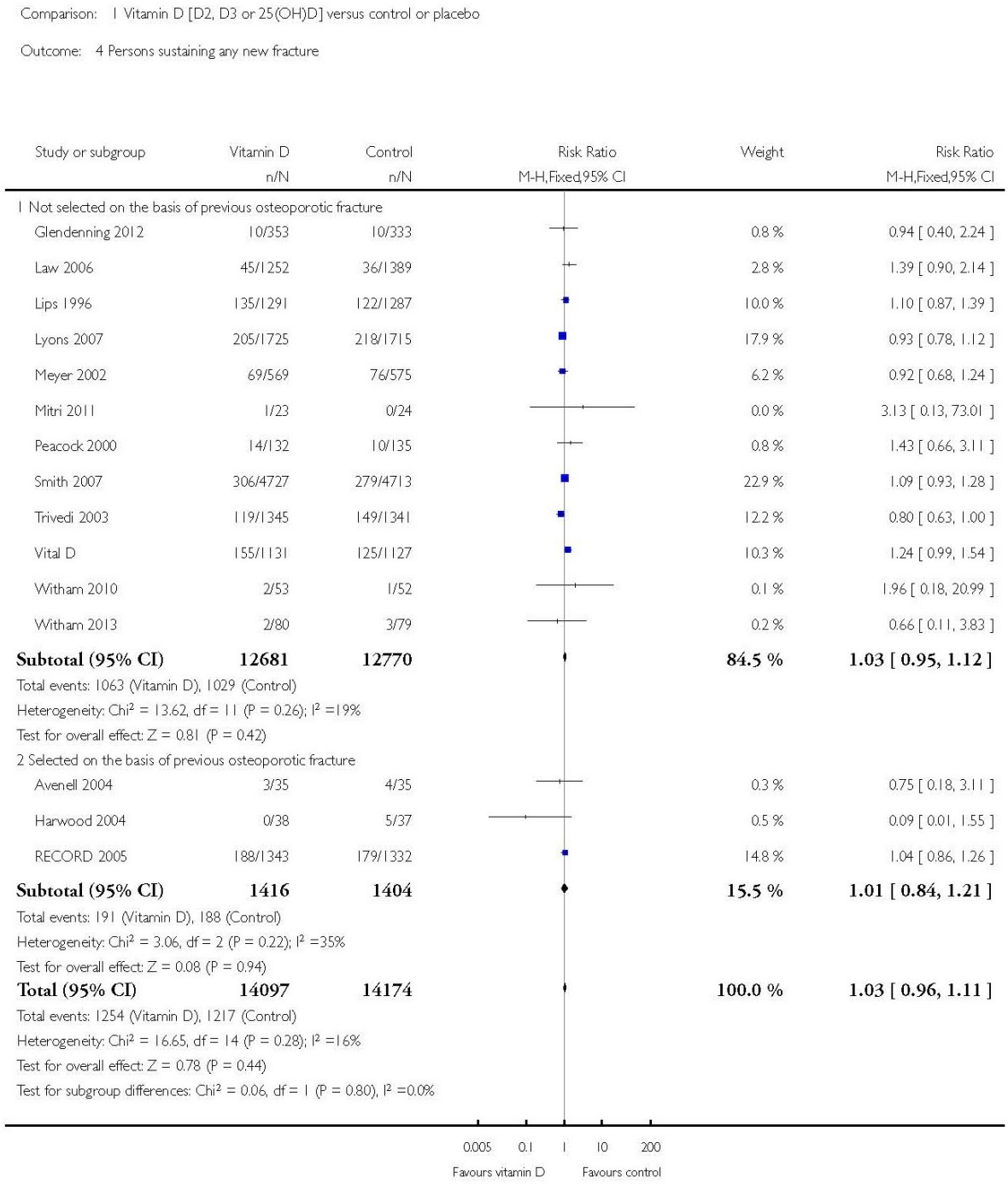


Figure 2.3: Forest plot on the effect of vitamin D supplementation on new fracture. Reproduced from [Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men (Review) Cochrane Database of Systematic Reviews 2014, Issue 4. Art. No.: CD000227] [5] with permission from John Wiley and Sons and Copyright Clearance Center

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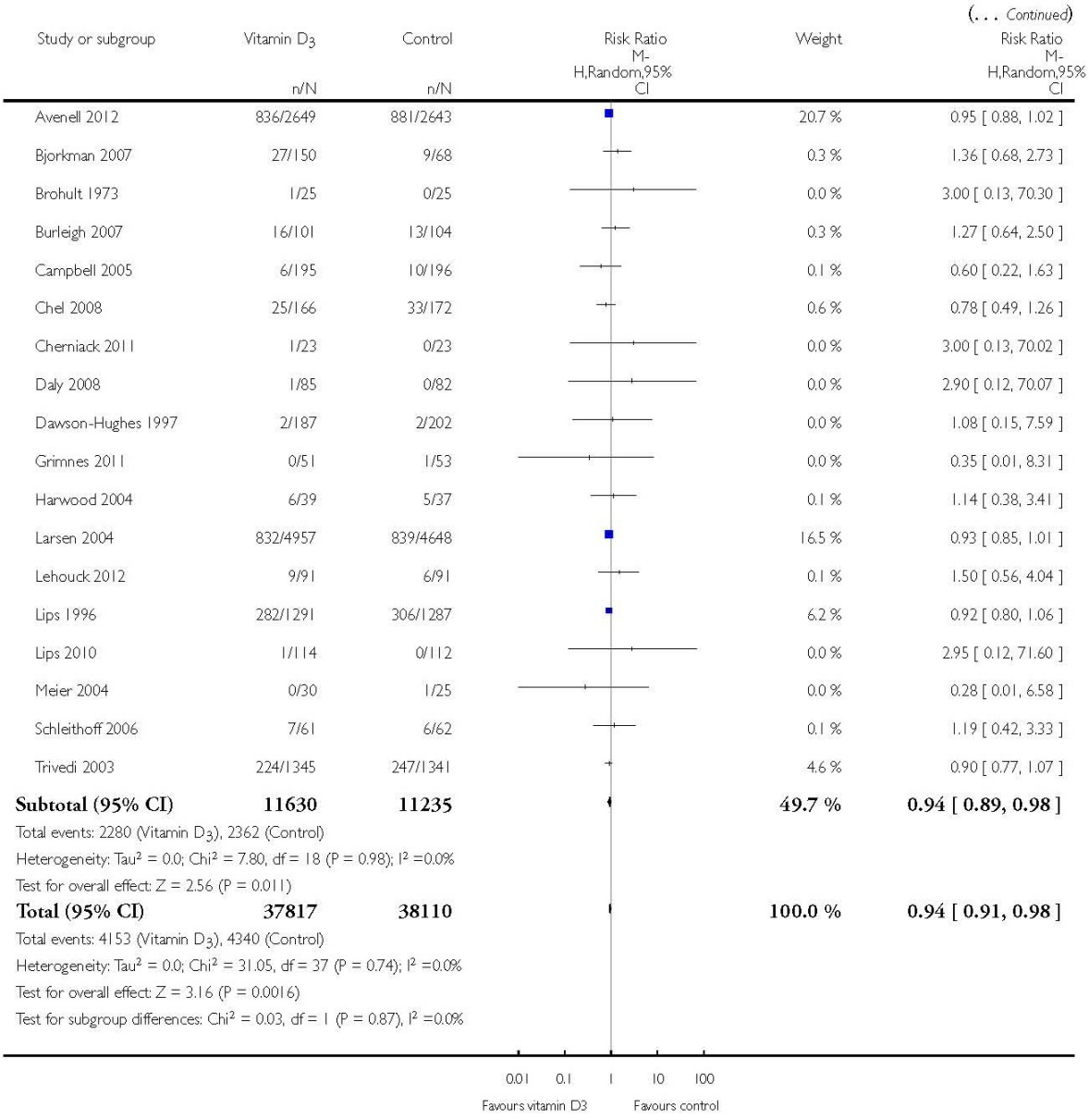

well as genetic variations affecting 25(OH)D concentrations [149]. One fact needs to be pointed out. In the pooled data reported for the meta-analyses, older men account for approximately 30% of the sampled participants. The underrepresentation of this group is an important gap in vitamin D and bone health research and more investigation is required to substantiate recommendations for older men. Even less is focused on older men in LTC.

2.1.7 Vitamin D Intake in Adults in North America

Since various recommendations for vitamin D intakes and supplementation for adults over the age of 50 y have been published by the IOM, Osteoporosis Canada, Health Canada and the US National Osteoporosis Foundation, vitamin D has been at the forefront of the public attention. However, reports of inadequate consumption of vitamin D in Canada [224-226] and the US [227] continue to prevail and international concerns of sub-optimal vitamin D intake were raised following recent observations through a systematic review of randomized controlled trials in Europe [228].

The Canadian Community Health Survey (CCHS, Cycle 2.2, Nutrition, 2004) [224] estimated the usual mean (±SE) dietary intake of vitamin D to be 212±16 IU in women (n=2610) and 252±16 IU in men (n=1520), both over the age of 70 y, living in the community. In men, these values are lower than the usual intake estimated for the 51-70 y group, but higher than for the 31-50 y group (Table 2.3). For the adults over 70 y, the mean results (±SE) in vitamin D intakes for the population of the province of Québec are slightly higher with 224±36 IU in women and 276±56 IU in men. Smaller studies with more days of dietary intake, such as the NuAge Study (n=404; 51% men; age range: 69-83 y) in Québec, suggest lower usual intakes with mean (±SD) dietary intakes of vitamin D of 172±92 IU and 212±136 IU in men [226].

Table 2.3: Vitamin D daily intake from food in adult > 31 years old, Canadian Community Health Survey (CCHS, 2004) [1]

	Men	Women
Age (year)	Dietary Intake (IU/d) (Mean±SEM)	Dietary Intake (IU/d) (Mean±SEM)
31-50	232 ± 8 n=2596	208 ± 12 n=2686
51-70	284 ± 20 n=2550	200 ± 12 n=3200
71 +	252 ± 16 n=1520	212 ± 12 n=2610

[1] Data source: Canadian Community Health Survey, Nutrition—Nutrient Intakes from Food Provincial, Regional and National Summary Data Tables, Cycle 2.2 (2004) http://www.hc-sc.gc.ca/fn-an/surveill/nutrition/commun/index-eng.php

In 2009, using the data provided by the Canadian Multicentre Osteoporosis Study (CaMos) longitudinal observational study of adults over the age of 25 y, Poliquin et al. [225] reported that Canadian women (n=6539; age: 63.1±1.8 y) consumed 108±116 IU (mean±SD) of vitamin D from diet alone and 224±236 IU of vitamin D from milk and supplements combined. The Canadian men included in this study (n=2884; age: 59.9±14.5 y) consumed 120±140 IU of vitamin D from diet alone and 224±236 IU overall. In 2013, the CaMos research team published the results of their longitudinal monitoring of vitamin D intakes in adult Canadians [229]. In the older adults of the CaMoS cohort, it can be observed that vitamin D intake from the diet alone has not changed after 10 years of follow-up, despite recommendations (Table 2.4). However, the supplement values have increased. The authors reported that the prevalence of supplement intake has increase from 26% to 52% during the course of the study, possibly due to the 2007 Canada's Food Guide to supplement adults over the age of 50 y with 400 IU/d of vitamin D. Nevertheless, the mean values of vitamin D intakes remain below the 600 IU and 800 IU/d suggested for these specific age groups by the IOM [230] and Osteoporosis Canada [214].

Low dietary intakes of vitamin D are not unique to Canadians. In the US, the Continuing Survey of Food Intakes by Individuals (CSFII) reported mean daily values (±SE) of 220±6 IU in men of > 71 y (n=674) whereas NHANES III documents mean daily values (±SE) of 236±6 IU in men of the same group (n=1255) [227]. Again, these values are well below the proposed DRIs for vitamin D in older populations. Furthermore, Moore and colleagues assessed that 29.7% of women over the age of 71 y consumed vitamin D-containing supplements whereas 26.0% of men consumed them. Nearly 75% of the vitamin D supplements used provided 400 IU of vitamin D [227], however combined with diet this would still not meet the RDA of 800 IU/d for those over 70 y of age. Studies are emerging with motivating data.

Table 2.4: Vitamin D daily Intake in the male sub-group of the Canadian Multicentre Osteoporosis Study (CaMos)[1]

Age (year)	n	Diet alone (IU/d) (Mean±SEM)	Diet + Supplements (IU/d) (Mean±SEM)
Baseline			
51-70	1373	116 ± 4	204 ± 8
71 +	745	116 ± 4	212 ± 12
Year 10			
51-70	708	108 ± 4	360 ± 28
71 +	660	120 ± 4	436 ± 28

[1]Data source: Zhou et al. [231]

The prospective Québec study NuAge reported that individuals over 68 years of age presenting 25(OH)D >75 nmol/L were taking an average of 356 IU of vitamin D from supplements. Ginter et al. [232] assessed vitamin D status and intakes in 224 seniors living in the community with an average of 72 years. The participants were deliberately chosen for their ethnical diversity. They documented a 25(OH)D concentration of 82.4 nmol/L and total vitamin D intake (diet + supplement) of 1086 IU/d. In this cohort, the supplements represented 85% of the daily intake of vitamin D. These studies show that adequate intake can help in achieving adequate vitamin D status in the older populations. Therefore, a better understanding of vitamin D sources is required to propose sufficient intake.

Main sources of vitamin D in the 2004 CCHS were milk products, contributing 49% of dietary vitamin D, followed by meat and meat-alternatives with 31.1% of the contribution [83]. In fact, the top foods sources to vitamin D intakes in the US and in Canada have been studies by the National Eating Trends service [233] with data collected from March 2006 to February 2008 for the United States and February 2007 to January 2008 for Canada. The top 3 food groups providing dietary vitamin D were milk (74 - 78 IU/d), meat, poultry and fish recipes (18.4 – 19.2 IU/d) complemented by fish and fish roe (16.4 – 17.2 IU/d). For Canadians, margarine (9.2 IU/d), eggs (3.6 IU/d) and egg recipes (3.2 IU/d) completed the list. For Americans, eggs (6 IU/d), all-family cereal sweetened or unsweetened (5.2 IU/d) follow. These differences are explained by fortification policies implemented in each country.

Since the CCHS was conducted in 2004, and the updated dietary information is not yet available, smaller community based studies help to fill this knowledge gap. For example, in a 2014 survey of Labrador and Newfoundland senior residents living in the community, Yan and

colleagues [234] looked at diet adequacy via a mailed modified version of the Hawaii food frequency questionnaire which had been validated for the Newfoundland population. Vitamin D intake for the complete sample (n=109; mean age: 73.5±6.45 y) was 340±188 IU/d. The older men in the cohort presented with similar daily values at 333±215 IU/d. The authors demonstrated that 68.5% of the studied population did not meet the Canadian daily recommendations of 600 IU/d for individuals of 51 to 70 y and 800 IU/d for individuals over the age of 70 y.

More recently, in a 2016 publication looking at dental health and nutritional status in Alberta, Pehowich et al. studied vitamin D and calcium intake over the course of 10 years (305 males – mean age of 49.2±19.3 y; 366 females – mean age of 48.1±19.7 y) using 24-hour food recall questionnaire using protocols developed by the NHANES [235]. Supplements were not considered in the analysis. The study revealed that the men of less than 50 years of age consumed 68.0±50.8% of the AI of vitamin D of 200 IU/d for their age group whereas men of more than 50 years of age consumed significantly less vitamin D with 37.9±29.3 percent of the AI of 400 IU/d at the time ($p < 0.05$). They also noted that the median dietary intake of vitamin D significantly decreased between 2003 and 2013 reflecting a change in consumption habits in this cohort. While the AI had not been replaced with new values for EAR and RDA, both of these studies highlight that dietary intakes of vitamin D by men remain below recommendations.

One exception to the presumably low vitamin D intakes in Canadian seniors living in the community is provided by the group of Ginter and al. [232]. They looked at community dwelling older adults from the Toronto area. The population included 224 adults of various ancestral origins (East Asians, Europeans and South Asians). As seen in the previous studies, the mean dietary vitamin D intake for the group was 168 IU/d. When values for the men were separated,

the mean dietary vitamin D intakes were 159 IU/d. The reason intakes were met was because this population used supplements intensely with vitamin D intakes coming from supplements with an average dosage of 917 IU/d for the complete cohort and 723 IU/d specifically for the men. This work demonstrates acceptability of supplement use among men as well as women and offers a realistic framework to enhance vitamin D status in LTC.

2.1.8 Vitamin D Sources and Intakes in Seniors in Long-Term Care Facilities

In Canada, the 2011 Census revealed that 4.9 million Canadians were over the age of 65 y. Over 7% of those seniors live in nursing homes, residences for seniors, chronic care hospitals or LTC facilities [48]. In 2014, there were an estimated 1,369,700 Americans residing in nursing homes and 41.6% were 85 y of age and over [236]. Bone health is a major concern in LTC facilities. In fact, risk of hip fractures is 1.8 times higher in LTC facilities than in the community [237] with crude hip fracture rates (person-year) of 32.1 per 100 men and 24.4 per 100 women over the age of 85 y. Although specific guidelines for bone health in seniors living in LTC facilities have emerged, frail seniors from the LTC population are often under-represented in clinical trials pertaining to bone health, osteoporosis and fracture prevention [220, 238].

Institutionalized Canadians present with insufficient vitamin D intake [61, 63, 64, 88], despite health care supervision. An analysis of micronutrients available on nontherapeutic menus in a long-term care facility in Ontario [239] demonstrated that the average content of vitamin D (356±211 IU/d) from the analyzed menus was insufficient to meet the requirements of this aged population. Super-menus were then developed to increase intake of faulty nutrients, however, vitamin D only increased to 448±211 IU/d which represented only 56% of the 800 IU/d requirements for this age group. This confirmed the 2013 results obtained by Viveky et al. [67]

in Saskatoon where the analyzed menus provided 196±60 IU/d of vitamin D. Wright-Thompson et al. [68] also documented that vitamin D dietary content of the menu served in an Ontario facility provided 342±286 IU/d, this was even after adapting to improve the food selection to meet the Ontario LTC standards.

Food menus and food selection, however, do not necessarily translate into intakes. For this reason, in 1999, Lengyel et al. studied the dietary intakes of 5 long-term care facilities in Saskatoon [64]. Using a 3-day weighed food audits as well as meal and snack observations, they documented the nutritional intakes of 45 residents (62% women) with a mean age (± SD) of 88±8 years. The vitamin D intake values were well below the AI, suggested at the time of the study for individuals over the age of 70 years, and were at 176±88 IU/d and 228±92 IU for women and men, respectively. In Vancouver, similar results were obtained in a cohort of 53 older adults (85% female) for whom 3-day food records were obtained [66]. The dietary intakes of vitamin D were of 254±91 IU/d for women and 187±87 IU/d for men. Mean total vitamin D intake was 473±360 IU/d for women and 316±239 IU/d for men when supplemental vitamin D was taken into consideration. In 2010, Hall and colleagues [63] also looked at the vitamin D content following 3-day tray audits in an Toronto LTC facility (n=30; mean age ±SD of 87.2±4.1 y). When considering nutritional supplements, such as Boost^{TM} or high-protein puddings, the daily average of vitamin D served was 414 IU. However, the consumed quantity was significantly less with 295 IU due to true consumption being 30% less than was actually served ($p< 0.001$). Those residents receiving a nutritional supplement were offered 480 IU of vitamin D per day and consumed 357 IU/d (74%). These Canadian studies were similar to Australian studies where the average 1-day plate waste of vitamin D intakes were observed to be as low as 40 IU in nursing homes and hostels in 2003 [240] and a median intake of vitamin D was

documented at 71.2 IU/d in 169 older residents [241] in 2007. Also, a review of meals served in a 120-bed facility in St-Louis (US) estimated the vitamin D provision to be approximately 200 IU/d [242]. With the new RDA recommendations at 800 IU of vitamin D daily, it appears that, even in the best of circumstances, provision of sufficient vitamin D intake solely based on foods would be challenging.

Moreover, considering that few foods are good sources of vitamin D, the poor consumption of foods providing vitamin D and the reduction of sun exposure in LTC, it is logical to think that institutionalized seniors might be increasingly affected by osteoporosis, osteomalacia and increased risk of fractures. The development of alternative food-based delivery systems is a promising solution.

2.1.9 Vitamin D Supplementation in Seniors

Given the limited food sources of vitamin D, the use of vitamin D supplements is encouraged by various agencies including Health Canada [223], Osteoporosis Canada [214] and the Canadian Cancer Society [54] to maintain bone health and prevent osteoporosis. In 2007, the revised Canada's Food Guide acknowledged the specific fact that aging Canadians needed more vitamin D and included a recommendation for a 400 IU supplement of vitamin D for individuals over 50 years of age [243]. This is important since reliance on diet and supplements is likely necessary to achieve and maintain healthy vitamin D status in Canada, particularly in winter. In community dwelling Canadians, daily consumption of various nutritional supplements was analyzed in 2010 and revealed that almost 45% men and 60% of women >70 years were consuming them. In addition to gender, the prevalence of seniors taking supplements was high regardless of socio-economic status showing a common concern for nutrition and health [244].

More recently, the American usage of supplements was analyzed by Kantor and colleagues [245] over 7 cycles of NHANES surveys. They reported that 72% of the surveyed individuals over the age of 65 y (58% in women and 45% in men). No change in overall consumption was observed, however vitamin D – in the form of multivitamin/ multimineral supplement – had a significant increase in consumption going from 5.1% in 1999-2000 to 19% in 2011-2012.

Vitamin D supplements have been tested in community-dwelling environments as well as in nursing homes and LTC facilities, with predominantly women participants, and with various formulations (tablets, oil, injections) with dosages varying from 400 IU/d to 300,000 IU/ 6mo (Table 2.5). In the community, the age of the cohorts ranged from 63 to 84 y. Participants in LTC were older with an age range of 81 to 89 y. High dosage studies (10 out of 13 reported) suggested the safety of such large regimens and would support a better compliance in supplementation. They have proven to efficiently raise 25(OH)D concentrations in older populations. However, meta-analyses have presented results conflicting effect of vitamin D, with or without calcium, on bone health [1, 3, 5, 6, 11] and potential risk of increase falls [217, 246]. The efficacy at improving vitamin D status was demonstrated with reduced daily doses [247, 248]. In LTC facilities, nursing supervision is ensured for distribution and intake of medication and supplements. Daily supplementation of vitamin D is feasible and compliance could be less of a challenge. However, a Saskatchewan cross-sectional study [249] of the prevalence of vitamin and mineral supplements prescription was conducted. Although mean prescription of overall supplements was 1.01 tablet per day, specific supplementation of vitamin D was observed to be prescribed in only 35.4% of the residents. Multivitamin pills were prescribed in 19.5% of the residents. Unfortunately, the report did not specify the dosages of vitamin D prescribed.

Furthermore, specific information regarding use of vitamin D supplements and dosage by Canadians in LCT in other regions or nationally is not available.

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults

Authors (year) Country Sample size (%M)	Age (y: SD)	Form	Interventions	Length	Baseline 25(OH)D (nmol/L)	Endpoint 25(OH)D (nmol/L)	Assay
In the Community							
Johnson et al. (1980) [250] US n=662 (26%)	>70	Oil	Vit D_3 - 2000 IU/d	10 mo	N/A	N/A	N/A
Lips et al. (1996) [251] NL	80 (6)	Tablets		3.5 y	Median (25th-75th centile)	Median (25th-75th centile)	HPLC Serum
n=2578 (26%)					Sub study 1 (n=270 women)		
			Vit D_3 - 400 IU/d		27 (19-36)	62 (52-70)	
			Placebo		26 (19-37)	23 (17-31)	
					Sub study 2 (n=46 Independent Seniors)		
					N/A	49 (41-62)	
						26 (18-35)	
					Sub study 2 (n=46 Nursing Home Seniors)		
					N/A	55 (45-60)	
						21 (15-26)	
Latham et al. (2003) [252]	79.1	Tablets		6 mo	Mean (95% CI)	Δ Median (95% CI)	RIA
NZ	(6.9)		Vit D_3 - 300,000 IU		37.5 (35.0-45.0)	22.5 (17.5-27.5)	
n=243 (47%)			Placebo		47.5 (40.0-52.5)	N/A	
			Resistance exercise		45.0 (37.5-52.5)	N/A	
			Attention control		40.0 (35.0-45.0)	N/A	

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) **Country** **Sample size (%M)**	**Age** **(y: SD)**	**Form**	**Interventions**	**Length**	**Baseline** **25(OH)D (nmol/L)**	**Endpoint** **25(OH)D(nmol/L)**	**Assay**
In the Community							
Trivedi et al. (2003)	76	Capsule		4 y	N/A	Mean (±SD)	N/A
UK [253]	(5)		Vit D_3 - 100,000 IU every 4mo			74.3±20.7	Serum
n=2686 (76%)			Placebo			53.4±21.1	
Dhesi et al. (2004)	77	IM inj.		6 mo	Mean (Range)	Mean (Range)	RIA
UK [254]	(6)		Vit D_2 - 600,000 IU		26.7 (25.5-28)	43.5 (41.3-46.5)	Serum
n=139 (22%)			Placebo		25.0 (23.7-26.3)	31.5 (28.5-34.5)	
Grant et al. (2005)		Tablets		24 mo	Mean (±SD)	Δ Mean (±SD)	HPLC
UK [255] RECORD Trial			Calcium (1000 mg/d) + Vit D_3 (800 IU/d)			Δ 24.00±17.25	Plasma
n=5292 (15%)			Calcium (1000 mg/d)			Δ 3.50±14.25	
n_{subset}=60 (N/A)			Vit D_3 (800 IU/d)			Δ 24.25±21.75	
			Placebo			Δ 7.75±18.00	
			Global		38±16.3		
Lyons et al. (2007)	84	Tablets		3 y			RIA
UK [256]	(7.53)		Vit D_2 - 100,000 IU every 4 ms			80.1	Serum
n=3400 (24%) n_{subset}=102			Placebo			54.0	
Smith et al. (2007)	79	IM inj.		16 mo	Mean		RIA
UK [257]	IQR		Vit D_2 - 300 000 IU/y		56.5	Increase of 21%	Serum
n=43 (36%)	77-83		Placebo		62.2		

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) **Country** **Sample size (%M)**	**Age (y: SD)**	**Form**	**Interventions**	**Length**	**Baseline 25(OH)D (nmol/L)**	**Endpoint 25(OH)D (nmol/L)**	**Assay**
In the Community							
Witham et al. (2010a)		Orally			Mean (±SD)		RIA
UK [258] n=105 (66%)	78.8 (5.6)		Vit D_2 - 100 000 IU/y	at 10wk	20.5±8.9	Δ 22.9±22.0	Serum
	80.6 (5.7)		Placebo		23.7±10.0	Δ 2.3±10.9	
			Vit D_2 - 100 000 IU/y	at 20wk		Δ 19.5±13.9	
			Placebo			Δ 1.3±13.4	
Witham et al. (2012)		Orally		4 mo	Mean (±SD)	Mean (±SD)	RIA
UK [259] n=58 (72%)	66.2 (13.0)		Vit D_2 - 100 000 IU/y	at 2 mo	38.7±17.6	54±15	Serum
	67.7 (6.9)		Placebo	at 2 mo	37.8±17.8	42±21	
Patients with Stroke			Vit D_2 - 100 000 IU/y	at 4 mo	38.7±17.6	51±22	
			Placebo	at 4 mo	37.8±17.8	40±19	

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) **Country** **Sample size (%M)**	**Age (y: SD)**	**Form**	**Interventions**	**Length**	**Baseline 25(OH)D (nmol/L)**	**Endpoint 25(OH)D (nmol/L)**	**Assay**
In the Community							
Witham et al. (2010c)		Vig. Oil		4 mo	Mean (±SD)	Mean (±SD)	ELISA
UK [260]	65.3		Vit D_3 - 100 000 IU/y	at 2 mo	41±14	63±20	Serum
n=61 (62%)	(11.1)						
	63.3		Vit D_3 - 200 000 IU/y	at 2 mo	48±21	79±31	
	(9.6)						
	66.7		Placebo	at 2 mo	45±17	54±20	
	(9.7)						
Patients with Db			Vit D_3 - 100 000 IU/y	at 4 mo	41±14	59±18	
			Vit D_3 - 200 000 IU/y	at 4 mo	48±21	76±30	
			Placebo	at 4 mo	45±17	53±20	
Binkley et al. (2011)	77	Capsule		12 mo	Mean (±SEM)	Mean (±SEM)	HPLC
US [91]	(65-88)		Vit D_3 - 1600 IU/d		29.9±2.5	39.0±2.4	Serum
n=64 (36%)			Vit D_2 - 1600 IU/d		32.0±2.1	38.1±2.0	
			Vit D_3 - 50,000 IU/month		36.2±2.1	45.2±3.3	
			Vit D_2 - 50,000 IU/month		31.1±2.2	34.7±2.3	

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) Country Sample size (%M)	**Age (y: SD)**	**Form**	**Interventions**	**Length**	**Baseline 25(OH)D (nmol/L)**	**Endpoint 25(OH)D (nmol/L)**	**Assay**
In the Community							
Papaioannu et al. (2011) [248]		Tablets		3 mo	Mean (95% CI) adj. Time of collect	Mean (95% CI) %change (95% CI)	RIA Serum
CAN	82.9		Vit D_2 - 50,000 IU +	at 1 mo	53.5 (42.3-64.8)	84.5 (73.7-95.3)	
n=65 (43%)	(8.7)		Vit D_3 - 1000 IU/d for 90 d			Δ 100.3% (59.3,141.3)	
	73.9		Vit D_2 - 100,000 IU +	at 1 mo	58.4 (47.3-69.5)	75.6 (64.5-86.8)	
Post hip fracture	(12.4)		Vit D_3 - 1000 IU/d for 90 d			Δ 82.5% (40.5, 124.5)	
	78.5		Placebo	at 1 mo	46.7 (34.8-58.6)	69.3 (58,4-80,2)	
	(10.3)		Vit D_3 - 1000 IU/d for 90 d			Δ 43.3% (-0.29, 86.8)	
			Vit D_2 - 50,000 IU +	at 3 mo	53.5 (42.3-64.8)	84.2 (73.6-94.9)	
			Vit D_3 - 1000 IU/d for 90 d			Δ 146.1% (83.8, 208.4)	
			Vit D_2 - 100,000 IU +	at 3 mo	58.4 (47.3-69.5)	73.3 (64.5-82.1)	
			Vit D_3 - 1000 IU/d for 90 d			Δ 68.1%(17.1, 119.1)	
			Placebo	at 3 mo	46.7 (34.8-58.6)	86.7 (77.6-95.9)	
			Vit D_3 - 1000 IU/d for 90 d			Δ 85.6% (30.2, 141.0)	
Bischoff-Ferrari et al.	78			12 mo	Mean (±SD)	Δ Mean (95%CI)	HPLC/MS
(2016) [217]	(5)		Vit D_3 - 24,000 IU/mo	6 mo	46.75±24.50	31.75 (26.5-37.25)	Serum
SZ			Vit D_3 - 60,000 IU/mo	6 mo	52.25±23.00	45.75 (40.5-51.25)	
n=200 (33%)			Vit D_3 - 24,000IU/mo +25(OH)D	6 mo	46.00±19.00	69.00 (63.5-74.50)	
with reported falls			Vit D_3 - 24,000 IU/mo	12 mo		29.25 (24.00-34.50)	
			Vit D_3 - 60,000 IU/mo	12 mo		48.00 (42.75-53.50)	
			Vit D_3 - 24,000 IU/mo +25(OH)D	12 mo		64.50 (59.00-69.75)	

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) **Country** **Sample size (%M)**	**Age (y: SD)**	**Form**	**Interventions**	**Length**	**Baseline 25(OH)D (nmol/L)**	**Endpoint 25(OH)D (nmol/L)**	**Assay**
In long-term care facilities							
Corless et al. (1985)		Tablets			Mean (±SEM)		N/A
UK	82.3		Vit D_2 - 9000 IU/wk		16.60±2.10	Nb<20 nmol/L =	Plasma
n=65 (22%)	(34)					21/32	
	82.6		Placebo		17.63±2.05	Nb<20 nmol/L =	
	(39)					23/33	
Meyer et al. (2002)		Cod Liver Oil		2 y	Mean (±SD)	Mean (±SD)	HPLC Serum
NO [261]							
n=1144 (24%)	84.4 (7.3)		Vit D_3 (440 IU/d)		47±26	64±21	
	85.0 (7)		Vit D_3 (20 - 40 IU/d)		51±33	46±20	
Law et al. (2006)	85	IM inj.		10 mo	Median (90th perc range)	Median (90th perc range)	ELISA
UK [262]							
n=3717 (24%)		before inj.	Vit D_2 - 100,000 IU every 3 ms	at 1 mo	47 (35-102)	82 (67-185)	
n_{subset}=18 (N/A)		before inj.		at 3 mo		74 (52-110)	
Lyons et al. (2007)	84	Tablets		3 y			RIA
UK [256]	(7.53)		Vit D_2 - 100,000 IU every 4 ms		N/A	80.1	Serum
n=3400 (55%)			Placebo			54.0	
n_{subset}=102							

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) **Country** **Sample size (%M)**	**Age (y: SD)**	**Form**	**Interventions**	**Length**	**Baseline 25(OH)D (nmol/L)**	**Endpoint 25(OH)D (nmol/L)**	**Assay**
In long-term care facilities							
Broe et al. (2007) US [263]	89 (6)	Tablets		5 mo	Mean (±SD)	Mean (±SD) n_{subset}=100	ELISA
n=124 (37%)			Vit D_2 - 200 IU/d		44.5±23.0		Serum
			Vit D_2 - 400 IU/d		51.8±29.0	Ranging from:	
			Vit D_2 - 600 IU/d		41.3±18.5	55 - 60	
			Vit D_2 - 800 IU/d		53.5±23.0		
			Placebo		53.0±28.5	74.9±14.7	
			Global		48.8±24.8		
Chel et al. (2008)	84	Tablets		4 mo	Mean (±SD)	Mean (±SD)	RIA
NL [247]	(6.3)	Tablets	Vit D_3 - 600 IU/d	at 2 mo	23.0±8.3	59.9±16.5	Serum
n=338 (22%)		Powder	Vit D_3 - 4200 IU/wk	at 2 mo	27.3±12.7	58.8±12.8	
			Vit D_3 - 18,000 IU/month	at 2 mo	23.8±8.0	44.8±14.1	
			Placebo	at 2 mo	25.2±12.1	24.3±11.2	
			Global		25.0±10.9		
			Vit D_3 - 600 IU/d	at 4 mo	23.0±8.3	69.9±17.8	
			Vit D_3 - 4200 IU/wk	at 4 mo	27.3±12.7	67.2±14.0	
			Vit D_3 - 18,000 IU/month	at 4 mo	23.8±8.0	53.1±15.9	
			Placebo	at 4 mo	25.2±12.1	25.5±12.0	

Table 2.5: Changes in 25(OH)D status assessed via dose-response vitamin D supplement interventions conducted in senior adults (cont'd)

Authors (year) **Country** **Sample size (%M)**	**Age** **(y: SD)**	**Form**	**Interventions**	**Length**	**Baseline** **25(OH)D (nmol/L)**	**Endpoint** **25(OH)D (nmol/L)**	**Assay**
In long-term care facilities							
Lips et al. (2010)		Tablets		4 mo	Mean (±SD)	Mean	HPLC
NL [264]	78(6.2)		Vit D_3 - 8400 IU/wk		35.2±13.7	65.5	Serum
n=226 (% N/A)			as 3x 2800 IU/wk				
			Placebo		34.2±11.0	33	
Veleva et al. (2014)	83			3 mo	Mean (±SD)	Mean (±SD)	RIA
NL [265]	(7)	Capsules	Vit D_3 - 5600 IU/wk	n=52		90±22	Serum
n=71 (35%)		Drops	Vit D_3 - 7500 IU/wk	n=19		41±18	
			Global		77±30		
Feldman et al. (2014)	85.0			12 mo		Mean (95% CI)	CLIA
CAN [266]	(7.7)		Vit D - 20,000/wk			102	Serum
n=236 (25%)						(98-106)	
Ginde et al. (2016)	81			12 mo	Mean (±SD)		N/A
US [246]	(10)		Vit D_3 - 100,000 IU/mo	3 mo	57.50±21.00	85.00	
n=107 (42%)			Standard doses:				
			Placebo with 400-1000 IU/d Placebo with 12,000 IU/mo	3 mo	57.50±24.75	67.50	
Respiratory			Vit D_3 - 100,000 IU/mo	11 mo	57.50±21.00	76.25	
infections			Standard doses:				
			Placebo with 400-1000 IU/d Placebo with 12,000 IU/mo	11 mo	57.50±24.75	63.75	

(%M): %Men; CAN: Canada; NL: The Netherlands; NO: Norway; SU: Switzerland; SW: Sweden; UK: United Kingdom; US: United States

2.1.10 Vitamin D Fortification Considerations for Use in Long-Term Care Facilities

High use of medications and supplementation can affect quality of life for seniors [267]. Polypharmacy can alter taste and smell which could lead to decreases in food intake [268]. The use of fortification in lieu of supplementation regimens might provide an alternate route to help in increasing vitamin D status in seniors living in LTC facilities.

Canadian legislation requires mandatory addition of specific amounts of vitamin D, known as fortification, in milk and margarine [269, 270]. Other fortified foods such as meal supplements, apple or orange juice and yogurts are now available on the market and provide amounts of vitamin D per serving similar to eggs. Dietary sources of vitamin D and 25(OH)D content could vary in content for several reasons: selected species, provenance, season, duration of storage, cooking method [70-72, 74, 140, 271] or industry fortification levels and quality control measures [75-77]. The foods need to be stable over time in vitamin D concentrations to ensure constant and reliable supplementation.

An association between vitamin D dose (food-based delivery systems or supplements) and serum 25(OH)D concentration has been observed by the Agency for Healthcare Research and Quality (Ottawa) via meta-analyses [18, 19]. Serum 25(OH)D concentration was reported to increase by 1 to 2 nmol/L with every additional 100 IU (2.5 μg)/d of vitamin D_3. The Agency for Healthcare Research and Quality (Tufts) [23] confirmed this relationship, but noted a different relationship when the baseline concentration was below or above 40 nmol/L and when the supplementation was less or more than 3 months showing a possible a non-linear relationship. Over the years, in various research settings, specially formulated foods (orange juice, cheese,

eggs, malted milks, yogurts, breads and dehydrated mushrooms) have been developed for use in clinical trials [61, 73, 78, 135, 138, 271-287] in the community or in LTC (Table 2.6).

A recent scoping review by Whiting and al. [288] looked at 14 studies pertaining to bone health and involving mature adults and older populations (≥ 50 years). Twelve of the 14 studies involved vitamin D (24 IU to 5000 IU/d) and calcium fortification. Unfortunately, many of the study protocols were limited by single-arm designs and the commonly combined fortification approach limited conclusions considering the impact of any specific nutrient alone. Only 1 intervention looked at the impact of vitamin D alone, using bread as carrier [271]. Other carriers were fortified with calcium or other nutrients such as iron or vitamin K.

Several interventions involved younger participants as the age ranges were 24 to 80 y in the community and 50 to 87 y in LTC. Studies took place over a course of 4 weeks to a maximum of 18 months and predominantly included women. The fortification regimens ranged from 100 IU/d to 5000 IU/d. However, the additional dietary intakes were not assessed. Of the 19 studies reported in Table 2.6, only 5 took place in LTC facilities with participants older than 50 y. Of those, only 2 studies included older men. Adolphe and al. [61] proposed fortification of pureed foods to 11 residents of a Saskatoon LTC facility. The research team added a multivitamin powder, including vitamin D, to 6 puréed foods (tomato-basil bread purée, beef purée, savoury-bread purée, chicken purée, chicken sandwich purée and vegetarian pasta purée). The fortification approach improved vitamin D intakes from 84±88 IU at baseline to 488±132 IU at the end of the intervention. The serum 25(OH)D values increased from 41±27 nmol/L at baseline to 66±11 nmol/L after 8 weeks of fortification. The fortification of 400 IU of vitamin D per day resulted in a mean increase of 25(OH)D concentration of 25 nmol/L which is in line with what was proposed by Heaney et al. [289]. Mocanu and colleagues [275] used bread fortified

Table 2.6: Interventions using foods as carrier for vitamin D fortification

Authors (Year) Country, Sample size	**Age (y: SD)**	**Dose (IU)**	**Ca (mg)**	**Length**	**Pre Fortification 25(OH)D (nmol/L)**	**Post Fortification 25(OH)D (nmol/L)**	**Assay**
In the Community							
Keane et al. (1998)		Milk		12 mo	Mean (Range)	Mean (Range)	ELISA
IR [290]	80.3	Vit D - 200 IU/500 mL	800	*Apr-Sept*	24.00 (13.75, 31.75)	54.25 (33.75, 94.50)	Serum
n=51 (24%)	(66-91)						
	78.1	Placebo - 4 IU/500 mL	600	*Apr-Sept*	25.00 (13.75, 32.25)	51.50 (34.75,102.50)	
	(65-92)				Mean (Range)	Δ Mean±SEM	
		Vit D - 200 IU/500 mL	800	*Oct-Apr*	24.00 (13.75, 31.75)	22.28±2.23	
		Placebo - 4 IU/500 mL	600	*Oct-Apr*	25.00 (13.75, 32.25)	6.75±2.58	
Toxqui et al. (2014)		Milk		4 mo	Mean±SD	Mean±SD	ELISA
ES [291]	24.7 (4.6)	Vit D - 200 IU/d + 15 mg iron			62.3±20.8	71.2±21.1	Serum
n=165 (0%)	24.8 (4.1)	15 mg iron			62.9±20.8	63.2±18.3	
	26.5 (3.8)	Placebo			57.1±17.8	54.9±16.0	
Tangpricha et al. (2003) [276]		Orange Juice		3 mo	Mean±SEM	Mean±SEM	ELISA
US	29.0	Vit D_3 - 1000 IU/d	350		37.0±8.0	94.0±20.0	Serum
n=26 (%N/A)	(9.0)	Placebo	350		50.0±10.0	73.0±8.0	
Bioavailability study		Whole/Skim Milk and margarine		Once	Mean±SEM	Mean±SEM	
n=18 (%N/A)	36.3 (10.0)	Vit D_2 - 25,000 IU				Peak at 12h: 74.0 Near Baseline at 72h	

Table 2.6: Interventions using foods as carrier for vitamin D fortification (Cont'd)

Authors (Year) Country, Sample size	Age (y: SD)	Dose (IU)	Ca (mg)	Length	Pre Fortification 25(OH)D (nmol/L)	Post Fortification 25(OH)D (nmol/L)	Assay
In the Community							
Daly et al. (2006a)		Milk		2 y	Mean±SD	Δ Mean±SD	RIA
AU (sub-study) [284]	62.9 (7.6)	Vit D_3 - 800 IU/d	1000		78.5±23.7	2.4±27.3	Serum
n=111 (100%)	62.8 (7.2)	No Placebo			79.2±22.9	-14.1±24.5	
Natri et al. (2006)		Bread		3 wk	Mean±SEM	Δ Mean±SEM	RIA
FI [271]	27.3	Wheat Vit D_3 - 400 IU/100g			29.0±3.0	16.3±6.6	Serum
n=41 (0%)	(0.6)	Rye Vit D_3 - 400 IU/100g			28.9±3.5	14.9±6.2	
	28.8	Vit D_3 suppl - 400 IU			29.6±2.6	19.5±10.1	
	(1.8)	Placebo			27.1±3.7	-0.3±4.0	
	31.1 (1.8)						
	29.0 (1.7)						
Kruger et al. (2010)		Milk		4 mo	Mean±SEM	Mean±SEM	RIA
NL [292]	27	Vit D_3 200 IU/d + Mg+Zn	1000		58.5±2.24	53.5±2.58	Plasma
n=82 (0%)	(20-35)	Vit D_3 200 IU/d + Mg+Zn + K	1000		68.2±2.28	60.7±2.65	
		Placebo			60.8±2.28	59.9±2.46	

Table 2.6: Interventions using foods as carrier for vitamin D fortification (Cont'd)

Authors (Year) Country, Sample size	Age (y: SD)	Dose (IU)	Ca (mg)	Length	Pre Fortification 25(OH)D (nmol/L)	Post Fortification 25(OH)D (nmol/L)	Assay
In the Community							
Biancuzzo et al. (2010) [273]		Orange Juice		11 wk	Mean±SD	Mean±SD	HPLC
US	41.4 (12.6)	Vit D_3 - 1000 IU/d	350		17.9±11.1	30.7±8.5	Serum
n=86 (31%)	40.1 (15.6)	Vit D_2 - 1000 IU/d	350		15.8±10.0	26.4±7.4	
	40.1 (18.0)	Vit D_3 caps -	350		19.6±11.1	28.0±11.0	
	38.9 (12.3)	1000IU/d	350		16.6±9.9	27.4±10.5	
		Vit D_2 caps - 1000IU/d	350		19.8±9.6	18.1±6.4	
		Placebo					
Daly et al. (2006b)		Milk		2 y	Mean±SD	Δ Mean (95% CI)	RIA
AU [285]	62.1 (7.7)	Vit D_3 - 800	1000		77.2±22.6	27.3 (18.7, 36.0)	Serum
n=167 (100%)	61.7 (7.7)	No Placebo			76.1±23.5		
Kukuljan et al.		Milk		18 mo	Mean±SD	Δ Mean (95% CI)	RIA
(2009; 2011)	61.7 (7.6)	Vit D_3 - 800	1000		90.5±29.9	7.4 (-3.7, 18.4)	Serum
AU [283, 293]	61.7 (7.7)	IU/d+Exer	1000		83.6±32.7	15.3 (5.8, 24.8)	
	60.7 (7.1)	Vit D_3 - 800 IU/d	1000		85.0±40.6	-16.0 (-27.9, -4.1)	
n=180 (100%)	59.9 (7.4)	Exercise	1000		85.7±40.3	-7.2 (-13.9, -0.5)	
		Placebo					

Table 2.6: Interventions using foods as carrier for vitamin D fortification (Cont'd)

Authors (Year) Country, Sample size	Age (y: SD)	Dose (IU)	Ca (mg)	Length	Pre Fortification 25(OH)D (nmol/L)	Post Fortification 25(OH)D (nmol/L)	Assay
In the Community							
Kanellakis et al. (2012) [294]		Yogurt		12 mo	Mean±SD	Mean±SD	ELISA
	62	Vit D_3 - 400 IU/d	800		22.8±5.9	24.4±5.2	Serum
n=173 (0%)	(5.8)	Vit D_3 - 400 IU/d	800+K1		23.2±4.8	25.7±5.0	
	Range	Vit D_3 - 400 IU/d	800+K2		21.5±5.5	25.1±5.0	
	(54-73)	Placebo			23.3±7.7	22.6±13.4	
Fisk et al. (2012) [281]		Malted Milk		1 mo	Mean±SD	Δ Mean (95% CI)	HPLC
UK							/MS
n=40 (40%)	24.4 (4.7)	D_2 sachet A: 192			48.0±26.6	4.9 (-2.3, 12.7)	Serum
	24.4 (3.9)	D_2 sachet B: 300			41.9±14.1	13.6 (4.1, 23.0)	
	30.5 (10.1)	D_3 sachet A: 208			31.3±22.1	11.9 (2.7, 21.2)	
	30.6 (10.6)	D_3 sachet B: 400			30.9±29.1	19.7 (9.4, 30.1)	
	24.1 (1.8)	Placebo			33.5±13.3	-3,4 (-8.2, 1.5)	
Bonjour et al. (2012)		Soft cheese		1.5 mo	Mean±SD	Δ Mean±SD	RIA
FR [279]	56.6	Vit D_3 - 100 IU/d	200		58.8±20.8	9.0±10.5	Serum
n=71 (0%)	(3.9)	Placebo	100		57.3±16.8	8.5±17.3	

Table 2.6: Interventions using foods as carrier for vitamin D fortification (Cont'd)

Authors (Year)	Age (y: SD)	Dose (IU)	Ca (mg)	Length	Pre Fortification 25(OH)D nmol/L	Post Fortification 25(OH)D nmol/L	Assay
In the Community							
Bonjour et al. (2015)		Yogurt		3 mo	Mean±SEM	Mean±SEM	ELISA
GB [277]	74.3	Vit D_3 - 400 IU/d	520		34.1±2.4	56.2±2.43	Serum
n=48 (0%)	(1.4)						
	72.8	Placebo	280		35.1±2.45	41.3±2.92	
	(1.6)						
Nikooyeh et al. (2016)		Bread		2 mo	Mean±SD	Mean±SD	HPLC
IR [282]	37.9	Bread Vit D_3 - 1000 IU/d + Plac			33.9±21.9	72.9±23.1	Serum
n=90 (54%)	(10.9)	Reg. Bread + Vit D_3 - 1000 IU/d			35.0±38.7	63.9±31.0	
		Reg. Bread + Plac			34.7±30.5	25.4±21.8	

Table 2.6: Interventions using foods as carrier for vitamin D fortification (Cont'd)

Authors (Year)	Age (y: SD)	Dose (IU)	Ca (mg)	Length	Pre Fortification 25(OH)D nmol/L	Post Fortification 25(OH)D nmol/L	Assay
In long-term care facilities							
Mocanu et al. (2009)		Bread		18 mo	Mean±SD	Mean±SD	HPLC
RO [275]	71	Vit D_3 - 5000 IU/bun					Serum
n=45 (38%)	(6.9)		320		28.5±10.8	125.6±38.8	
Adolphe et al. (2009)		Pureed Foods		2 mo	Mean±SD	Mean±SD	LCMS
CAN [61]	> 50	Vit D 160 IU/d x 4 food items			41±21	66±11	Serum
n=11 (85%)		(~640 IU/d)					
Bonjour et al. (2009)		Soft cheese		1 mo	Mean±SD	Mean±SD	RIA
FR [272]	84.8	Vit D_3 - 100 IU/d	320		13.75±4.25	15.75±4.25	Serum
n=35 (0%)	(8.8)						
Bonjour et al. (2011)		Soft cheese		1.5 mo	Mean±SEM	Mean±SEM	RIA
FR [278]	86.9	Vit D_3 - 100 IU/d	151		22.0±18.8	31.25 (±N/A)	Serum
n=21 (0%)	(6.3)	Placebo	118				
Bonjour et al. (2013)		Yogurt		2 mo	Mean±SEM	Mean±SEM	ELISA
FR [280]	85.8	Vit D_3 - 400 IU/d	800	at 1 mo	19.2±1.2	39.3±3.3	Serum
n=59 (0%)	(1.2)						
	85.1	Placebo	280	at 1 mo	16.2±0.6	20.1±2.6	
	(1.3)						
		Vit D_3 - 400 IU/d	800	at 2 mo	19.2±1.2	44.6±2.5	
		Placebo	280	at 2 mo	16.2±0.6	21.4±2.7	

AU: Australia; CAN: Canada; ES: Spain; FI: Finland; FR: France; GB: Great Britain; IR: Ireland; NL: The Netherlands; RO: Romania; UK: United Kingdom; US: United States

with 5000 IU/bun to successfully increase 25(OH)D concentrations from 28.5±10.8 nmol/L to 125.6±38.8 nmol/L without negatively affecting serum calcium. They reported an improvement in hip and lumbar spine BMD [275]. In a 3 year follow up report, the improvement remained present in the lumbar spine BMD only [295].

In 2014, Shakur and colleagues [296] looked at different modelling scenarios to simulate the addition of vitamin D to foods in Canada, beyond the current mandatory food fortification. The modelling was aimed at providing vitamin D benefits while mitigating the potential risks of excess. They proposed yogurt and cheese fortification and increased milk fortification in a model designed to possibly double vitamin D intakes in Canadians. In the United Kingdom, similar work has been done to assess the impact of fortification of wheat flour and milk [297]. To date, Health Canada has not moved forward with such recommendations.

2.2 Vitamin D Status in Seniors and Implications to Bone Health

Serum 25(OH)D is a reflection of both endogenous synthesis and dietary sources. Traditionally, optimal vitamin D status was based on concentrations required to achieve bone health. In 1997, Chapuy et al. [56] presented epidemiological results looking at vitamin D status and PTH. Although a non-linear relationship was observed between PTH concentrations and lower 25(OH)D concentrations, a plateau was observed at approximately 36 pg/mL of PTH and 78 nmol/L of 25(OH)D. Lower vitamin D status was associated with increased PTH which can lead to bone resorption and the increase risk of fractures. This plateau has been used by medical societies that define the optimal serum value for vitamin D at > 75 nmol/L [58]. The rationale for this concentration is being challenged in the light of other potential health benefits associated

with vitamin D status. More randomized clinical trials are required to suggest higher intake recommendations or serum concentrations [21, 69].

Vitamin D is relevant to osteoporosis and osteomalacia in the adult population. Frailty of the bone structure will lead to fractures, mainly of the hip, wrist and vertebrae by thinning and loss of trabeculae and thinning of cortices. Osteoporosis is known to be a progressive disease affecting more than 2 million Canadians. Over the age of 50 y, one in four women and one in eight men is suspected to be affected by the disease. It commonly grows silently until the first fracture [298]. In the mid '90s, the CaMos group began recruiting men and women over the age of 25 y. This large randomly selected population-based prospective study looked at osteoporosis in the Canadian population. CaMos documented: 1) the burden of osteoporosis and fracture; 2) factors associated with osteoporosis and fracture; and 3) measured the health and economic consequences of osteoporosis and fracture for Canadians. This study provides information on prevalence of osteoporosis [299], occurrence of fractures [300], as well as considerable quality of life impacts in Canadians living at home [301].

Health Canada reports 12,000 hip fractures in 2003, in free living adults aged 60 or more, whereas the number rose to 25,000 for thigh, knee, ankle, lower leg or foot fractures and 40,000 for shoulder, arm, wrist and hand fractures [302]. In the community, hip fractures occurred at a median age of 80 years and can result in permanent impact on mobility and loss of independence. It is estimated that more than 10% of the community dwelling population will need to move to an institution following a hip fracture [303] and 66% of individuals reporting a hip fracture in the 2003 CCHS mentioned being afflicted by arthritis or rheumatism. The reality of individuals living in a LTC facility is quite different with regard to social and neighborhood surroundings, daily activities, rehabilitation and adaptive aids, dietary intake and supervision by

health care staff. The adults in LTC are often frailer and present a fracture rate that is 2 to 4 times higher than those living in the community [220]. Hip fractures are one of the leading cause of hospitalization [304] in nursing home residents. Fractures also cause pain leading to agitation or reduced mobility in this clientele. In 2015, recommendations for preventing fractures in LTC were published for Canada [220]. They included, alongside vitamin D and calcium recommendations, recommendations for hip protectors, exercises, revision of medications and assessment of environmental hazards that could increase the risk of falls and fractures. Hence, a comprehensive evaluation of the resident in LTC is required to prevent fractures.

2.2.1 Role of Vitamin D in Calcium Metabolism in Aging

Well known roles of $1,25(OH)_2D_3$ include serum calcium and phosphate homeostasis. Its action is observed in the intestine, the kidneys and in bone. VDR are expressed throughout the small and large intestine segments. Absorption of calcium has been documented in both proximal and distal areas of the intestine [305] as well as in the colon [306]. Recent work in CYP27B1 *null* mice demonstrated that $1,25(OH)_2D_3$ regulates a large number of genes involved in calcium absorption [307].

The involvement of $1,25(OH)_2D_3$ in calcium transport is multifaceted in both the intestine and the kidney. In the intestine, the increase uptake of calcium is supported by the fact that $1,25(OH)_2D_3$ promotes the expression of the TRPV6 calcium channel, the calbindin-D9k protein and the CaATPase present in the plasma membranes cells known as PMCA1b. In the kidney, passive reabsorption of calcium occurs in the proximal tubule. However, $1,25(OH)_2D_3$ acts jointly with PTH to improve calcium reabsorption in the renal distal tubule. There again, $1,25(OH)_2D_3$ stimulates the expression of calcium channel TRPV5, calbindin-D9k and calbindin-

D28k as well as PMCA1b which will also enables active transport of calcium [308, 309]. Calcium from the bone structure is also mobilized back into the circulation to maintain homeostasis. Increased bone turnover and mineralization of bone is observed in TRV6P *null* mice on a low calcium diet [310] whereas severe hypercalciuria and change in bone structure has been documented in TRV5P *null* mice [311] demonstrating their implication in calcium homeostasis.

Aging leads to several physiological changes and metabolism of vitamin D is affected. Intestinal absorption of calcium is reduced which results in hyperparathyroidism [312]. However, synthesis of $1,25(OH)_2D_3$ in response to PTH declines with age [313]. Also, in aging, a progressive degeneration of the renal function associated to a decrease glomerular filtration rate occurs [314]. Renal production of CYP24A1 increases with age which increases the degradation of $1,25(OH)_2D_3$ [315]. Coupled with a decrease in efficacy of 1 α-hydroxylation, the overall synthesis of $1,25(OH)_2D_3$ is reduced [316]. It is suspected that the reduction in synthesis of $1,25(OH)_2D_3$ might be triggered by the increase of FGF23 in the senescence of the kidney [317]. Lastly, reabsorption of phosphate by the kidney appears to be decreased, as observed in aged rats [318].

2.2.2 Role of Vitamin D and Bone Health in Aging

Decreased mineral density, osteoporosis and osteomalacia can result from an inadequate bone remodeling process where osteoclast activity supersedes osteoblast compensation [50, 319]. Osteoporosis is a skeletal disorder characterized by decreased bone strength predisposing to augmented risk of fracture [320]. Vitamin D has mostly been studied in aging women with several meta-analyses presenting only 30% of the pooled participants being males [21]. In

looking at bone health, important work has been done with Canadian [321-326] and American cohorts [85, 327-329] living at home.

Although predominantly perceived as a women's disease, osteoporosis will affect 1 in 5 Canadian men over the age of 50 y [330]. Over 25% of the 30,000 hip fractures in Canada are in men [330]. In the United States, the National Osteoporosis Foundation affirms that 25% of men over the age of 50 y will suffer from osteoporosis. Moreover, mortality risk is increased 2- to 3-fold in men suffering major fractures when compared to women [331-333]. Osteoporosis Canada states that 37% of men who suffered a hip fracture will die within the following year [330]. Unlike in women, bone loss occurs gradually after the age of sixty years in men [334]. The reported bone loss rate is 0.5% to 1% per year [335]. The national data are based on population living in the community and could be a poor surrogate to reflect the needs of the very old population living in LTC facilities.

2.2.3 Role of Vitamin D in Musculoskeletal Health and Falls

Accumulating evidence suggests that maintenance of vitamin D status is related to prevention of sarcopenia. Sarcopenia is a syndrome characterized by the progressive and generalized decrease in muscle mass and muscle strength [336]. Low vitamin D status has been associated to muscular weakness and pain [337, 338], declining physical performance, mobility, functional capacity and quality of life [336, 339-341]. It is often reported in older populations alongside frailty [342, 343]. Frailty is defined as a state of vulnerability leading to functional deterioration [343]. In the InChianti prospective study, individuals presenting vitamin D status > 50 nmol/L were able to recovered from a prefrail status, which was defined has being affected by

only 1 or 2 of the following conditions: unintentional weight loss, exhaustion, sedentariness, muscle weakness, and slow speed walking [344].

Sarcopenia appears as a major cause of frailty and vitamin D is believed to have an important impact on this aging syndrome [343-346]. Concentration of 25(OH)D of less than 50 nmol/L was associated with reduced muscle mass [347] and physical function [341] in older populations. In older women, vitamin D supplementation was reported to augment muscle fiber size [348, 349], increase intramyonuclear vitamin D receptors [348, 350] and improved strength and functional capacities [264, 338, 351]. Nevertheless, meta-analyses present inconsistent results when looking at vitamin D and muscle strength. Some show positive effects of vitamin D supplementation on strength [352, 353] or no impact of supplementation [354, 355]. However, in the meta-analysis by Beaudart et al., 3 studies involved only men (n=159), whereas 13 studies representing women (n=4173) were tallied. This is also noted in the systematic review of the literature prepared by Rosendahl-Riise and colleagues [354]. More research will be required to explain the possible mechanisms at play in both men and women.

2.3 Densitometry

Bone mineral density (BMD) is measured using densitometry techniques to help determine bone health and is used for the diagnosis and management of treatment of osteoporosis. Dual-energy X-ray absorptiometry (DXA) is an X-ray technique using two distinct photo-electric peaks necessary to discriminate between bone and soft tissue [356]. DXA provides extremely low radiation exposure. It allows for analysis of axial and appendicular sites [357]. DXA is considered as the gold standard for bone mineral density assessment [358] but it is highly dependent of the quality of the scans, the analysis and the interpretation of the results.

Comparing bone mineral density results, reported in g/cm^2, to mean normal BMD values in a healthy and young population of the same sex (reference population) help quantifying the fracture risk of an individual. Calculation of the number of standard deviations above or below the mean BMD of the reference population is known as the T-score. World Health Organization (WHO) has defined osteoporosis as a T-score at the femoral neck at or below -2.5. A Z-score of -2.5 or less, which is a comparison of the BMD results at the femoral neck to a population of the same sex and same age as the patient, could also raise suspicion of osteoporosis [357]. The WHO FRAX® [359, 360], the fracture assessment tool, is using the T-score at the femoral site, along with clinical risk factors to predict the 10 year probability of an individual to sustain an osteoporotic fracture. It has been calibrated and validated for Canada [214, 359]. In the 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada, BMD should be measured in patients over the age of 50 y, if they present certain risk factors such as fragility fracture, prolonged glucocorticoid use, low weight, current smoking or high alcohol intake [214]. Peripheral DXA can also measure appendicular sites such as wrists or heal [356]. This measure is not used in the assessment of the risk of osteoporotic fractures. Another photon absorption technique, the quantitative computed tomography (QCT) is a densitometry measurement providing a three-dimensional measurement of bone density. Also known as volumetric bone density (vBMD), it permits the differentiation of trabecular and cortical bone. It is measured in mg/cm^3. The localization of the region of interest for assessment of vBMD is done with a scout view and a 8 to 10 mm slice of selected bone is measured [356]. In 2011, the International Society for Clinical Densitometry (ISCD) has stated that QCT of the hip was providing comparable information to the DXA measurements and that the analysis of the total femur trabecular BMD by QCT can predict hip fractures, but not vertebral fractures, as well as

DXA in post-menopausal women. However, QCT presents a 50 to 300 times more radiation exposure [361]. Peripheral QCT (pQCT) can provide vBMD of distal bones such as the radius and the tibia. The radiation exposure in pQCT is similar to DXA [357]. In the very old population living in LTC facilities, BMD measurement could be challenging as mobility and accessibility to equipment might be difficult. Peripheral measures are an avenue that should be considered in research to better document BMD in this population.

BRIDGE 1

Vitamin D has predominantly been studied in aging women with several meta-analyses presenting only 30% of the pooled participants being males [21]. In looking at bone health, important work has been done with Canadian [321-326] and American older cohorts [85, 327-329] living at home. In contrast, the proposed research is aims to bridge the knowledge gap and is based on the advanced aging male population of veterans living in Ste-Anne's Hospital, a long-term care facility located Ste-Anne de Bellevue (QC). Being 97% male, this group of veterans provides a pool of very old males with an average age of 88 years and a unique opportunity to look at vitamin D intakes and relationship to vitamin D status. Given the paucity of data in this clientele, it is required to assess the vitamin D and calcium intake prior to the elaboration of a randomized controlled trial. Furthermore, it is essential to document if a seasonal effect would be seen in bone and mineral metabolism. Access to densitometry evaluations is limited in LTC facilities and the measurement of bone biomarkers as surrogate measures of bone health would be clinically relevant. As a number of bone biomarkers could be used to monitor bone health and that limited data is published to determine pertinence and correlation with vitamin D intake, the study of these markers in this very old population will bridge an important knowledge gap.

CHAPTER 3 – MANUSCRIPT 1

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Title: Insufficient vitamin D intake and low vitamin d status in men over 80 y of age: intervention is required to meet dietary targets in long-term care facilities

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3.1 Abstract

Introduction: Vitamin D is important to bone health. This study examined vitamin D intake and status in institutionalized elderly men in relation to biomarkers of bone metabolism and functional indicators.

Materials and Methods: Elderly male veterans were studied in Phase I (n=40) for 16 weeks (April, June, August 2008) and Phase II (n=30) for another 16 weeks (October and December 2008 and February 2009) for dietary vitamin D using 5 day menu selection (Phase I) and using 3 x 3-d weighed food records (Phase II). Anthropometric data, Mini-Mental State Evaluation (MMSE) scores and sun exposure were collected. Functional capacity was assessed using the Frail Elderly Functional Assessment Tool (FEFA) and handgrip strength. Biochemistry included serum 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), osteocalcin (OC) and C-terminal telopeptides of Type 1 collagen (CTX). Mixed model ANOVA and Pearson correlations analyses were used.

Results: Participants were relatively healthy (Age: 85±3 y (Mean ±SD), BMI: 26.1±4.3 kg/m^2, MMSE: 25±5, FEFA: 13±8, handgrip strength: 22±8 kg). Sixty-six percent (280±120 IU) of the planned dietary vitamin D was consumed. Vitamin D came mainly from fortified milk and meal supplements and 33% took pill supplements (400-800 IU/d). Serum 25(OH)D concentration rose by summer (Phase I: 60.9±24.4, 68.2±24.6 and 76.1±22.4 nmol/L, respectively) and declined thereafter (Phase II: 57.7±24.1, 62.9±30.7 and 61.3±29.2 nmol/L). PTH was lower in spring compared to late summer through winter whereas CTX and OC did not change. Serum 25(OH)D was correlated to BMI, but not to indicators of functional status.

Conclusions: In long-term care, vitamin D from foods and supplements fails to meet recommendations of 800 IU (20 μg) for those over 70 y.

3.2 Introduction

Aging is associated with reduced bone health, reduced mobility and increased need for help in accomplishing activities of daily living [47]. Vitamin D is considered an important nutrient for its role in bone health with well-known consequences of deficiency leading to osteomalacia and osteoporosis in the elderly [362]. It is predicted that in 2025, more than 20% of Canadians will be over the age of 65 y [47]. These statistics are very similar to other countries. For example, by 2040, 21 % of the population will be aged 65 years or older in the United States [363] and will represent 36.1% of the population in Japan [364].

For most people, values of 25-hydroxyvitamin D (25(OH)D) above 50 nmol/L are sufficient to maintain bone health [21]. However, low vitamin D status demarked by circulating 25(OH)D below 50 nmol/L has been reported in community dwelling [226, 365-367] and institutionalized elderly [61, 62, 337]. In the community, a wide range of values have been reported. A large cohort study looking at bone health in American men reported blood levels of 25(OH)D to be 62.5 ± 19.8 nmol/L with 2.9% below the deficiency cut-off of 30 nmol/L [85]. The Canadian Health Measures Survey reported that men over the age of 70 years presented with 25(OH)D values of 71 ± 27.5 nmol/L with 10% of them below 37.5 nmol/L [368]. More recently, elderly men living in the community in Australia were reported to have levels of 25(OH)D as low as 42 nmol/L [369]. Less is known about those in institutions. In long-term care facilities where 25(OH)D was assessed, values ranging from 26 to 40 nmol/L were seen despite a supervised environment. Low status may be ascribed to vitamin D intakes below recommendations [61, 63, 64, 66, 68, 88, 337], in some cases as low as 120 IU/d. Such intakes are exceptionally low in view of the newly updated recommendations by Institute of Medicine (IOM) Dietary Reference Intakes (DRI) for Calcium and Vitamin D [21]. The IOM modified the recommendation from an

Adequate Intake (AI) to now include Estimated Average Requirements (EAR) and Recommended Dietary Allowances (RDA). For adults of >70 year, a daily EAR of 400 IU (10 μg) and RDA of 800 IU (20 μg) were set in comparison to the previous AI of 600 IU (15 μg).

Vitamin D intake and serum 25(OH)D levels have been reported to be both below dietary and status targets in institutionalized elderly populations of women and men. This deserves further attention since better 25(OH)D status has been associated with better leg strength and function [370], handgrip strength [371], general physical activity and daily living activity [338, 372]. Furthermore, low serum concentration of 25(OH)D has been identified as a risk factor for long-term care facility admission [373]. Moreover, bone turnover is a constant physiological phenomenon and although aging is associated with higher resorption than formation, vitamin D should be provided in sufficient amounts to ensure normal parathyroid hormone (PTH) concentrations and reduce associated morbidity and mortality risks [374, 375]. Nonetheless, information specifically regarding very old males (aged 80+) is scarce and impacts on biomarkers of bone metabolism are not as well studied.

The primary objective of this 1 year prospective cohort study was to evaluate vitamin D intake and 25(OH)D concentration in a long-term care population of elderly male veterans (aged 80+y) across all seasons. Our secondary objective was to track changes in biomarkers of bone metabolism including PTH, osteocalcin and C-terminal telopeptides of Type 1 collagen (CTX) over the year as well as changes in Frail Elderly Functional Assessment (FEFA) and the Mini-mental State (MMSE) tool scores in association with vitamin D status. It was hypothesized that all participants would present with low serum 25(OH)D (< 50 nmol/L) regardless of sampling time, that total intake of vitamin D would be below recommendations and that 25(OH)D concentration would be significantly related to functional tests of daily living.

3.3 Methods

This prospective observational cohort study was conducted in elderly males living at Ste-Anne's Hospital, a long-term care facility (Veterans Affairs Canada, Montreal, QC; 46°N). Phase I took place from spring to summer (16 weeks; n=40) whereas Phase II captured the fall to winter period (16 weeks) for 30 of the 40 original participants. Of the 10 participants who did not continue in Phase II, 4 died, 3 had a significant cognitive decline and the other 2 did not wish to participate. In view of the study objectives, all veterans over the age of 70 years were eligible including those with stable chronic diseases, receiving oral and enteral feeding modes. There were 8 exclusion criteria: 1) end-stage (ie prognosis of less than 4 months) conditions and palliative care, 2) end-stage renal disease due to altered vitamin D metabolism, 3) use of vitamin D analogues, 4) end-stage liver disease, 5) untreated hyperparathyroidism, 6) active cancers, 7) metabolic bone diseases except for osteoporosis and osteomalacia and, 8) any acute condition that would exclude any oral intake of food. The completion of the 3 month study phase or, change in clinical status preventing continuance in the study was considered the end-point (Figure 3.1). The study was approved by the Institutional Review Board of the Faculty of Medicine of McGill University and Ste-Anne's Hospital Scientific Board. Consent forms stated voluntary participation, right to withdraw at any time without consequences and respect of privacy. Competency to consent was validated via medical records or confirmed with the treating physician. When competent, the patient signed the consent form for himself. If participant was unfit to consent, the legal representative (mandatary, curator or tutor) signed, as per required by Article 21 of Quebec Civil Code.

Participants were measured for weight using a standard balance, calibrated yearly at the hospital. Height was obtained from the military medical chart and confirmed using knee-height

measuring caliper (Seca 207 model, Seca Corp, MD, US) using algorithms adjusted for age and sex calculations, then body mass index (BMI; kg/m^2) was calculated. A nurse met with participants to complete a MMSE at baseline (unless the medical chart had a MMSE score dated less than 3 months prior to the study) and at the end of each phase of the study. The FEFA scores were obtained at midway and final assessments. Handgrip strength (Hydraulic hand dynamometer, Jamar®) was performed at midway and final assessments of Phase II (Fall-Winter). Subjects were instructed to squeeze the handle as hard as they could and were encouraged for 20 seconds each trial. The maximum reading of each trial was recorded. Measurements were done in triplicates, using the non dominant arm and average values were used. The time used to read and record the data (approximately 15 sec.) was used as a rest period.

In both segments of the study, fasted blood samples were obtained every 8 weeks, between 0630 and 0730 h. Routine biochemistry were immediately measured at the hospital using Vitros 250E (Ortho Clinical Diagnostics, Johnson & Johnson, version 250) and Symex XT-2000i (Sysmex, version XT-2000i/XT-1880i) auto analyzers. This laboratory participates in the ISO (Norm 15189 – Medical Laboratories) quality assurance program. Sample aliquots were stored at – 80°C until further testing at McGill. Serum total 25(OH)D, PTH, OC were measured using chemiluminescent immunoassays (Liaison; DiaSorin, Minnesota) and serum CTX by colorimetric immunoassay (IDS Inc, Arizona). This laboratory participates in the DEQAS (Vitamin D External Quality Assessment Scheme) program and consistently reports results within 25% of the ALTM (All-Laboratory Trimmed Mean). Controls were in range with specifications of each assay. Intra-assay variability ranges were 0.1% - 8.1% for 25(OH)D,

0.18% - 12.4% for PTH, 0% - 5.8% for OC and 0% - 14.8% for CTX. Inter-assay variability ranges were 5.3% - 15.8% for 25(OH)D, 1% - 7.7% for PTH and 1.8% - 7.3% for OC.

Main food sources of vitamin D were examined in Phase I using 5 days of hospital menus at each time point, for all participants. In Phase II, actual food intake was assessed using weighed food records for 3 non consecutive days, including a weekend day, in October, December and February. All foods were weighed before being served and leftover weights were deducted to obtain actual intake. For Phase II, a database including all detailed recipes cooked in the Production Center of the Hospital was used to determine nutritional composition. The Canadian Nutrient File 2007b and menu management software ProMenu was used to generate nutrient intakes to reflect the period of study. Nutritional values of market foods were included to complete the missing nutritional values of certain items. Intakes were compared to the various DRI values.

3.4 Statistical Analysis

Continuous variables were expressed as means ±SD or median (range), if non-normally distributed. Categorical variables were expressed as n (%). All data were checked for normality using D'Agostino & Pearson omnibus normality test; when normality criteria were not met, data were log transformed or a nonparametric test was used. Levene's test was used to determine homogeneity of variances. The relationships among time and vitamin D intake with 25(OH)D, PTH, OC and CTX were assessed using a mixed model ANOVA, controlling for random effect of age. Tukey-Kramer was used as post hoc test. Relationships between 25(OH)D and other measures were tested using Pearson correlation analyses. Statistical significance was set at $p \leq$

0.05, and all p values presented are 2 tailed. Data were analyzed using Statistical Analysis System, version 9.2, statistical software (SAS Institute Inc., Cary, N.C.).

3.5 Results

In general, the participants were of healthy body weight for age, in good mental status and had routine serum biochemistry within the normal range (Tables 3.1 and 3.2). Thirty-three percent of participants were receiving a supplement containing vitamin D (n=6 received 400 IU/d, n=2 received 600 IU/d and n=2 received 800 IU/d). Sunlight exposure was minimal for most participants due to limited outdoor activities or because hats, long sleeves and pants were worn regularly. From April to August, 73% (29) of participants were able to go outside for an average period of 41 min per day (Range: 2-180 min/d). Thirteen participants were less than 15 min. outdoors every day. Eighty percent (80%) wore hats, 53% wore long sleeves and 75% wore long pants. Although ultraviolet beta (UVB) radiation is minimal in the fall to winter months, sunlight exposure was observed. During that period, only 21.4% participants went outdoors, and all of them wore hats, long sleeves and long pants.

In Phase I, the analysis of the foods identified as the main sources of vitamin D provided on the hospital tray (average of 3 x 5 days of proposed menus) revealed a mean dietary vitamin D of 240±160 IU/d. In Phase II, the nutrient intake was obtained via average weight of all foods of 9 days of intake (3 days at baseline, 3 days at midway and 3 days at final assessments) using detailed recipes. No difference was seen among days, therefore the mean of 9 days of intake was used (Table 3.4). The diet was well balanced for macronutrients. The vitamin D content of all foods served was 440±200 IU/d, however, only 66% of that was consumed. At the time of the study, the recommendation for vitamin D was an AI set at 600 IU revealing that the assessed

menu could not met these recommendations with food and meal supplements alone. In Phase II, actual intakes were compared to DRI values; only 1 participant (3%) exceeded the AI of 600 IU with food and meal supplements alone in October, December and February. Similarly, when comparing to the 2011 revised DRIs, only 1 participant (3%) reached the new 800 IU/d RDA value with food alone (February only). Three participants (10%) met the EAR of 400 IU in October and December whereas 4 participants (14%) met the EAR in February with food and meal supplements alone. The main sources of dietary vitamin D (52%) were fortified milk, enriched meal supplements and milk-based soups and margarine.

In Phase II, only 33% of participants were receiving vitamin D from tablets to enhance their exogenous intake, providing an average additional 530±160 IU/d. Pill supplement dosages ranged from 400 IU/d to 800 IU/d. When considering actual food-derived vitamin D intakes and the additional intake of vitamin D from pill supplementation, 27% (8/30) of participants met the AI of 600 IU/d in October and December whereas 24% (7/29) met the AI in February. With the revised IOM values, 40% (12/30) of participants met the EAR value at every time points. The RDA was met by13% (4/30) of participants and by 10% (3/30) in October and February.

In Phase I of this prospective study, mean serum 25(OH)D concentrations were above 50 nmol/L in April, June and August (Table 3.3) for this elderly institutionalized population. The proportion of participants presenting with 25(OH)D concentrations above 50 nmol/L increased from 72.5% in April to 77.5% in June and 88.9% in August. In April, 10% of the participants were deficient (<30 nmol/L). However, in June and August no participant was deficient (Figure 3.2). In Phase II, the proportions of participants presenting with 25(OH)D concentration above 50 nmol/L were 60.0% in October, 65.5% in December and 67.9% in February. Deficiency was

observed in 10.0%, 10.3% and 17.9% of the population in October, December and February, respectively.

Biomarkers of bone metabolism over a year are shown in Table 3.3. In the months of April, June and August, mean 25(OH)D, PTH and OC concentrations were within their respective normal ranges. During the fall to winter segment of the study PTH levels were indicative of secondary hyperparathyroidism. Although 25(OH)D and PTH concentrations fluctuated over time, CTX and OC did not show significant changes throughout this 1 year follow up study, reflecting a lack of change in bone turnover during the year.

In Phase I, PTH and OC demonstrated a positive correlation (Pearson r = 0.32; p=0.044) only in April whereas 25(OH)D was negatively correlated to PTH (Pearson r = -0.398 p=0.016) in the month of August. Body mass index was correlated with 25(OH)D concentrations in April (Pearson r = - 0.32; p=0.019). No other relationships were observed in this phase.

In the fall to winter period (Phase II), where 25(OH)D was at its minimal value, 25(OH)D was negatively correlated with PTH in October, December and February (Pearson r = -0.584; p=0.001, Pearson r = -0.519; p=0.004 and Pearson r = -0.440; p=0.019 respectively). CTX was positively correlated to OC at all 3 time points assessed in Phase II (Pearson r = 0.546; p=0.002, Pearson r = 0.523; p=0.004 and Pearson r = 0.636; p=0.001, respectively). Correlations between 25(OH)D and biomarkers of bone health and function in February, when vitamin D status is dependent on exogenous sources, are presented in Figure 3.3. No other relationships were observed among bone biomarkers.

No correlations were seen between serum 25(OH)D concentration and handgrip strength, MMSE nor FEFA scores. However, the FEFA scores were negatively correlated to the handgrip

strength (Pearson r = - 0.43; p=0.034) in February demonstrating that handgrip strength is a reflection of functional mobility and the upper body strength is required for adequate daily activity. Vitamin D intake in Phase II was positively correlated to energy intake, protein, calcium, phosphorus and potassium intakes ($p<0.010$), but was not correlated to 25(OH)D concentrations.

Table 3.1**:** Characteristics of participants (Mean ±SD)

	Phase I (n=40)		Phase II (n=30)	
	Mean*	SD	Mean*	SD
Age, y	85.2	3.2	84.9	3.6
Weight, kg	76.0	12.7	74.7	13.2
BMI, kg/m^2	26.1	4.1	26.0	4.3
MMSE (/30)	23.0	7.0	24.0	3.0
FEFA (/55)	13.0	8.0	13.0	8.0
Number of prescriptions**	11.0	5.0	11.0	5.0
Handgrip (kg)	N/A		10.0	3.6

* Stable over time, within phases. ** Including vitamin tablet supplements.

FEFA: Frail Elderly Functional Assessment; MMSE: Mini Mental State Evaluation;

N/A: not assessed.

Table 3.2: Serum biochemistry of participants (Mean ±SD)

	Normal Range[1]	Phase I (n=40)		Phase II (n=30)	
		Mean*	SD	Mean*	SD
Glucose (nmol/L)	4.1 - 5.9	5.2	1.1	5.2	0.9
Albumin (g/L)	35 - 50	35	4	36	4
Phosphate (mmol/L)	0.81 - 1.45	1.14	0.17	1.20	0.16
Total calcium (mmol/L)	2.10 - 2.55	2.27	0.09	2.23	0.08
Ionized calcium (mmol/L)	0.95 - 1.15	1.04	0.05	1.03	0.04
Total cholesterol (mmol/L)**	0 - 6.2	4.1	0.9	4.3	0.8
Triglycerides (mmol/L)**	0 - 2.26	1.58	0.70	1.59	0.81

[1]References values of Ste-Anne's Hospital Laboratory.

* Stable over time, within each phase;

** Total cholesterol and triglycerides were only obtained at baseline and final assessments in Phase I and in Phase II.

Table 3.3: Biomarkers of bone metabolism in elderly veterans living in long-term care facility (Mean ±SD)

	Normal Range		Serum 25(OH)D* (> 50 nmol/L)		Serum 1-84 PTH (1.1 – 7.5 pmol/L)		Serum OC (2.4 – 7.9 nmol/L)		Serum CTX (ng/L) ----	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
	Month	n					Phase I			
Baseline	April	40	60.9[ac]	24.4	6.4[a]	3.3	5.1	2.8	941	533
Midway	June	40	68.2[a]	24.6	7.4[ad]	6.6	4.6	2.2	915	529
Final	August	36	76.1[b]	22.4	7.5[b]	3.2	5.2	3.2	828	419
		n					Phase II			
Baseline	October	30	57.7[c]	24.1	7.9[bd]	3.2	5.0	2.8	834	379
Midway	December	29	62.9[ac]	30.7	10.4[cd]	4.6	4.8	3.3	899	458
Final	February	28	61.3[c]	29.2	8.4[b]	3.7	5.2	3.0	920	593

Means followed by different superscript lowercase letters, within columns, differ ($p<.05$), mixed model ANOVA, controlling for random effect of age and using Tukey-Kramer adjustment for multiple comparisons. Values were log transformed for statistical analyses, but are presented in original units.

* Minimal ultraviolet beta radiation in the fall to winter period.

25(OH)D: 25-hydroxyvitamin D; PTH: parathyroid hormone; OC: osteocalcin; CTX: C-terminal telopeptides of Type 1 collagen.

Table 3.4: Average daily intake in elderly living in long-term care facility

(Phase II - 9 days; Mean ±SD)

Intake per day	DRI[1]	Provided on Tray		Consumed	
		Mean	SD	Mean	SD
Energy, kcal	3067[2]	2522	547	1744	351
Protein, g (%Energy)	56 (10-35%)	102	23 (16%)	69	16 (15%)
CHO, g (%Energy)	130 (45-65%)	333	76 (51%)	233	56 (51%)
Fiber, g	30 g*	22	8	14	6
Fat, g (%Energy)	--- (20-35%)	90	24 (31%)	62	15 (31%)
Vitamin D, IU[4]	800 IU[3]	440	200	280	120
Calcium, mg	1200 mg[3]	1488	632	1013	424
Phosphorus, mg	700 mg	1837	569	1255	376
Sodium, mg	1200 mg*	3936	939	2512	653
Potassium, mg	4700 mg*	4010	969	2643	736

[1]Values are RDA; when followed by (*) values represent Adequate Intake (AI).

[2] Estimated Energy Requirement (EER): For males, subtract 10 kcal/d for each year above 19.

[3] New DRI values (IOM, 2011): RDA: Vitamin D, 800 IU and Calcium, 1200 mg;

EAR: Vitamin D, 400 IU and Calcium, 1000 mg.

[4] Does not include pill supplement.

Figure 3.1: Recruitment for Phase I and Phase II

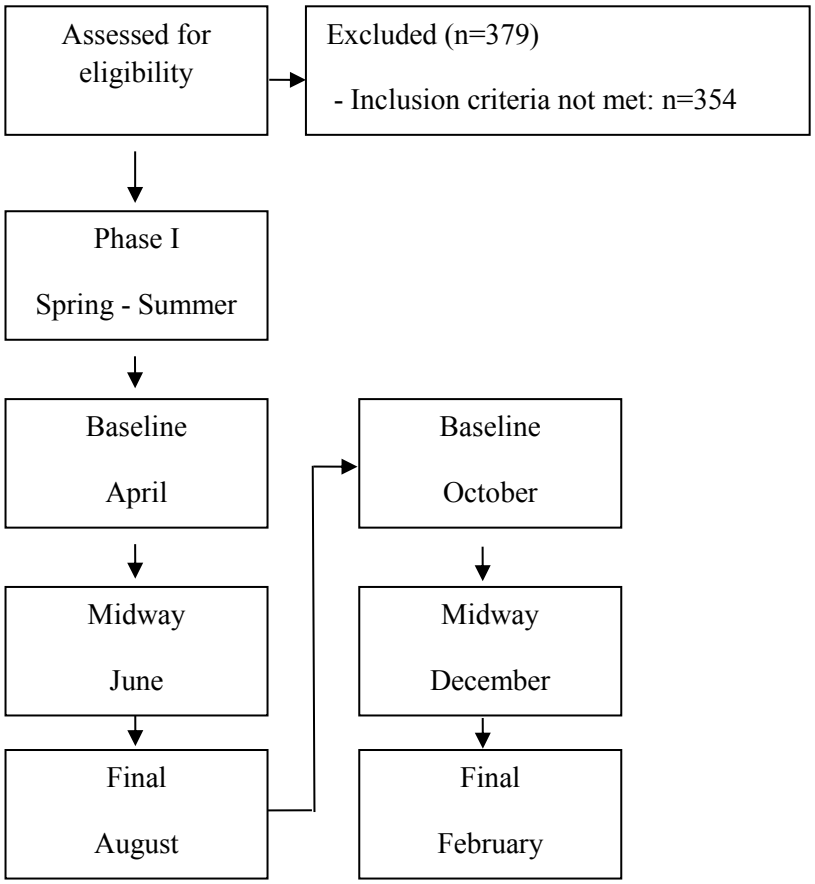


Figure 3.2: Proportion (%, CI 95%) of participants according to 25(OH)D concentration during 1 year follow-up

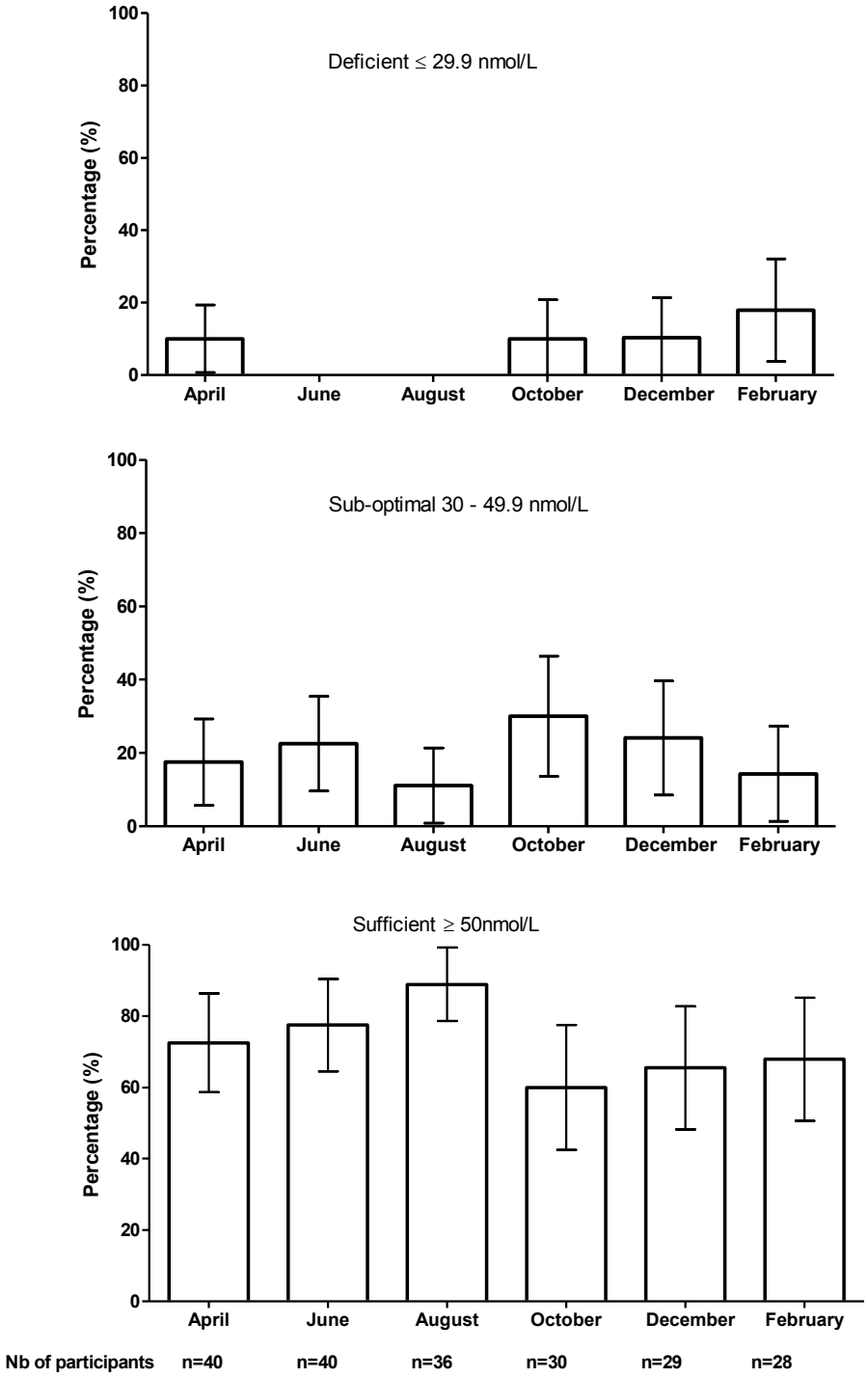
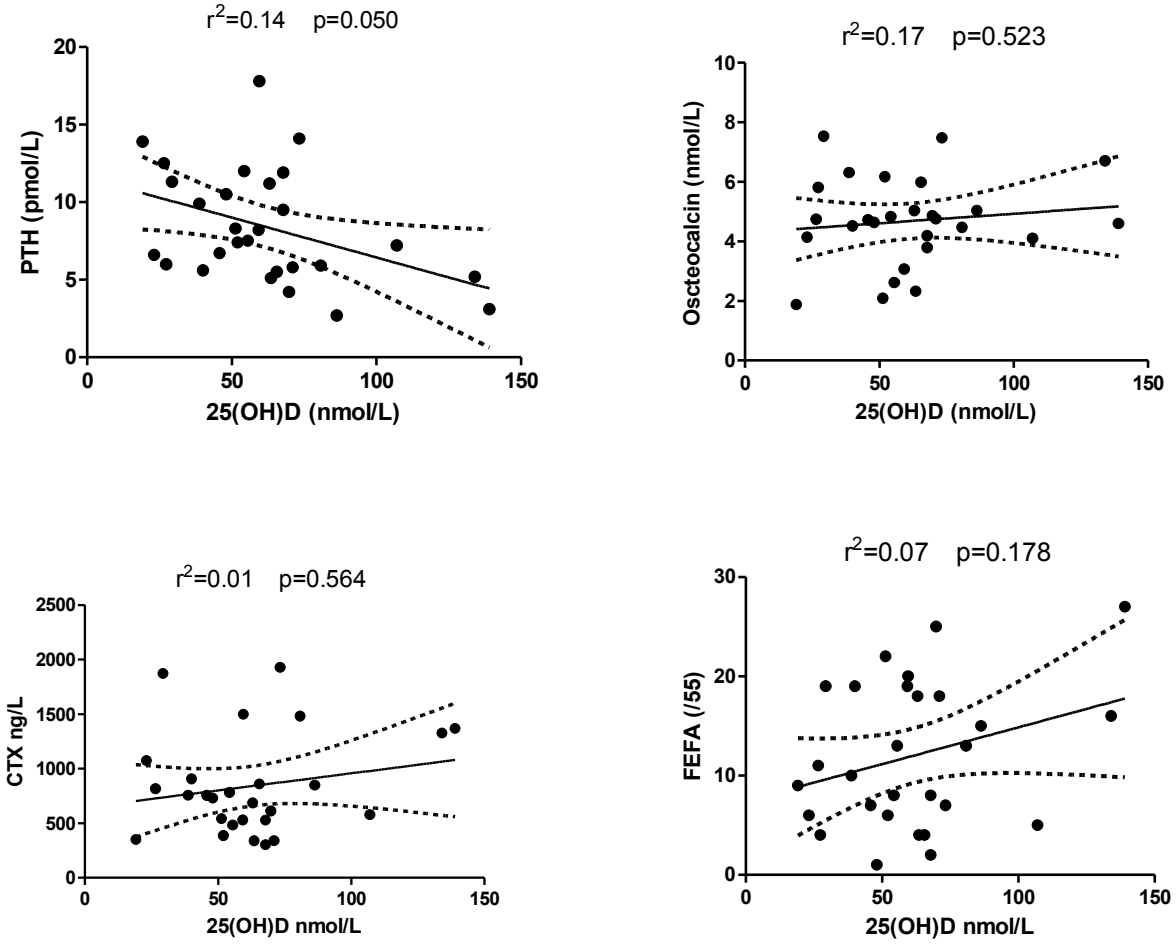


Figure 3.3: Linear regression between 25(OH)D and PTH, OC, CTX and FEFA in February, when vitamin D status is dependent on exogenous sources



Values were log transformed for statistical analyses for 25(OH)D, PTH, OC and CTX, but are presented in original units.

3.6 Discussion

This study was carried out in an elderly cohort of males living in a long-term care facility of Montreal (QC, Canada; 46°N). Despite their advanced aged, the biological markers revealed a healthy population presenting with normal values for glucose, albumin, phosphate, total and ionized calcium as well as for total cholesterol and triglycerides. Participants maintained BMI, cognitive and functional status and number of medications taken over the year of study. The average serum concentrations of 25(OH)D were relatively good during the summer months in this elderly cohort when compared to the targets (25(OH)D >50 nmol/L) recently set by the IOM [21]. Only 34.5% and 32.1% were below 50 nmol/L of 25(OH)D in December and February when 25(OH)D is dependent on exogenous sources alone. These observations concur with other reports in nursing home facilities [61, 62, 337]. However, this report is the first to provide a yearly profile of 25(OH)D status in institutionalized elderly men over 80 y as well as rigorous dietary assessment. Despite limited direct exposure to UVB, a small seasonal effect was observed in June and August where no participant presented 25(OH)D values below 30 nmol/L as observed in other studies of elderly [62, 365, 367].

Although Canada's Food Guide (Health Canada) suggests that 400 IU (10 μg) of vitamin D be taken as a supplement by individuals > 50 y, only 33% of participants were receiving supplements at the time of the study. Thus the majority of vitamin D intake was from diet. In Phase II, nutritional intake over time was stable and confirms the presence of a routine regarding food preferences. These results complement previous observations revealing low average intake of vitamin D in the elderly living in a Canadian long-term care facility [61, 63, 66, 376], but provides new knowledge regarding food sources and status of vitamin D across a year. The main sources of vitamin D for this cohort were fortified milk, meal supplements and vitamin from

tablets. Only one participant met the AI value of 600 IU, which prevailed at the time of the study, with food and meal supplements alone. With the new DRI values, 10 to 14% of participants reached the EAR with food and meal supplements. However, the actual intake from food was sufficient to meet the RDA for one participant in the month of December only.

The implications of not meeting recommended intakes of vitamin D extend beyond vitamin D status alone. In this study, PTH was elevated often throughout the course of the study but was only significantly elevated during the early winter segment of the study. As in the younger adult, PTH is known to increase with declining 25(OH)D status [377]. However, hyperparathyroidism is also associated with higher morbidity and mortality in the elderly [57, 374, 375]. OC and CTX did not significantly change over time in this group. This aligns with the modest changes in PTH and that the values were on average elevated for the majority of the year.

Other possible functional indicators of vitamin D status were explored in this study such as handgrip strength and function in daily living tasks. Contrary to previous observations in community-dwelling seniors with vitamin D deficiency, the FEFA [338] did not associate well with vitamin D intake or vitamin D status. It is possible that the 25(OH)D concentrations observed in our cohort were above a threshold for influencing functionality. Vitamin D status above 50 nmol/L compared to lower status positively associates with physical performance, handgrip strength in community dwelling elderly [378] as well as with functional capacity [338]. However, it is negatively associated with frailty [379, 380]. Dose response studies are required to clarify this association and determine if a threshold exists as related to optimal performance [21].

Although this study is a comprehensive look at vitamin D nutrition in elderly men living in a long-term care facility, the fact that it is entirely composed of elderly veterans and is of small

sample size might hinder the extrapolation of our results to other elderly. It is also possible that participants ate more since they were aware that meal intake was documented, therefore increasing vitamin D intake. However, the similar BMI in both study phases and only 66% of vitamin D consumed suggests this was not the situation.

In summary, this study provided detailed data on food intake, vitamin D status and bone biomarkers for relatively healthy, well monitored, very old male veterans living in a long-term care facility over a year. This study underscores the importance of not only planning intakes to meet needs, but to observe actual food intakes since only 66% of the food was consumed. In this advanced aged population, vitamin D intake was positively correlated with vitamin D status during winter months. The main contributors of vitamin D in the diet of this long-term care facility were vitamin D fortified milk and meal supplements and tablet supplements. The newly published DRI values for vitamin D in the healthy population above 70 y have changed from an AI value of 600 IU (15 μg) to an EAR of 400 IU (10 μg) and RDA of 800 IU (20 μg) [21]. Reaching these recommendations with foods or meal supplements alone will be a challenge in the institutionalized elderly. This study adds to the mounting evidence of insufficient intake of vitamin D by food and meal supplements alone as well as 25(OH)D concentration values below 30 nmol/L for up to 18% of this population in the winter months. Although vitamin D status was sufficient for a majority of participants, PTH was elevated and above the normal range in the fall and winter months suggesting that higher intakes could be beneficial. Future research should thus provide information including data on calcium and bone metabolism in elderly individuals living in long-term care facilities within higher vitamin D status ranges. Vitamin D dose-response studies in the elderly population should also provide information to reduce the knowledge gap in

elderly males with regard to benefits of achieving vitamin D recommendations on health outcomes other than bone health.

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BRIDGE 2

Previous work has documented poor intake of vitamin D and calcium as well as poor vitamin D status in the elderly living in long-term care (LTC) facilities in Canada. The basis of the Institute of Medicine updated DRIs for vitamin D is that providing sufficient vitamin D to reach 50 nmol/L is important to bone health, and that a number of side effects could occur if vitamin D is consumed in too large of an amount. However, the Endocrinology Society stated that a level of 75 nmol/L of vitamin D was necessary to achieve the skeletal and non skeletal benefits of vitamin D. Information regarding vitamin D metabolism is scarce in the old population living in LTC facilities. Randomized controlled trials are required to assess the impact of various levels of vitamin D supplementation on vitamin D status along with the impact of vitamin D status on other related biomarkers such as calcium, PTH, ionized calcium and phosphate, in order for these very old men with the ultimate objective to maintain bone health. The following chapter is an analysis of the impact of 4 amounts of vitamin D (2000 IU/d for 8 weeks and 0 IU/d, 500 IU/d and 1000 IU/d for 24 weeks) to achieve and maintain serum 25(OH)D in elderly men in LTC using a novel loading phase followed by a dose-response phase. This design was not only unique but required as dose-response studies in the very old are lacking. Had only one dose been tested, the contribution to the advancement of knowledge would have been limited. Furthermore, as quality of life is key in the LTC facility clinical management of this clientele, the use of newly fortified foods is also an innovative treatment avenue. The acceptance of these foods was tested to further reinforce the importance of striving to maintenance of quality of life even in this advanced age setting.

CHAPTER 4 – MANUSCRIPT 2

Title: Impact of vitamin D_3 supplementation on maintaining 25-hydroxyvitamin D in men over 80 y of age living in a long-term care facility: a randomized placebo-controlled trial of 3 doses of vitamin D_3

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Clinical Trial Registry number and website: This research project was registered under the identification number NCT01437696 with ClinicalTrials.gov (www.clintrial.gov)

4.1 Abstract

Introduction: Falls and fractures are major concerns in long-term care (LTC) facilities. Vitamin D is considered a key nutrient for bone health. This study assessed the impact of 2000 IU/d of vitamin D_3 on serum 25-hydroxyvitamin D (25(OH)D) as well as bone biomarkers and the sufficient maintenance dose.

Materials and Methods: Men (>80 y) from Ste-Anne's Hospital were included in a 2 phase study where they first received 2000 IU/d of vitamin D_3 (Run In period) in the form of tablets for the first 8 weeks. For the second segment, the participants were randomized in a single-blind fashion to one of 3 groups following a 1:1:1 ratio: Placebo 0 IU/d group, 500 IU/d group of vitamin D_3 and 1000 IU/d of vitamin D_3 group for 24 weeks. Anthropometric data and Disease Activity Score (DAS28) values were collected. Dietary intakes were measured every 8 weeks and consumption of fortified foods was ascertained by weekly weighed food waste. Biochemistry included serum 25(OH)D and parathyroid hormone (PTH), every 4 weeks.

Results: Participants were 89.9±3.3 y of age and had a Body Mass Index (BMI) of 26.3±4.6 kg/m^2. Mean dietary intakes reported were 1700 kcal/d where 15.5% of energy was provided by proteins. At Wk -8, vitamin D and calcium did not meet Dietary Reference Intakes (DRIs) for adults of > 70 y of age. Dietary intake of calcium did not vary over time. Mean serum values of 25(OH)D was 56.1±13.8 nmol/L for the entire cohort. All groups increased and reached an overall serum 25(OH)D concentration of 68.2±12.2 nmol/L. Serum PTH, calcium, ionized calcium and phosphate remained within normal ranges throughout the course of the 32 weeks.

Conclusions: A Run In period of 8 weeks increased serum vitamin D_3 concentrations > 65 nmol/L. The well appreciated fortified foods, containing either 500 IU/d or 1000 IU/d of vitamin D_3, were successful at maintaining 25(OH)D concentrations >75 nmol/L in older men in LTC.

4.2 Introduction

The 2011 Canadian Census reported that 7.1% of all seniors over the age of 65 y resided in nursing homes, residences for seniors, chronic care hospitals or long-term care (LTC) facilities. Nearly 30% of these seniors are 85 y and over [48]. In 2014, there were an estimated 1,369,700 Americans residing in nursing homes and 41.6% were 85 y and over [236]. Bone health is a major concern in LTC facilities. The risk of hip fractures is 1.8 times higher in LTC facilities than in the community [237] with crude hip fracture rates (person-year) of 32.1 per 100 men and 24.4 per 100 women over the age of 85 y. Vitamin D_3 is regarded as important for bone health and healthy aging [214, 220, 381] and specific guidelines for bone health for LTC residents are emerging [219, 382]. However, meta-analyses providing conflicting results regarding the impact of vitamin D in prevention of fractures and achievement of bone health [1-3, 5, 6, 10, 11, 16, 19, 49, 222, 381]. Frail seniors from the LTC population are often under-represented in trials on bone health, osteoporosis and fracture prevention [1, 5, 6, 11, 238].

It is incorrectly presumed that health care and nutritional intake is optimized in LTC facilities. Vitamin D intakes in LTC facilities range from 104 to 295 IU/d [62-64, 66-68, 240, 241] and thus, are well below recommendations of 400 to 800 IU/d [382]. Lam et al. designed super-menus aiming at meeting Recommended Dietary Allowances (RDA) for micronutrients. They were successful, with 3 exceptions: potassium and vitamin E and D [65]. It is a challenge to provide sufficient vitamin D by food alone in LTC environment. However, using multivitamin powder to fortify pureed foods Adolphe et al. [61] achieved a mean intake of 488±132 IU of vitamin D by the end of the study; close to the Estimated Average Requirement (EAR) value of 400 IU/d, but well below the RDA of 800 IU/d. These studies highlight the difficulty in providing sufficient vitamin D by food alone in LTC.

Supplementation is often necessary to meet recommended intakes and attain 25-hydroxyvitamin D (25(OH)D) concentrations to support bone health in aging [383-386]. This is exemplified by a study of LTC residents (83.2±8.7 y, 31% male) where 49% were prescribed supplements [386]. In this retrospective chart review, serum 25(OH)D concentrations followed a dose-response to supplemental intakes of ≤ 400 IU/d (72.8±22.2 nmol/L), 401-800 IU/d (98.9±26.3 nmol/L) and > 800 IU/d (96.0±26.2 nmol/L). Despite this evidence and the recommendations for older adults [21, 49], prescription of vitamin and mineral supplement for fracture prevention in LTC facilities can be as low as 31.3 % for vitamin D and 26.2 % for calcium [249, 387, 388]. Furthermore, practitioners in LTC facilities are not consistent in supplementation practices [389]. As an alternative to supplements, a trial using vitamin D fortified pureed foods (400 IU vitamin D/d), elevated serum 25(OH)D by 25 nmol/L in LTC residents [61], suggesting that diet can enhance intakes effectively.

With the exception of two publications focusing on older men in LTC facilities [63, 385], important knowledge gaps exist for the older male population in LTC regarding vitamin D intakes and status. Canadian clinical practice guidelines [382] in LTC state that individuals at high risk of vitamin D deficiency can safely use supplements of 800 to 2000 IU/d. To the authors' knowledge, no placebo-controlled RCT in LTC facilities exists on the vitamin D requirements needed to maintain serum 25(OH)D concentration above 75 nmol/L for very old men. Therefore, this LTC study was designed to first improve vitamin D status in men ≥ 80 y of age through an 8-wk run-in period using a tablet-supplementation approach (2000 IU/d), followed by a 24-wk randomized placebo-controlled dose-response trial of specially formulated foods containing 0 IU (Placebo), 500 IU or 1000 IU of vitamin D_3 to test for the dosage required

to maintain 25(OH)D status in a healthy range. Secondly, we aimed to assess acceptability and preference for the specially fortified foods as opposed to tablets supplementation.

4.3 Methods

4.3.1 Participants

During the month of October 2011, male Veterans living at Ste-Anne's Hospital, a LTC facility in Québec (Veterans Affairs Canada, 46°N) were invited to participate in a study lasting 32 weeks. Excluded criteria were: serum 25(OH)D > 75 nmol/L, end-stage renal disease (calculated creatinine clearance, < 15 mL/min), hyperparathyroidism due to cancer or metabolic bone disease with the exception of osteoporosis and osteomalacia, if they could not take food orally, or if they had a poor prognosis of less than 4 months.

After providing written informed consent, ninety-two male Veterans were screened using the Mini-Mental State Evaluation (MMSE) survey and for serum 25(OH)D concentration (Liaison, DiaSorin). Those with MMSE scores above 18/30 and 25(OH)D <75 nmol/L were included in this single-blinded randomized dose-response trial. Participants were blinded to the study groups of the trial as were all the researchers with the exception of the one who prepared the fortified foods (IG). An Intent-to-treat (ITT) analysis was used. The reporting of this trial conforms with the recommendations of the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement on randomized control trials [390] (Figure 4.1). The study was approved by the Institutional Review Board of McGill University, Therapeutic Product Directory of Health Canada (Letter of no objection) and the Scientific Committee of Ste-Anne's Hospital (Veterans Affairs Canada).

4.3.2 Safety

Monthly safety monitoring of 25(OH)D and PTH concentrations was incorporated in the design as little is known about vitamin D_3 supplementation at 2000 IU/d for 8 wk or 1000 IU/d for 24 wk in this very old population. At any time in the study (run in period and RCT), participants presenting with 25(OH)D concentrations below 30 nmol/L or above 125 nmol/L were tested a second time with a second blood draw. Once confirmed, the participant's physician prescribed adequate supplementation for those presenting deficiency. For those in excess, the trial supplementation was stopped by the research team and the physician was informed. Participants were followed as intent-to-treat (ITT).

4.3.3. Study Design

This 32-wk study includes 2 distinct segments. In November 2011, the 8-week Run-In period commenced (before after design Phase I; Week -8 to Week 0). All vitamin D supplement prescriptions were stopped by physicians and all participants were administered 2 daily tablet supplements of 1000 IU of vitamin D_3 (for a total of 2000 IU/d). In January 2012 (RCT Phase II; Week 0 to Week 24), all vitamin D tablet supplementation was ceased. Participants were then randomized in a single-blind fashion to one of three groups following a 1:1:1 ratio: one daily serving of foods containing either no vitamin D_3, (Placebo 0 IU/d group), 500 IU of vitamin D_3 (500 IU/d group), or 1000 IU of vitamin D_3 (1000 IU/D group) for a period of 24 weeks. As inflammation could also influence vitamin D, the rheumatoid arthritis Disease Activity Score (DAS28) were calculated and used to form blocks of randomization. The DAS28 questionnaire determines the level of inflammation (tenderness and swelling) of 28 joints namely hand knuckles, wrists, elbows, shoulders and knees. The DAS28 score also includes erythrocyte

sedimentation rate (ESR) and general health rating. Participants of each of the 4 levels of activity (remission, low, moderate and high) were considered as a block. Those who declined the additional testing for the DAS28 assessment were denoted as an additional "Refusal" block (Figure 4.1). Blinding of participants, medical, nursing and food service staff was achieved by the use of color coded labels on food containers.

4.3.4 Tablet-based Supplements

In the Run-in period, tablet-based supplements used by the institution's pharmacy were administered. For the first 4 weeks, Swiss Herbal vitamin D_3 supplements were used followed by 4 weeks of Webber vitamin D_3 supplements due to a change in institutional ordering. The Vitamin D_3 dose of 2000 IU was chosen as a loading dose to elevate the 25(OH)D serum concentration up to 75 nmol/L. This dose was considered to be safe for the extremely old population as it remained below the IOM (2011) tolerable upper intake level of 4000 IU, which is the maximum usual daily intake level at which no risk of adverse effect is expected. Adherence to the study protocol was ascertained by the daily distribution of tablets and monitoring of consumption by nursing staff.

4.3.5 Dietary Assessment and Compliance

During the RCT segment of the study, one researcher (IG) prepared the fortified foods. Fortified food samples included muffins, puddings and smoothies. Recipes were standardized (weighed ingredients) and a taste panel, including kitchen staff and clinicians, confirmed the lack of distinctive taste or appearance amongst fortified and placebo samples. All fortified foods were available in the same shape, texture and flavor for each level of fortification (Placebo, 500 IU and 1000 IU). They were designed to be of small portion sizes (12 g for muffins and 30 mL for

puddings and smoothies) to minimize possible impact on appetite. Aside from being close to the two most commonly offered dosages of vitamin D on the market (400 IU and 1000 IU), the selection of the level of fortification was based on previous results showing that vitamin D intake from food in this population was 300 IU/d on average [391]. A supplementation of 500 IU was considered adequate to meet the RDA recommendation of 800 IU/d for this cohort. The fortification level of 1000 IU/d enabled the dose-response. The vitamin D content of the fortified foods was verified to be 107.4±13% using HPLC. Samples of vitamin D_3-fortified muffins, puddings and smoothies (n = 40) were randomly selected across the 24 weeks of the trial. Vitamin D_3 content was extracted and analyzed based on a method that was developed at Health Canada (Longueuil, QC) [392] and proven to be comparable to international laboratory techniques [393]. Minor modifications were made to optimize the methodology for high concentrations of vitamin D_3 in the fortified food samples, in comparison to foods analyzed in the original method. All chemicals used in the study were of HPLC grade. Solid phase extraction (SPE) was carried out using the 12-port Visiprep SPE vacuum manifold from Supelco. Mega Bond Elut silica columns were attached onto the manifold for SPE (120 μm, 1 g/ 6 mL, Part #14256008). An HPLC system was equipped with an ultraviolet diode-array detector (UV-DAD) (Varian Prostar model 335). The HPLC system included an autosampler (Varian model 410), a solvent delivery module (Varian model 210), and a fraction collector (Varian model 701). Column temperature was controlled at 30°C by a column heater (Eppendorf CH-30). Galaxie Chromatography Data System (version 1.9.302.530) was used to handle data for analysis. External validation of vitamin content of a subset of fortified foods (n = 21) was also undertaken at the Health Canada laboratories during the intervention period of the study and using the same methodology as previously published [394].

Consumption of the fortified foods was monitored by weighed food waste measurement, on a weekly basis during RCT phase of the study. Meal consumption was determined by 3-day dietary intakes every 8 weeks. Nutritional content of all consumed foods was analyzed and calculated using the ProMenu Software (CMR Progiciels, v.6.1.44).

At the end of the study, appreciation and acceptability of the fortified foods was assessed via individualized interviews conducted by one of two researchers. Interest in receiving specially formulated foods containing vitamin D_3, appreciation of the various items offered during the protocol and preference in supplemental form (tablets versus specially formulated foods) were assessed using a 5-point Likert scale, with options including strongly disagree, tend to disagree, neither agree nor disagree, tend to agree, and strongly agree. To aid participants' memory recall, a print out of photos of the fortified foods was presented. Questionnaires were blinded for data entry.

4.3.6 Blood Sampling, Vitamin D Status, Parathyroid Hormone, Calcium, and Phosphate

Fasted venous blood was collected between 06h00 and 08h00. Routine biochemistry, including calcium and phosphate as safety procedures, were measured every 8 weeks in the hospital laboratory, using the Vitros 250E (Ortho Clinical Diagnostics, Johnson & Johnson, version 250) and Sysmex XT-2000i (Sysmex, version XT-2000i/XT-1880i) auto analyzers. Erythrocyte sedimentation rate (ESR) was reported as the distance travelled by red blood cells after 1 hour in a column of anticoagulated blood, under the influence of gravity. The laboratory adheres the International Organization for Standardization (ISO) (Norm 15189 – Medical Laboratories) quality assurance program. Serum sample aliquots were stored at -80° until further testing at McGill laboratory. Serum 25(OH)D and PTH were measured every 4 week (excluding

RCT-week20) by researchers (SA, CB) not in contact with participants (School of Dietetics and Human Nutrition, McGill University, Canada), using a chemiluminescence immunoassay that detects both 25(OH)D_2 and 25(OH)D_3 metabolites (LIAISON 25-OH Vitamin D TOTAL Assay, DiaSorin). The laboratory maintains certification with the Vitamin D External Quality Assessment Scheme (www.deqas.org). Controls were within the specified range for each assay. Inter-assay coefficient of variation was 7.2% for 25(OH)D and 11.2% for PTH based on triplicate testing a pooled serum sample.

4.3.7 Anthropometric Measurements

Monthly weights were obtained (kg) to the nearest 0.5 kg by nursing staff and admission height (m) obtained from medical chart. Body mass index (kg/m^2) was calculated by dividing the weight in kilograms by the square of height in meters.

4.3.8 Data Analyses

The estimated sample size was 17 per group based on 25(OH)D status in this institution [385]. Assumptions for calculations were an anticipated rise of 25 nmol/L in 25(OH)D in the 1000 IU/d intervention, as 25(OH)D values are often below 50 nmol/L in this older population with SDs of 25 up to 30 nmol/L. An alpha of 0.05 and power of 80% were set. Allowing for 10-15% attrition, we recruited 20 participants per group.

Participant characteristics were compared using ANOVA and are presented as mean ± SD. All data were audited and tested for normality using the Kolmogorov-Smirnov test. Nonparametric data were log-transformed when required (e.g., 25(OH)D and PTH). Homogeneity of variance was assessed by using the Bartlett test. Differences among groups over time were analyzed using a mixed model ANOVA for repeated measures, controlling for age and

BMI, with post hoc Bonferroni adjustment to compare biochemistry, dietary intakes and general characteristics among the 3 fortification groups. Data were analyzed based on intention-to-treat (ITT) for all outcomes. Statistical analysis of the data was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC), with a significance level of 0.05 used for all analyses including after adjustment for multiple comparisons testing.

4.4 Results

4.4.1 Demographic Characteristics

At baseline, participants were 89.9±3.3 y of age (range: 80.8–96.8 y) and presented with an average BMI of 26.3±4.6 kg/m^2. No changes in weight, BMI and MMSE were observed over the study (Table 4.1).

4.4.2 Dietary Characteristics

Dietary intakes (Table 4.1) show balanced macronutrient intakes for all groups that remained stable over the course of the study. At baseline, a mean intake of 1700 kcal/d was consumed by participants. Protein, carbohydrate and fat provided 15.5%, 54.0% and 31.7% of total energy, respectively. Similarly, dietary calcium intake was not different over time or among groups at any time point of the study.

At the time of recruitment (Wk -8), the daily total intakes of vitamin D and calcium did not meet the DRI values for adults > 70 y of age (Table 4.1). For calcium intake from food alone, 38% of participants met the EAR and 25% of participants met the RDA. When food intake of calcium and calcium supplements were considered, 52% were able to achieve the EAR whereas 38% met the RDA. For vitamin D intake from food alone, 12% of participants met the EAR and

1.7% of participants met the RDA. Nearly half of the participants (48.3%) were not receiving vitamin D supplementation. Thirty-eight percent (38%) were receiving 400 IU or 600 IU/d vitamin D supplements and only 15% were receiving 1000 IU/d of vitamin D supplement or more. When dietary intake of vitamin D and supplements were considered, 47% were able to achieve the EAR whereas 25% met the RDA.

4.4.3 Biochemical Assessments

Serum values of 25(OH)D obtained at screening averaged 56.1±13.8 nmol/L for the 60 participants who met all the entrance criteria. Twenty participants were below the 50 nmol/L mark (Placebo group: 6; 500 IU group: 8; 1000 IU group: 6) which is the target value set as sufficient by the IOM to maintain bone health in healthy seniors living in the community. No differences among groups were identified. During recruitment, only 31.5% of the sampled participants were excluded for having 25(OH)D values above 75 nmol/L (Figure 4.1). Also, average serum PTH (Figure 4.2b), phosphate and ionized calcium concentrations were all within normal ranges for this age group. PTH shows an effect of time between week -8 and week 8 as well as between week 0 and week 8 for the 500 IU group only.

Over the 8 week Run-in period, the 2000 IU/d tablet supplementation increased serum 25(OH)D concentration. All groups increased from their respective baseline value with no differences among groups after supplementation. The overall mean 25(OH)D was 68.2±12.2 nmol/L after the 8 wk supplement period (Figure 4.2a). Average serum PTH (Figure 4.2b), calcium (Normal range: 2.10 to 2.55 mmol/L), ionized calcium (Normal range: 0.90 – 1.15 mmol/L) and phosphate remained within the limits of the normal ranges at all time points. Only one participant in the Placebo group had mild hypercalcemia with a value of 2.56 mmol/L at

RCT0. Serum total calcium was low in 4 individuals (3 individuals in group of 500 IU/d at each time point and 1 in group of 1000 IU/d at RCT24).

At the beginning of the dose-response trial (0 wk), all vitamin D_3 tablet supplements were stopped. The 60 participants previously randomized into 3 groups (Placebo, 500 IU fortified foods and 1000 IU fortified foods) were not different in serum 25(OH)D concentration at wk 0 of the RCT. However, five participants remained below the 50 nmol/L mark (Placebo group: 1; 500 IU group: 3; 1000 IU group: 1). By week 8 of the RCT, serum 25(OH)D values in the Placebo group declined to values no longer different from -8 wk. In the 500 IU and 1000 IU intervention groups, values remained constant from week 0 to week 24, with no difference amongst these groups. By the end of the trial (week 24), the placebo group had a mean 25(OH)D value of 56.2±10.6 nmol/L, the 500 IU group had a mean 25(OH)D value of 74.0±16.5 nmol/L and the 1000 IU group had a mean 25(OH)D value of 79.6±17.2 nmol/L. Both intervention groups maintained higher 25(OH)D values than the Placebo group (Figure 4.2a). In the placebo group, 1 participant surpassed 75 nmol/L of serum 25(OH)D, in the 500 IU group, 8 participants surpassed 75 nmol/L of serum 25(OH)D and in the 1000 IU group, 10 participants surpassed 75 nmol/L of serum 25(OH)D. Eight participants were now below the 50 nmol/L mark, none of which were in the 1000 IU/d group (Placebo group: 6; 500 IU group: 2; 1000 IU group: 0). PTH remained stable and within normal range over the course of the RCT phase (Figure 4.2b). Fortified foods were stopped for one participant of the 1000 IU group as the serum value of 25(OH)D reached more than 125 nmol/L. Assessments were maintained to meet the ITT conditions.

We obtained a response rate of 82.7% to the preferences questionnaire. Non-respondents included 8 that were unable to recall their meal consumption due to poor memory, and 1

participant declined to participate in the survey. Results from the preference questionnaire indicate that 79.1% of the participants were interested in consuming vitamin D_3-fortified foods, and 81.4% of participants stated that they preferred receiving the specially fortified foods over taking tablet supplements in order to meet their daily requirements. While 97% of the participants indicated that they usually consumed all of the vitamin D-fortified food, only 3 participants admitted that they would consume less of their regular meal in order to compensate for consuming the fortified food. However, 95% of participants that received puddings or smoothies indicated that the 30 mL portion size was adequate for those two items.

Table 4.1: Baseline characteristics of participants (Mean ±SD)

		Vitamin D_3 Intervention Group						p-value		
	Wk	n	Placebo – 0 IU/d Mean ± SD	n	500 IU/d Mean ± SD	n	1000 IU/d Mean ± SD	Group	Time	Interaction
Age (y)								0.6967	**<.0001**	0.8709
	-8	*(n=20)*	89.9 ± 3.8 [A]	*(n=20)*	89.9 ± 2.5 [A]	*(n=20)*	89.1 ± 3.6 [A]			
	0	*(n=19)*	90.1 ± 3.8 [B]	*(n=20)*	90.2 ± 2.5 [B]	*(n=19)*	89.4 ± 3.6 [B]			
	24	*(n=18)*	90.7 ± 4.0 [C]	*(n=19)*	90.7 ± 2.6 [C]	*(n=17)*	89.6 ± 3.7 [C]			
Weight (kg)								0.7115	0.7776	0.9908
	-8	*(n=20)*	77.0 ± 11.8	*(n=20)*	75.2 ± 14.4	*(n=20)*	77.7 ± 14.4			
	0	*(n=19)*	77.3 ± 12.4	*(n=20)*	75.7 ± 14.6	*(n=19)*	77.0 ± 14.1			
	24	*(n=18)*	78.3 ± 12.3	*(n=19)*	73.7 ± 14.6	*(n=17)*	78.6 ± 14.6			
BMI (kg/m^2)								0.9922	0.8688	0.9993
	-8	*(n=20)*	26.3 ± 4.1	*(n=20)*	26.4 ± 5.4	*(n=20)*	26.1 ± 4.3			
	0	*(n=19)*	26.3 ± 4.0	*(n=20)*	26.5 ± 5.5	*(n=19)*	26.1 ± 4.1			
	24	*(n=18)*	26.7 ± 4.1	*(n=19)*	25.8 ± 5.4	*(n=17)*	26.7 ± 4.6			
Energy (kcal/d)								0.0665	0.4477	0.3615
	-8	*(n=20)*	1707 ± 271	*(n=20)*	1610 ± 375	*(n=20)*	1841 ± 387			
	0	*(n=19)*	1608 ± 286	*(n=20)*	1617 ± 369	*(n=19)*	1840 ± 366			
	24	*(n=18)*	1719 ± 346	*(n=19)*	1610 ± 375	*(n=17)*	1735 ± 366			
Protein (g/d)								0.5185	0.8979	0.2131
	-8	*(n=20)*	66.1 ± 13.2	*(n=20)*	62.8 ± 19.5	*(n=20)*	71.5 ± 18.3			
	0	*(n=19)*	63.1 ± 16.1	*(n=20)*	64.6 ± 18.9	*(n=19)*	72.4 ± 17.8			
	24	*(n=18)*	68.8 ± 18.3	*(n=19)*	68.0 ± 21.2	*(n=17)*	68.0 ± 17.0			
Calcium Intake (mg/d)								0.8685	0.6748	0.4090
	-8[1]	*(n=20)*	1046 ± 437	*(n=20)*	1152 ± 487	*(n=20)*	1104 ± 437			
	0[2]	*(n=19)*	1022 ± 524	*(n=20)*	1060 ± 507	*(n=19)*	1125 ± 366			
	24[3]	*(n=18)*	1090 ± 362	*(n=19)*	1132 ± 459	*(n=17)*	1047 ± 264			
Vitamin D Intake (IU/d)								**<.0001**	**<.0001**	**<.0001**
	-8[1]	*(n=20)*	494 ± 380 [a]	*(n=20)*	769 ± 526 [b]	*(n=20)*	562 ± 383 [ac]			
	0[2]	*(n=19)*	2310 ± 218 [d]	*(n=20)*	2337 ± 222 [de]	*(n=19)*	2348 ± 200 [df]			
	24[3]	*(n=18)*	295 ± 124 [g]	*(n=19)*	869 ± 345 [bh]	*(n=17)*	1316 ± 84 [i]			

[1]-8 wk includes diet and prescribed tablet supplementation; [2] 0 wk includes diet and 2000 IU tablet supplementation.
[3] 24 wk includes diet and specially formulated fortified food; Mixed Model ANOVA for repeated measures, controlling for age and BMI; Values with different superscripts are different, $p<0.05$; Capital superscripts show effect of time.

Figure 4.1: Consort diagram

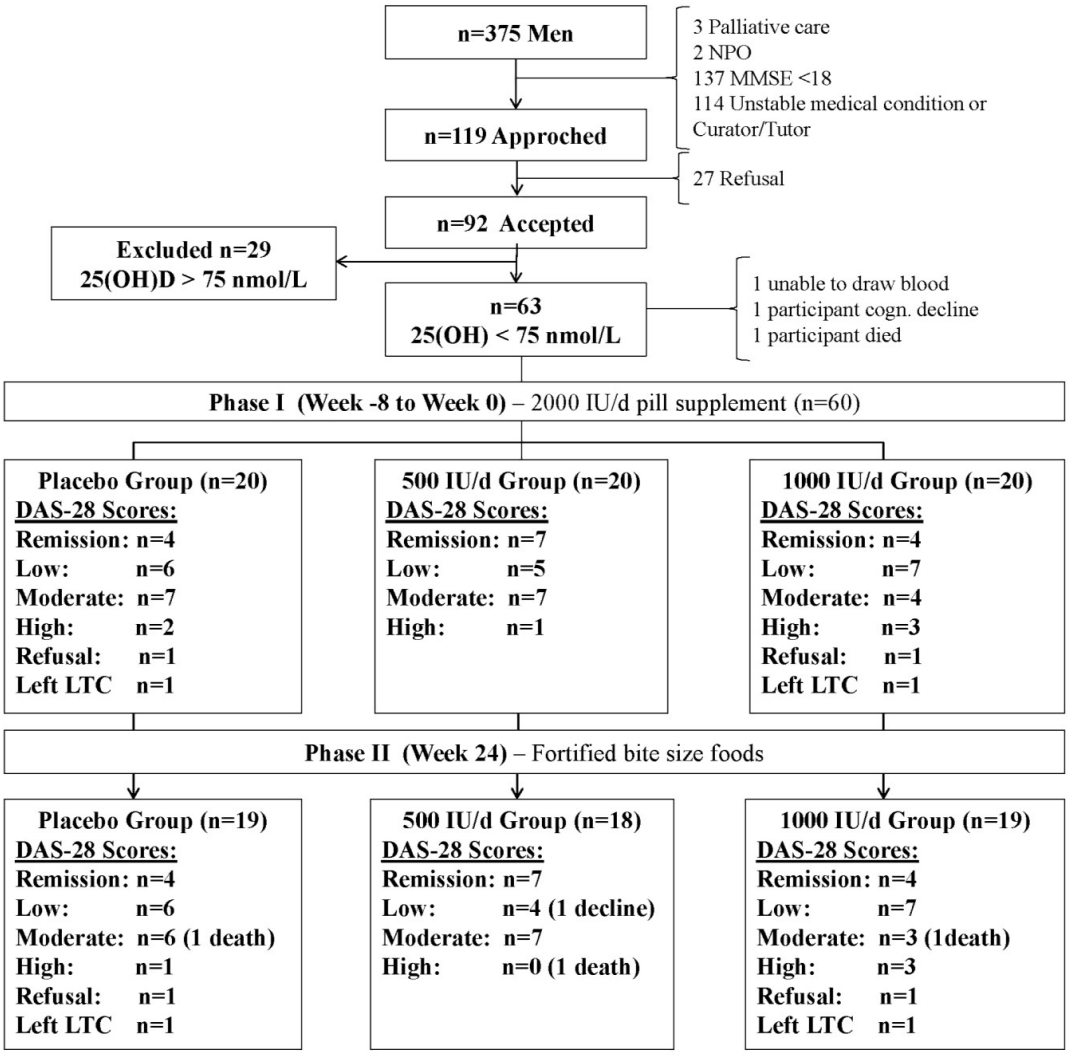
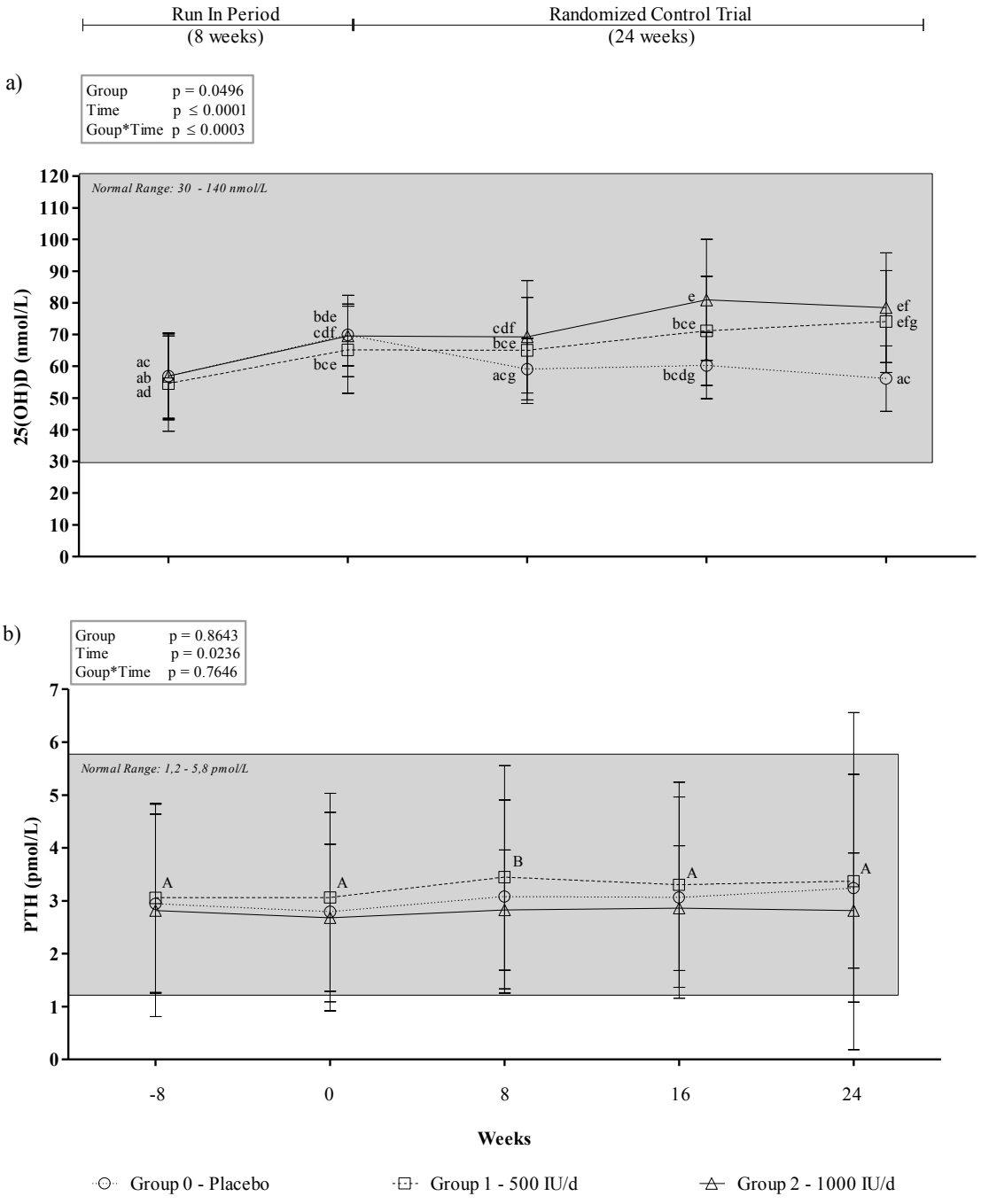


Figure 4.2: Vitamin D (nmol/L) and PTH (pmol/L) concentrations in older Veterans following 8 weeks of pill supplementation and 24 weeks of specially fortified foods (placebo, 500 IU and 1000 IU) (November 2011 to July 2012)



Mixed Model ANOVA for repeated measures, controlling for age and BMI;
Values with different superscripts are different, $p<0.05$; Capital superscripts show effect of time.

4.5 Discussion

Research regarding the amount of vitamin D needed to support healthy vitamin D status targets in advanced aging is limited. The primary objective of this study was to assess the required vitamin D intake necessary to maintain 25(OH)D status in a cohort of older men living in a LTC facility of Montréal (Québec, Canada; 46°N). As experts of the National Osteoporosis Foundation [216] and the National Osteoporosis Society [211] have proposed a loading doses to achieve 25(OH)D adequacy of 50 nmol/L of 25(OH)D, the 2 phase design allowed for the dose-response assessment of a typical tablet-based supplements (2000 IU/d; week 0) and 3 levels of food fortification (Placebo, 500 IU and 1000 IU; week 24). To our knowledge the design of this study presented an innovative model to assess, not only the required additional vitamin D intake needed to reach a desired 25(OH)D status, but also the amount of vitamin D intake needed to maintain the newly achieved 25(OH)D concentration in the older population. All of the supplements were consumed under supervision with very high compliance. Based on maintenance of serum 25(OH)D over the trial period, a dosage of either 500 or 1000 IU/d was observed to be adequate to support the IOM target of 50 nmol/L. However, no dosage across the entire study was able to support achievement or maintenance of the 75 nmol/L target suggested for bone health in aging in 100% of the participants [58].

In these older men, the tablet supplementation of the Run In period improved serum 25(OH)D with an average value raising above 65 nmol/L. On average, PTH, calcium and phosphate values remained in the normal ranges and were not negatively impacted by the supplementation regimen of 2000 IU/d. However, and perhaps due to its duration, the 8 week regimen of 2000 IU/d tablets of vitamin D_3 did not result in mean 25(OH)D concentration of more than 75 nmol/L suggested by some research teams [58]. This could be ascribed to the

highly variable response to supplementation in aging [17]. Thus the tempered response to supplementation, in part, could be also ascribed to the advanced age of the participants. The capsule-based supplementation elevated serum 25(OH)D to an average above 65 nmol/L with only 8.6% reaching 75 nmol/L and an estimated increment of 1 nmol/L per 40 IU of vitamin D. While trials have demonstrated an improvement of serum 25(OH)D in older adults with various vitamin D supplementation levels using vitamin D_3 ranging from 24 to 5000 IU/d [19, 395-397], Whiting et al. showed that serum 25(OH)D concentration could be raised by 2 nmol/L when only 40 IU of vitamin D was added in groups where baseline values were below 75 nmol/L. This was similar to what was reported by McKenna et al. [398]. However, it was more than double what was presented by Heaney et al. [289] where 40 IU of vitamin D was calculated as being necessary to raise serum 25(OH)D by 0.7 nmol/L. Participants had a baseline serum concentration of 70 nmol/L and were receiving more than 1000 IU/d of vitamin D. Whiting et al. [397] included studies supplementing between 200 to 800 IU/d of vitamin D and noted that higher dosages produced lower rate constants. They also suggest that adding 400 IU of vitamin D_3 to the diet, through food fortification, could improve 25(OH)D status in depleted adults. However, the requirements to maintain vitamin D status once the stores are replenished have not, to the best of the authors' knowledge, been tested.

The RCT segment of the study enabled testing of 3 levels of vitamin D intake in support of maintaining 25(OH)D status in institutionalized extremely old male seniors in a LTC facility. After only 8 weeks, the 25(OH)D concentration values of the Placebo group reverted back to the baseline values. Despite the professionally established and supervised menu, vitamin D intake from food alone (295±124 nmol/L) was below the EAR in 88% of the participants, in agreement with previous research [61, 63, 64, 66-68, 240, 241, 391], and could not maintain vitamin D

status beyond 8 wk. While the IOM report (2011) [17], Canada's Food Guide (2007) [223] and Osteoporosis Canada (2010) [49] all recommend use of a vitamin D supplement for the population over the age of 50 y or at risk of fractures, these guidelines were not designed specifically for use in LTC.

The vitamin D fortified foods were successful in maintaining vitamin D status with as little as 500 IU of additional vitamin D on a daily basis. Considering the difficulty to develop menus that incorporate sufficient vitamin D to meet the needs of the population over the age of 70 y [65, 67, 68], these products could be added to other fortified foods, such as fortified bread [275], purred foods [61], soft cheese [272, 278] and yoghurt [280], that have been successful at raising 25(OH)D concentrations in seniors living in LTC. The fortified foods were easily distributed on the breakfast tray, well accepted and appreciated by the participants and, did not affect overall dietary patterns nor biochemistry profiles other than improve 25(OH)D concentrations. These results are in accordance with the review by Lam et al. suggesting that micronutrient fortification is a worthwhile approach to improve nutritional status in LTC [399]. Still, a gap in knowledge exists in the literature regarding the impact of 25(OH)D supplementation in very men living in LTC and the markers of mineral metabolism. Malihi et al. [400] reported that long-term vitamin D supplementation could increase the risk of hypercalcemia (37 studies; RR: 1.54; 95% CI: 1.09, 2.18; P=0.01) and hypercalciuria (14 studies; RR: 1.64; 95% CI: 1.06, 2.53; p=0.03). These consequences were not related to vitamin D dose nor associated to baseline 25(OH)D concentration, duration, or calcium supplementation. No case of hypercalcemia was observed in this RCT in response to the tablet-based or food-based supplements.

Several proposed guidelines in prevention and management of osteoporosis suggest loading doses of vitamin D followed by a lower maintenance dose in vitamin D depleted seniors [58, 211, 214, 216]. This was effectively achieved with this RCT. However, considering the advanced age of this cohort, the possible comorbidities, the general understanding that systematic testing for 25(OH)D status in LTC is unwarranted [214, 220] and, the possible risks of ill-effects of long term vitamin D supplementation, these results support that a loading period for supplementation of vitamin D would improve vitamin D status and that a maintenance regimen of a lower dosage consistent with the RDA is suitable to preserve status.

The information emerging from this study of 60 very old men living in LTC is valuable for bone health and osteoporosis research since the majority of studies are conducted in women (ratio 1 male for 3 females). As research continues to demonstrate, vitamin D intake and 25(OH)D concentrations are known to be deficient or low in LTC residents. Osteoporosis and fractures pose an important health risk to seniors living in LTC and it appears further guidelines are deemed necessary [401]. Recently, a mandatory vitamin D supplementation has been suggested for all LTC facility residents in France [221]. Yet, scientific data to support vitamin D requirement to maintain serum 25(OH)D, specific to institutionalized males in the oldest segment of the populations, are nonexistent. In the past decade, large prospective cohort studies have filled part of this knowledge gap on bone health in men living in the community. Although the MrOs study [85], the InChianti study, [86] and the CHAMP study [87] are good examples of these efforts, however, very old men in LTC remain an under studied population. In addition, polypharmacy is a concern for the older population and should be considered from the perspectives of supplement-medication interactions [402, 403] and quality of life. Important use of medication due to comorbidities has been reported in the community [404] as well as in LTC

facilities [405, 406]. Therefore, an additional mandatory tablet supplementation regimen might expose these seniors to more health risks and drug interactions and should be considered with caution.

Limitations of our study include that this was a single blind study. Also, the data might be not representative of all older male living in institutionalized facilities since, at the time of the study, Ste-Anne's Hospital was federally funded and offered services to veterans only.It is also possible that total dietary intake of vitamin D could have been underestimated as evening snacks were assessed, but participants had access to foods, and although we inquired about them, memory was sometime failing and recall could have been affected. Finally, the recipes for the fortified foods were not commercially produced and this could have affected the desired concentrations, despite our efforts to control for variability throughout our 24 week study.

4.6 Conclusion

In conclusion, after 8 weeks of a tablet supplementation of 2000 IU/d, very old institutionalized men were on average able to reach a serum 25(OH)D concentration over 65 nmol/L. Furthermore, those who consumed a food fortified supplement containing either 500 to 1000 IU/d of vitamin D were on average able to maintain a 25(OH)D status over 75 nmol/L for 6 months. The use of foods fortified in vitamin D was greatly appreciated by this older cohort and could represent an appealing alternative to reduce polypharmacy in LTC facilities and increase vitamin D status.

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The authors' contributions were as follows—IG and HAW: conceived the study and obtained funding, coordinated and led the data collection, oversaw laboratory analyses, conducted the data analysis, provided technical oversight and input into all aspects of the study and had primary responsibility for the final content, drafted the research manuscript; SA, CB and SJP: conducted laboratory analyses; ML: designed and validated the vitamin D food appreciation questionnaire, led interviews and wrote final report; and all authors: contributed to the review and revision of the manuscript and read and approved the final manuscript. All other authors declared no conflicts of interest related to this study.

BRIDGE 3

The gold standard for measuring bone mineral density (BMD) and diagnosis of osteoporosis is dual-energy X-ray absorptiometry (DXA). The latest Position Statement of the International Society for Clinical Densitometry (ISCD) has maintained that central DXA is the best approach to diagnosis of osteoporosis using a T-score of -2.5 of the femoral neck. Furthermore, the reference standard for the T-score is the white female of 20 – 29 y obtained from the NHANES III database. Continuously assessing technological changes to improve the study of BMD and fracture risk, the ISCD has recently determined that peripheral quantitative computed tomography (pQCT) of the ultra-distal radius could predict fragility fractures of the hip, but not vertebral fractures, in postmenopausal women. In men, more evidence is required to support this position statement. Given the frail state and the reduced mobility of the older population in long-term care facilities, as well as their limited access to DXA for diagnosis or serial BMD measurements, complementary tools to assess BMD and muscle strength would be beneficial in assessing BMD and help to enlighten practices towards prevention of falls and fractures. Potential tools would be handgrip strength, markers of bone turnover and peripheral measures of BMD. The group of very old men (veterans) living at Ste-Anne's Hospital provides a well-controlled setting to study markers of bone turnover and changes in BMD measured with pQCT or peripheral DXA in response to vitamin D supplementation.

CHAPTER 5 – MANUSCRIPT 3

Title: Impact of vitamin D on musculoskeletal health of men in advanced aging: a randomized placebo-controlled dose-response trial in long-term care

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Clinical Trial Registry number and website: This research project was registered under the identification number NCT01437696 with ClinicalTrials.gov (www.clintrial.gov)

5.1 Abstract

Introduction: Frail older men are underrepresented in studies assessing the impact of vitamin D_3 supplementation on bone mineral density (BMD), bone strength and body composition. Increased risk of fractures with age warrants further trials towards improving musculoskeletal health outcomes. We aim to test the impact of vitamin D_3 supplementation on BMD, and markers of bone turnover and muscle function in advanced aging in men.

Material and Methods: Men (n=60) over the age of 80 y were included in an 8-week loading period followed by a 24-week, randomized, parallel placebo-controlled dose-response trial (RCT) in a long-term care facility in Québec, Canada. Vitamin D_3 supplementation (2000 IU/d tablet-based) for 8 weeks was followed by Placebo or vitamin D_3 supplementations (500 IU or 1000 IU food-based) for 24 weeks. At the beginning and end of the RCT, detailed nutritional intake, serum 25-hydroxyvitamin D (25(OH)D) concentrations, biomarkers of bone metabolism and volumetric bone mineral density (vBMD) of the non-dominant forearm was measured using peripheral quantitative computed tomography (pQCT; 4% and 66% forearm). Areal bone mineral density (aBMD) was measured at the distal 1/3 forearm using peripheral dual-energy X-ray absorptiometry (pDXA). Muscle and fat area, and muscle density were measured at the 66% forearm. Handgrip strength (Hydraulic hand dynamometer, Jamar®) was performed at baseline and 24 wk of the RCT.

Results: Participants were 89.9±3.3 y of age (range: 80.8-96.8 y) with a BMI of 26.3±4.6 kg/m^2. Groups were comparable at week -8 and at week 0 of the RCT. Although the increase of serum 25(OH)D concentrations were maintained by the 500 IU/d and the 1000 IU/d supplementation regimens, no change in biomarkers of bone metabolism were seen after 24 weeks for any of the

groups. Distal forearm aBMD increased only in the 1000 IU group (0.017±0.004 g/cm^2, $p<0.004$). Cortical vBMD of the 66% radius in the 1000 IU group decreased (6.02±19.11, $p<0.044$) whereas cortical cross-sectional area increased over time (4.50±1.07, $p<.0001$) without interaction. Muscle area (-117±206 mm^2, $p=0.002$) declined over time, without interaction. Muscle density, fat and muscle density and fat:muscle ratio did not vary by time or intervention group. Handgrip strength was maintained only in the 1000 IU group (placebo: -1.3±0.5; 500 IU: -1.8±0.5; 1000 IU: 0.3±0.6 kg, $p<0.05$).

Conclusions: This study demonstrates that achieving and maintaining higher vitamin D status is associated with improved aBMD and maintenance of muscle strength. Whether these improvements lead to prevention of falls and fracture requires further study.

5.2 Introduction

Worldwide, the human population is aging. In Canada, the 2011 Census revealed that 4.9 million Canadians were over the age of 65 y, representing 15% of the population [48]. In the United States, the number of individuals over the age of 60 years has doubled since 1940, reaching 20% of the population in 2015 [407]. Increased frailty and sarcopenia in seniors are associated to functional decline [343]. Risks of falls and fractures increase with age and could be a result of reduced bone mass or reduced muscle strength [354, 408]. The World Health Organization (WHO) study on global aging and adult health (SAGE) reported that the proportion of fall related injuries is now 65.7% in low- and middle-income countries such as China, Russia, India, Ghana and Mexico. In Australia, individuals aged 65 y and over were hospitalized for fall-injuries 3.5 times more than the 45 to 64 year old group. In Canada, 85% of all hospitalizations are ascribed to the 65 years and over age group [407]. Thus far, public health advisory efforts are strongly focused on community-living post-menopausal women to reduce the socio-economic burden of such injuries. However, both older male and female populations are living longer and often require the full support of long-term care (LTC) facilities.

Osteoporosis is defined by the National Institutes of Health (NIH) as a skeletal disorder that is characterized by a systematic impairment of bone strength predisposing a person to an increased risk of fragility fracture [409]. Frail seniors often experience low-energy falls from a standing height or lower which could result in fracture of the distal radius (also described as “fragility fracture’) due to the natural protective extension of the forearm. The distal radius is the most common long-bone fracture site [410]. In the United States, nearly 15% of emergency visits by the senior population were related to upper extremity fractures [411]. In Canada, the incidence of fracture of the proximal radius, ulna or both (per 10,000 person per year) are similar

for all age groups between 18 to 79 years of age (4.62 – 5.53) but the group of 80 years or more have almost double the incidence rate of 8.70 per 10,000 persons per year (95% CI: 6.24-11.16) [412]. Fourteen percent (14%) of individuals suffering from a wrist fracture will subsequently fracture within 3 years [413]. Older women presenting with distal radius fracture are known to experience important functional declines [414] and fractures are associated with increased risk of morbidity and mortality [415].

Frailty, frequent falls and fractures in older adults are often associated with poor vitamin D status [416-419]. Vitamin D and calcium are recognized as important contributors to bone health as a result of maintenance of bone mineral density (BMD) and reduction in risk of fracture [17, 58]. However, meta-analyses on the topic of vitamin D supplementation and muscle function are equivocal, one suggests that in older adults (>65 y), muscle strength is improved by vitamin D supplementation [352, 420] whereas a more recent systematic review of vitamin D supplementation (with or without calcium) in community dwelling adults > 65 y of age found no improvement of handgrip strength and only small improvements in the timed-up-and-go test [354]. Likewise, vitamin D supplementation is reported to reduce the rate and risk of falls in those with low vitamin D status [420], whereas high supplementation bolus dosages associate with increases in falls [217]. The majority of the studies in aging has been conducted in the community and might not fully capture advanced aging. Dose-response studies are required to ascertain the impact of vitamin D supplementation on BMD, falls and fractures, especially in advanced aging in men in LTC.

Given the paucity of data on the impact of vitamin D_3 supplementation on peripheral BMD, muscle mass and function in very old men living in a LTC facility, the goal of this study

was to test the impact of vitamin D_3 supplementation on BMD, and markers of bone turnover and muscle function in advanced aging in men.

5.3 Methods

5.3.1 Participants

During the month of October 2011, male Veterans living at Ste-Anne's Hospital, a LTC facility in Québec (Veterans Affairs Canada, 46°N) were invited to participate in a trial lasting 32 weeks. Veterans were excluded if they presented with 25(OH)D > 75 nmol/L, baseline end-stage renal disease (calculated creatinine clearance, < 15 mL/min), hyperparathyroidism due to cancer or metabolic bone disease with the exception of osteoporosis and osteomalacia, if they could not take food orally, or if they had a poor prognosis of less than 4 months. Participants were not excluded on the basis of medication, including glucocorticoids therapy, as they were prescribed to be inhaled.

After providing written informed consent, n=92 male Veterans were screened using a Mini-mental state evaluation (MMSE) survey and for serum 25(OH)D concentration (Liaison, DiaSorin). Those presenting MMSE scores above 18/30 and 25(OH)D <75 nmol/L were included in this single-blinded randomized dose-response trial. The reporting of this trial conforms with the recommendations of the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement on randomized control trials [390] (Figure 5.1). The study was approved by the Institutional Review Board of McGill University, Therapeutic Product Directory of Health Canada (Letter of no objection) and the Scientific Committee of Ste-Anne's Hospital (Veterans Affairs Canada).

5.3.2 Safety

As reported in a previous publication [385], monthly safety monitoring of serum 25(OH)D, PTH, total calcium, and phosphate concentrations were incorporated in the design as little is known about vitamin D_3 supplementation at 2000 IU/d for 8 wk or 1000 IU/d for 24 wk in this very old population. At any time in the study (run in period and randomized clinical trial), serum was drawn and tested a second time if participants had 25(OH)D concentrations below 30 nmol/L or above 125 nmol/L. Once confirmed, adequate supplementation was started by the participant's physician for the former group or supplementation was stopped by the research team for the later and physician was informed. Participants were followed as intent-to-treat (ITT).

5.3.3 Study Design

This 32-wk study includes 2 distinct segments. In November 2011, the 8-week Run-In period commenced (Phase I; Week -8 to Week 0). All vitamin D supplement prescriptions were stopped by physicians and all participants were administered 2 daily tablet supplements of 1000 IU of vitamin D_3 (for a total of 2000 IU/d). In January 2012 (Week 0 of Phase II), all vitamin D tablet supplementation was ceased. Participants were then randomized to one of three groups following a 1:1:1 ratio: one daily serving of foods containing either no vitamin D_3, (Placebo group), 500 IU of vitamin D_3 (500 IU/d group), or 1000 IU of vitamin D_3 (1000 IU/d group) for a period of 24 weeks. Randomization was done in blocks based on the Disease Activity Scores (DAS28). The DAS28 questionnaire determines the level of inflammation (tenderness and swelling) of 28 joints namely hand knuckles, wrists, elbows, shoulders and knees. The DAS28 score also includes erythrocyte sedimentation rate (ESR) and general health rating. Participants

of each of the 4 blocks of levels of DAS28 (remission, low, moderate and high) were created to enable a 1:1:1 ratio in each group of food fortification. Those who declined the additional testing for the DAS28 assessment were randomized as an additional "Refusal" block (Figure 5.1). The study groups were single-blinded as one researcher (IG) prepared the food-based products. Blinding of participants, medical, nursing and food service staff and all but one researcher (IG) was achieved by the use of color coded labels on food containers.

The estimated sample size was 17 per group based on 25(OH)D status in this institution that had a high variability and 25(OH)D concentrations below 50 nmol/L [385]. The estimate was based on an anticipated 25 nmol/L change in 25(OH)D to bring status into a healthy range assuming a SD of 25-30 nmol/L, alpha of 0.05 and power of 80%. Allowing for 10-15% attrition, we recruited 20 participants per group.

5.3.4 Tablet Supplements

In the Run-in period, tablet supplements used by the institution's pharmacy were administered. For the first 4 weeks, Swiss Herbal vitamin D_3 supplements were used followed by 4 weeks of Webber vitamin D_3 supplements due to a change in institutional ordering. The vitamin D_3 dose of 2000 IU was chosen as a loading dose to elevate the 25(OH)D serum concentration up to 75 nmol/L. This dose was considered to be safe for the extremely old population as it remained below the US Institute of Medicine (2011) tolerable upper intake level of 4000 IU, which is the maximum usual daily intake level at which no risk of adverse effect is expected. Adherence to the study protocol was ascertained by the daily distribution of tablets and monitoring of consumption by nursing staff.

5.3.5 Dietary Assessment and Compliance

During the RCT segment of the study, one researcher (IG) was in charge of preparing fortified foods thus, needed to be aware of the supplementation intervention for each participant. Details regarding the fortified food samples are shown elsewhere (Manuscript 2). In brief, other food sources of vitamin D were calculated using 3-day dietary intakes, every 8 weeks. Consumption of the fortified foods was monitored weekly by weighed food waste measurement during RCT phase of the study. Nutritional content of all consumed foods was analyzed and calculated using the ProMenu Software (CMR Progiciels, v.6.1.44).

5.3.6 Blood Sampling, Vitamin D Status, Parathyroid Hormone, Calcium, and Phosphate

Fasted venous blood was collected between 06h00 and 08h00. Routine biochemistry – including total calcium and phosphate - were measured every 8 weeks by hospital laboratory, using the Vitros 250E (Ortho Clinical Diagnostics, Johnson & Johnson, version 250) and Sysmex XT-2000i (Sysmex, version XT-2000i/XT-1880i) auto analyzers. Erythrocyte sedimentation rate (ESR) was reported as the distance travelled by red blood cells after 1 hour in a column of anticoagulated blood, under the influence of gravity. The laboratory adheres the International Organization for Standardization (ISO) (Norm 15189 – Medical Laboratories) quality assurance program. Serum sample aliquots were stored at -80°C until further testing at McGill laboratory. Serum 25(OH)D and PTH were measured every 4 week (excluding RCT-week20) at the School of Dietetics and Human Nutrition (McGill University, Canada), using a chemiluminescence immunoassay that detects both $25(OH)D_2$ and $25(OH)D_3$ metabolites (LIAISON 25-OH Vitamin D TOTAL Assay, DiaSorin). The laboratory maintains certification with the Vitamin D External Quality Assessment Scheme (www.deqas.org). Controls were in

range with specifications of each assay. Inter-assay coefficient of variation (CV) was 7.2% for 25(OH)D and 11.2% for PTH based on triplicate testing a pooled serum sample. Serum osteocalcin, C-terminal telopeptides-1 (CTX), Receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) were measured at McGill (School of Dietetics and Human Nutrition, McGill University, Canada) at week -8, RCT-week0 and RCT-week24. Serum osteocalcin was measured, using a chemiluminescence immunoassay (Osteocalcin Assay, DiaSorin). Serum C-terminal telopeptide of Type 1 collagen (CTX) was measured by the serum Crosslaps enzyme-linked immonuabsorbent assay (Immunodiagnostic Systems Ltd, Boldon, UK). Serum RANKL (Human RANKL Single Plex) and osteoprotegerin (Human Bone Magnetic Beads) were measured using Milliplex^{TM} Map (Luminex Corporation, Austin, Tx). The inter-assay coefficients of variation (CV) were 8.1% for OC, 5.3% for CTX, 14.5% for OPG and 8.3% for RANKL.

5.3.7 Anthropometric Measurements

Monthly weights were obtained (kg) to the nearest 0.5 kg by nursing staff and admission height (m) obtained from medical chart. Body mass index (kg/m^2) was calculated by dividing the weight in kilograms by the square of height in meters.

5.3.8 Peripheral Quantitative Computed Tomography

A scan of the nondominant forearm was obtained using peripheral quantitative computed tomography (pQCT; Stratec XCT2000) with a standard data acquisition protocol and a voxel size of 0.4×0.4×2.4 mm. Phantom scans were done each study day and were within 1% of known values. A scout scan was conducted to visualize the cortical end plate of the radius and the reference line was placed at the horizontal plateau. Cross-sectional slices were obtained at 4 and

66 % of the radius length, proximal from the reference line. One investigator (TH) analysed all scans using the manufacturer's software package (Stratec Medical, version 6.0). Scans were further analysed a postiori by a second investigator (CV), 3 participants were not included in the analyses due to artefacts in the images. Scans of the 4 % site radius were analysed, with a density threshold of 180 mg/cm^3 to separate bone from surrounding soft tissue, contour mode 1, peel mode 1 and trabecular bone cross-sectional area (CSA) at 45%. At the 66 % site, the outer contour of the radius was detected at a threshold of 711 mg/cm^3, to separate cortical from trabecular bone. Muscle tissue was differentiated from subcutaneous tissue and bone using contour mode1 and peel mode 1 and by selecting voxels with a density greater than 40 mg/cm^3 and less than 280 mg/cm^3. Muscle density was calculated by dividing the total muscle content by muscle area. Radial bone properties at the distal site (4 %) were measured for total bone area (mm^2) and bone mineral densities (Total vBMD and Trab vBMD, mg/cm^3). Measures of the bone properties of the shaft of the radius (66 %) included cortical area (mm^2) and density (vBMD, mg/cm^3). The resistance to torsion at the radial diaphysis site was analyzed using the polar density weighed section modulus (SSI_p, mm^3). Muscle density (mg/cm^3) and area (mm^2) were measured at the 66% forearm as indicators of muscle quality and cross-sectional area. Coefficient of variation for the bone parameters after repositioning ranged from 1.2% to 11.1% at 4% site and ranged from 0.5% to 6.6 % at the 66% radius site.

5.3.9 Dual-energy X-ray Absorptiometry

Scans of the non-dominant distal one-third radius and ulna provided areal bone mineral density (aBMD; g/cm^2) using peripheral dual-energy x-ray absorptiometry (pDXA; PIXI; Effective dose of less than 0.1 mSV; GE Medical Systems Lunar, Madison, WI). The pDXA

forearm phantom with a known density (0.433 g/cm^2) yielded a coefficient of variation of 0.01% (30 scans) over the study duration.

5.3.10 Handgrip Strength

Handgrip strength (Hydraulic hand dynamometer, Jamar®, Lafayette, IN, US) was performed at wk 0 and 24 of the RCT. Subjects were instructed to squeeze the handle as hard as they could and were encouraged for 20 seconds each trial. The maximum reading of each trial was recorded. Measurements were done in triplicate, using the non-dominant arm and average values were used. The time used to read and record the data (approximately 15 sec.) was used as a rest period.

5.3.11 Data Analyses

Participant characteristics at baseline were compared using ANOVA and are presented as mean ± SD. All data were audited and tested for normality using the Kolmogorov-Smirnov test. Nonnormal data were log-transformed when required (Eg: OC, CTX, OPG and RANKL). Homogeneity of variance was assessed by using the Bartlett test. Differences among the 3 study groups over time were analyzed using a mixed model ANOVA for repeated measures, controlling for random effects of age in all models, and additionally DAS28 for handgrip strength, followed by post hoc Tukey-Kramer tests adjusted for multiple comparisons. Data were analyzed based on intention-to-treat (ITT) for all outcomes. Statistical analysis of the data was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC), with a significance level of 0.05 after adjustment for multiple comparisons used for all analyses.

5.4 Results

This cohort of male veterans was 89.9±3.3 y of age (range: 80.8–96.8 y; ±SD) and presented with a BMI of 26.3±4.6 kg/m^2. The study took place from the months of October 2011 to June 2012 in a LTC facility where sunlight exposure was minimal for the participants due to mobility limitations. At week -8, the 3 groups consumed comparable amounts of energy, protein, phosphorus and calcium through a balanced diet (Table 5.1). Mean intake of energy was 1719±356 kcal/d with 16% of the energy provided by protein (67±17 g/d). Dietary phosphorus intake was 1209±372 mg/d on average. The Estimate Average Requirements (EAR) of 580 mg/d for phosphorus was met by all but one participant. Only 12 participants did not meet the Recommended Dietary Allowances (RDA) of 700 mg/d of phosphorus for this age group. Mean dietary intake of calcium was 994±424 mg/d from diet alone. Additional forms of supplements increased the mean intake to 1101±449 mg/d. Even with supplementation, the EAR of 1000 mg/d for calcium was not met by 52% of participants. The RDA of 1200 mg/d of calcium for this age group was met in only 38% of the participants. At week-8, mean intake of dietary vitamin D was 291±144 IU/d from food alone. Only 50% of the participants were receiving supplementation with 33% of them receiving 400 IU/d, 5% receiving 800 IU/d and 12% receiving 1000 IU/d or more. This was associated with a mean 25(OH)D concentration of 56.1±13.8 nmol/L and PTH 2.9±1.8 pmol/L for the cohort. No differences in any other dietary were observed during the trial.

The 2000 IU tablet supplementation provided from week -8 through week 0 increased the average 25(OH)D concentrations for the 3 groups above 65 nmol/L. For the RCT groups randomized a priori, there were no differences among groups for serum 25(OH)D before or after the 8 week tablet-supplementation period. During the RCT segment of the study, where fortified

foods were used to provide the vitamin D supplementation instead of tablets, serum 25(OH)D concentration was maintained by the 500 IU/d and the 1000 IU/d supplementation regimens but declined in the placebo group (Table 5.2).

Mean serum PTH, phosphate and total calcium values were within normal ranges at every time point and remained stable. Hypocalcemia was measured in 4 participants (1 in group of 500 IU/L at each time point, for a total of 3 individuals; 1 in group of 1000 IU/L at RCT24) and mildly elevated total calcium was reported in only one participant in the Placebo group at RCT0 (2.56 mmol/L; normal range: 2.10-2.55 mmol/L). None of the biomarkers of bone resorption or bone formation significantly changed over the course of the study and no interactions were observed between groups overtime (Table 5.2).

At the epiphysis region (4% site), a reduction of total vBMD was observed over time, without interaction among groups. Furthermore, a significant decline of trabecular vBMD was reported over time for the 500 IU/d group (Table 5.3). A decline in diaphysis vBMD (66% site) was observed in the 1000 IU/d group (-6.02±19.1 mg/cm^3, $p<0.044$). However, the same group had an increase in total cortical area (4.50±1.07 mm^2, $p=0.0002$). Muscle area declined significantly over time (-117±206 mm^2, $p=0.002$), without interaction. Muscle density, fat and muscle density as well as fat to muscle area ratio did not vary by time or by intervention group (Table 5.3). The mean aBMD of the distal 1/3 radius increased only in the 1000 IU/d vitamin D_3 group at the end of the trial (0.017±0.004 g/cm^2). Handgrip strength declined in the Placebo group and 500/d IU vitamin D_3 groups but not the 1000 IU/d group (-1.3±0.5 kg in Placebo group, -1.8±0.5 kg in 500 IU/d group and 0.3±0.6 kg in the 1000 IU/d group, $p<0.05$) (Figure 5.2). During the course of the RCT, 2 participants sustained fractures: 1 wrist fracture in the 500 IU/d Group and 1 fatal hip fracture in the 1000 IU/d group.

Table 5.1: Characteristics of participants (Mean ±SD)

		Vitamin D_3 Intervention Group						p-values		
Wk	n	Placebo	n	500 IU/d	n	1000 IU/d		Group	Time	Interaction
Age (y)								0.6967	**<.0001**	0.8709
-8	*(n=20)*	89.9 ± 3.8 [A]	*(n=20)*	89.9 ± 2.5 [A]	*(n=20)*	89.1 ± 3.6 [A]				
0	*(n=19)*	90.1 ± 3.8 [B]	*(n=20)*	90.2 ± 2.5 [B]	*(n=19)*	89.4 ± 3.6 [B]				
24	*(n=18)*	90.7 ± 4.0 [C]	*(n=19)*	90.7 ± 2.6 [C]	*(n=17)*	89.6 ± 3.7 [C]				
Weight (kg)								0.7115	0.7776	0.9908
-8	*(n=20)*	77.0 ± 11.8	*(n=20)*	75.2 ± 14.4	*(n=20)*	77.7 ± 14.4				
0	*(n=19)*	77.3 ± 12.4	*(n=20)*	75.7 ± 14.6	*(n=19)*	77.0 ± 14.1				
24	*(n=18)*	78.3 ± 12.3	*(n=19)*	73.7 ± 14.6	*(n=17)*	78.6 ± 14.6				
BMI (kg/m^2)								0.9922	0.8688	0.9993
-8	*(n=20)*	26.3 ± 4.1	*(n=20)*	26.4 ± 5.4	*(n=20)*	26.1 ± 4.3				
0	*(n=19)*	26.3 ± 4.0	*(n=20)*	26.5 ± 5.5	*(n=19)*	26.1 ± 4.1				
24	*(n=18)*	26.7 ± 4.1	*(n=19)*	25.8 ± 5.4	*(n=17)*	26.7 ± 4.6				
Energy (kcal/d)								0.0665	0.4477	0.3615
-8	*(n=20)*	1707 ± 271	*(n=20)*	1610 ± 375	*(n=20)*	1841 ± 387				
0	*(n=19)*	1608 ± 286	*(n=20)*	1617 ± 369	*(n=19)*	1840 ± 366				
24	*(n=18)*	1719 ± 346	*(n=19)*	1542 ± 376	*(n=17)*	1735 ± 366				
Protein (g/d)								0.5185	0.8979	0.2131
-8	*(n=20)*	66.1 ± 13.2	*(n=20)*	62.8 ± 19.5	*(n=20)*	71.5 ± 18.3				
0	*(n=19)*	63.1 ± 16.1	*(n=20)*	64.6 ± 18.9	*(n=19)*	72.4 ± 17.8				
24	*(n=18)*	68.8 ± 18.3	*(n=19)*	68.0 ± 21.2	*(n=17)*	68.0 ± 17.0				
Phosphorus (mg/d)								0.5381	0.7892	0.4097
-8	*(n=20)*	1136 ± 373	*(n=20)*	1203 ± 394	*(n=20)*	1289 ± 349				
0	*(n=19)*	1105 ± 406	*(n=20)*	1224 ± 460	*(n=19)*	1318 ± 325				
24	*(n=18)*	1159 ± 371	*(n=19)*	1203 ± 394	*(n=17)*	1220 ± 294				
Calcium Intake (mg/d)								0.8685	0.6748	0.8889
-8[1]	*(n=20)*	1046 ± 437	*(n=20)*	1152 ± 487	*(n=20)*	1104 ± 437				
0[2]	*(n=19)*	1022 ± 524	*(n=20)*	1060 ± 507	*(n=19)*	1125 ± 366				
24[3]	*(n=18)*	1090 ± 362	*(n=19)*	1132 ± 459	*(n=17)*	1047 ± 264				
Vitamin D Intake (IU/d)								**<.0001**	**<.0001**	**<.0001**
-8[1]	*(n=20)*	494 ± 380 [a]	*(n=20)*	769 ± 526 [b]	*(n=20)*	562 ± 383 [ac]				
0[2]	*(n=19)*	2310 ± 218 [d]	*(n=20)*	2337 ± 222 [de]	*(n=19)*	2348 ± 200 [df]				
24[3]	*(n=18)*	295 ± 124 [g]	*(n=19)*	869 ± 345 [bh]	*(n=17)*	1316 ± 84 [i]				

[1] -8 wk includes diet and prescribed tablet supplementation; [2] 0 wk includes diet and 2000 IU tablet supplementation.
[3] 24 wk includes diet and specially formulated fortified food; Mixed Model ANOVA for repeated measures, controlling for age.
Values with different superscripts are different, $p<0.05$; Capital superscripts show effect of time.

Table 5.2: Biomarkers of calcium and bone metabolism (Mean ±SD)

		Vitamin D_3 Intervention Group						p-value		
Wk	n	Placebo	n	500 IU/d	n	1000 IU/d		Group	Time	Interaction
25-Hydroxyvitamin D (nmol/L)								0.0358	0.0001	**<0.0004**
-8	*(n=20)*	56.9 ± 13.3 [ae]	*(n=20)*	54.6 ± 15.0 [a]	*(n=20)*	56.9 ± 9.7 [a]				
0	*(n=19)*	69.9 ± 9.7 [bf]	*(n=20)*	65.2 ± 13.8 [be]	*(n=19)*	69.6 ± 12.8 [bd]				
24	*(n=18)*	56.2 ± 10.6 [ag]	*(n=19)*	74.0 ± 16.5 [cdf]	*(n=17)*	79.6 ± 17.2 [c]				
PTH (pmol/L)								0.6641	0.6946	0.0663
-8	*(n=20)*	2.946 ± 1.693	*(n=20)*	3.055 ± 1.786	*(n=20)*	2.817 ± 2.008				
0	*(n=19)*	2.796 ± 1.875	*(n=20)*	3.063 ± 1.970	*(n=19)*	2.680 ± 1.389				
24	*(n=18)*	3.241 ± 2.154	*(n=19)*	3.372 ± 3.188	*(n=17)*	2.816 ± 1.087				
Total calcium								0.7681	0.2161	0.2434
-8	*(n=20)*	2.29 ± 0.10	*(n=20)*	2.25 ± 0.10	*(n=20)*	2.27 ± 0.05				
0	*(n=19)*	2.29 ± 0.11	*(n=20)*	2.28 ± 0.12	*(n=19)*	2.28 ± 0.07				
24	*(n=18)*	2.29 ± 0.11	*(n=19)*	2.28 ± 0.13	*(n=17)*	2.26 ± 0.09				
Phosphate								0.4091	0.3642	0.1179
-8	*(n=20)*	1.20 ± 0.14	*(n=20)*	1.13 ± 0.18	*(n=20)*	1.15 ± 0.10				
0	*(n=19)*	1.20 ± 0.15	*(n=20)*	1.17 ± 0.21	*(n=19)*	1.16 ± 0.10				
24	*(n=18)*	1.19 ± 0.14	*(n=19)*	1.17 ± 0.18	*(n=17)*	1.12 ± 0.12				
Osteocalcin (nmol/L)								0.7312	0.7183	0.0654
-8	*(n=20)*	5.092 ± 2.447	*(n=20)*	4.390 ± 4.684	*(n=20)*	3.469 ± 1.832				
0	*(n=19)*	4.832 ± 2.230	*(n=20)*	4.646 ± 4.371	*(n=19)*	3.423 ± 1.909				
24	*(n=18)*	4.480 ± 2.158	*(n=19)*	4.161 ± 3.593	*(n=17)*	3.332 ± 2.746				
C-Telopeptides (nmol/L)								0.7698	0.9892	0.1201
-8	*(n=20)*	0.7952 ± 0.5196	*(n=20)*	0.5166 ± 0.3265	*(n=20)*	0.6062 ± 0.3775				
0	*(n=19)*	0.7690 ± 0.4419	*(n=20)*	0.5381 ± 0.3436	*(n=19)*	0.5977 ± 0.3645				
24	*(n=18)*	0.7038 ± 0.4305	*(n=19)*	0.5944 ± 0.4311	*(n=17)*	0.5830 ± 0.3906				
RANKL (pg/mL)								0.3108	0.6444	0.6704
-8	*(n=20)*	35.05 ± 38.72	*(n=19)*	43.15 ± 73.17	*(n=20)*	33.18 ± 43.39				
0	*(n=19)*	36.66 ± 38.85	*(n=20)*	37.88 ± 55.47	*(n=19)*	33.61 ± 44.63				
24	*(n=18)*	31.97 ± 36.32	*(n=19)*	79.98 ± 217.3	*(n=17)*	34.75 ± 34.91				
Osteoprotegerin (pg/mL)								0.6973	0.2604	0.7199
-8	*(n=20)*	972 ± 277	*(n=20)*	1059 ± 365	*(n=20)*	956 ± 330				
0	*(n=19)*	990 ± 335	*(n=20)*	1169 ± 630	*(n=19)*	1078 ± 423				
24	*(n=18)*	980 ± 278	*(n=19)*	1010 ± 278	*(n=17)*	946 ± 294				
RANKL/OPG ratio								0.3958	0.6240	0.8525
-8	*(n=20)*	0.040 ± 0.051	*(n=20)*	0.035 ± 0.053	*(n=20)*	0.040 ± 0.061				
0	*(n=19)*	0.044 ± 0.063	*(n=19)*	0.043 ± 0.094	*(n=19)*	0.034 ± 0.045				
24	*(n=18)*	0.040 ± 0.064	*(n=19)*	0.077 ± 0.205	*(n=17)*	0.047 ± 0.012				

Mixed Model ANOVA for repeated measures, controlling for age; Values with different superscripts are different, $p<0.05$.

Table 5.3: Bone mineral density and muscle parameters of the non-dominant forearm (Mean ±SD)

			Vitamin D_3 Intervention Group						p-values		
	Wk	n	Placebo	n	500 IU/d	n	1000 IU/d	Group	Time	Interaction
Radius (mm)										
	0	*(n=19)*	263.9 ± 9.7	*(n=20)*	259.5 ± 9.3	*(n=18)*	260.6 ± 17.5	0.5215		
pQCT – Radius Epiphysis										
Total vBMD (mg/cm^3)	*0*	*(n=19)*	297.8 ± 73.8 ^{A}	*(n=19)*	303.7 ± 91.6 ^{A}	*(n=18)*	279.0 ± 50.5 ^{A}	0.5422	**0.0364**	0.5057
	24	*(n=17)*	288.1 ± 78.4 ^{B}	*(n=19)*	295.1 ± 87.9 ^{B}	*(n=17)*	273.2 ± 50.0 ^{B}			
Trab vBMD (mg/cm^3)	*0*	*(n=19)*	195.1 ± 65.2 ^{A}	*(n=19)*	193.2 ± 62.0 ^{A}	*(n=18)*	182.5 ± 45.0 ^{A}	0.7558	**0.0081**	0.4541
	24	*(n=17)*	193.5 ± 62.4 ^{B}	*(n=19)*	184.4 ± 56.4 ^{B}	*(n=17)*	181.0 ± 44.7 ^{B}			
Total Area (mm^2)	*0*	*(n=19)*	444.9 ± 68.6	*(n=19)*	452.1 ± 85.8	*(n=18)*	455.2 ± 77.2	0.9506	0.0689	0.4312
	24	*(n=17)*	469.5 ± 95.8	*(n=19)*	465.7 ± 87.6	*(n=17)*	465.4 ± 70.5			
pQCT – Radius Diaphysis										
Cortical vBMD (mg/cm^3)	*0*	*(n=19)*	1059 ± 51 ^{ab}	*(n=18)*	1066 ± 68 ^{ab}	*(n=18)*	1063 ± 47 ^{a}	0.6956	**0.0196**	**0.0442**
	24	*(n=16)*	1051 ± 46 ^{ab}	*(n=19)*	1066 ± 68 ^{ab}	*(n=16)*	1055 ± 44 ^{b}			
Total Area (mm^3)	*0*	*(n=19)*	184.6 ± 22.6 ^{A}	*(n=18)*	170.1 ± 25.2 ^{A}	*(n=18)*	177.0 ± 27.3 ^{A}	0.3068	**0.0001**	0.1350
	24	*(n=16)*	187.4 ± 26.1 ^{B}	*(n=19)*	175.6 ± 27.0 ^{B}	*(n=16)*	186.8 ± 31.9 ^{B}			
SSI_P	*0*	*(n=19)*	396.7 ± 83.8	*(n=18)*	360.8 ± 82.0	*(n=19)*	387.0 ± 110.7	0.4678	0.0816	0.2009
	24	*(n=16)*	407.0 ± 98.1	*(n=19)*	366.2 ± 84.5	*(n=17)*	403.2 ± 106.1			
Muscle Area (mm^2)	*0*	*(n=19)*	3203 ± 558 ^{A}	*(n=18)*	3085 ± 389 ^{A}	*(n=18)*	3124 ± 615 ^{A}	0.5838	**0.0002**	0.6485
	24	*(n=16)*	3116 ± 617 ^{B}	*(n=19)*	2940 ± 480 ^{B}	*(n=16)*	3098 ± 523 ^{B}			
Muscle Density (mg/cm^3)	0	*(n=19)*	69.58 ± 4.34	*(n=18)*	67.99 ± 4.12	*(n=19)*	67.21 ± 5.99	0.2175	0.7888	0.9855
	24	*(n=16)*	69.18 ± 4.34	*(n=19)*	67.66 ± 2.84	*(n=17)*	68.04 ± 5.19			
Fat and Muscle Density	0	*(n=19)*	54.87 ± 8.95	*(n=18)*	53.71 ± 6.87	*(n=19)*	53.78 ± 6.87	0.8233	0.3586	0.4610
	24	*(n=16)*	54.31 ± 9.67	*(n=19)*	53.73 ± 7.54	*(n=17)*	54.00 ± 6.47			
Fat to Muscle Area Ratio	0	*(n=19)*	37.54 ± 23.00	*(n=18)*	36.78 ± 14.98	*(n=19)*	33.50 ± 10.46	0.8193	0.6653	0.3215
	24	*(n=16)*	37.85 ± 20.22	*(n=19)*	37.04 ± 16.35	*(n=17)*	34.21 ± 11.45			
Bone to Muscle Area Ratio	0	*(n=19)*	6.72 ± 1.27 ^{A}	*(n=18)*	6.56 ± 1.47 ^{A}	*(n=19)*	6.65 ± 1.11 ^{A}	0.9500	**0.0002**	0.6412
	24	*(n=16)*	6.86 ± 1.21 ^{B}	*(n=19)*	6.86 ± 1.67 ^{B}	*(n=17)*	6.93 ± 1.30 ^{B}			

One participant in the Placebo and one participant in the 500 IU groups were excluded at 4% due to movement artifact; Mixed Model ANOVA for repeated measures, controlling for age; Values with different superscripts are different, $p<0.05$; Capital superscripts show effect of time.

Figure 5.1: Consort diagram

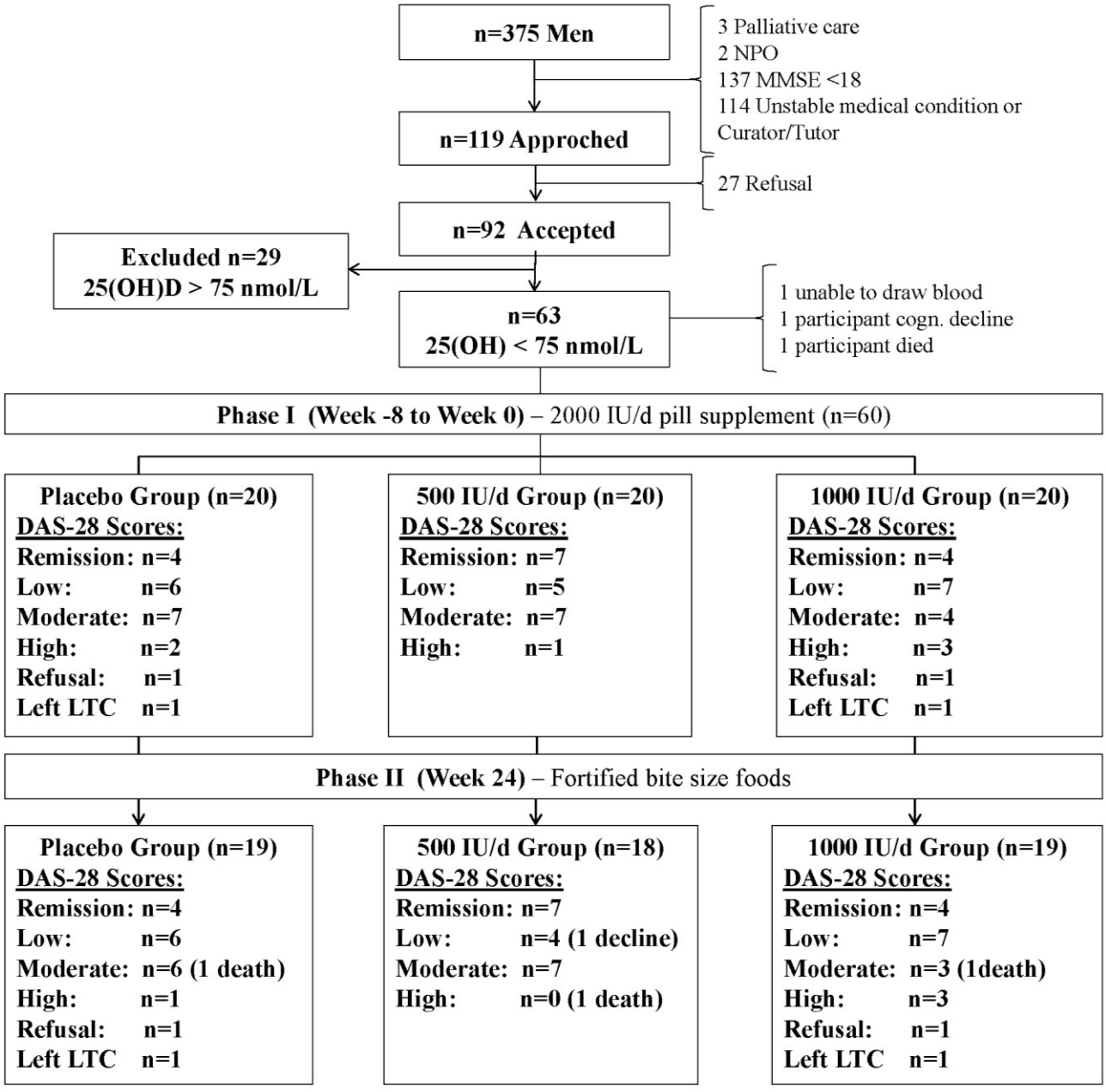
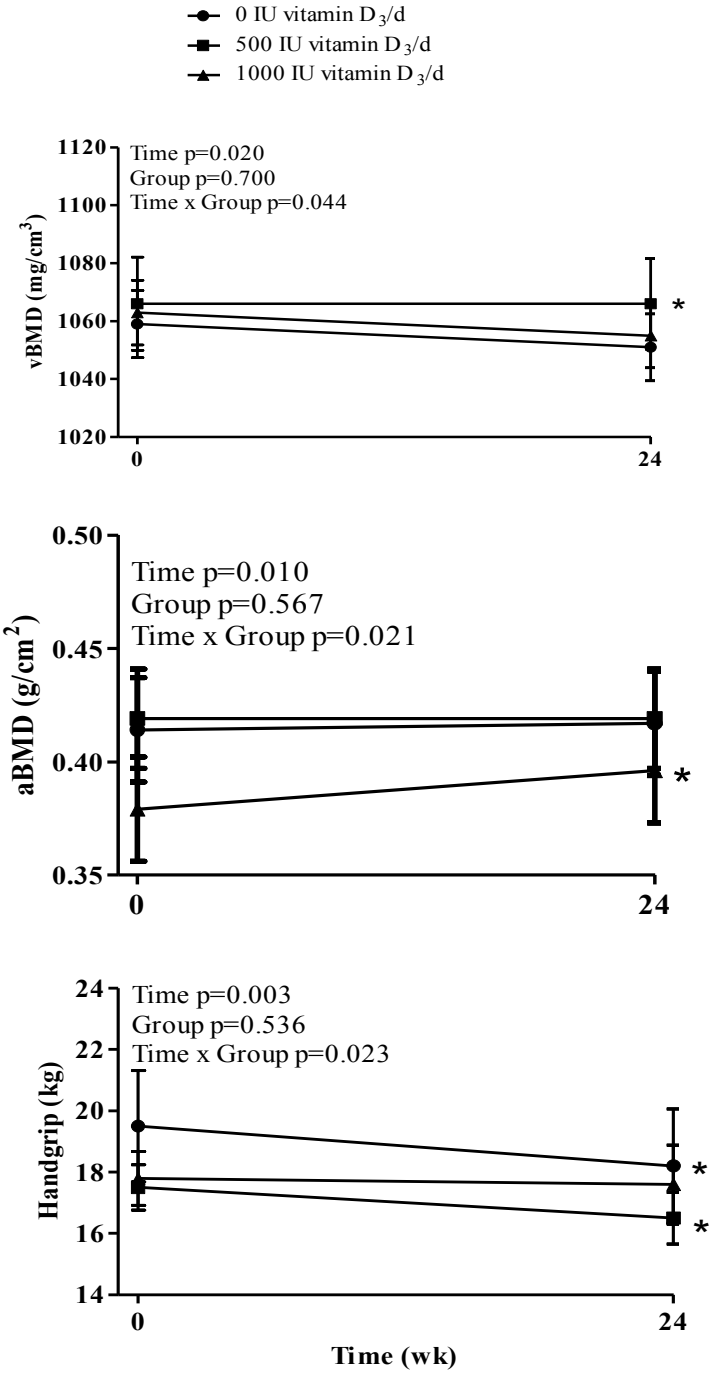


Figure 5.2: Forearm vBMD – 66% diaphysis (top), distal pQCT (center) and handgrip strength (bottom) in men > 80 y of age randomized to placebo, 500 or 1000 IU vitamin D_3/d over 24 weeks.

* $p<0.05$: Differences over time within group.



5.5 Discussion

Osteoporosis and bone health was historically studied in women and thus a care gap for fragility fractures in men exists [324]. The present study recruited very old men exclusively and thus, provides a rare portray of bone health in Caucasian male octogenarians and nonagenarians living in a LTC facility. The benefits of improving and maintaining vitamin D status through supplemental approaches were observed only at the 66% site of the diaphysis of the forearm aBMD and handgrip strength of the group receiving 8 weeks daily supplementation of 2000 IU followed by 24 weeks of 1000 IU of vitamin D_3. Based on the low trabecular vBMD, the increase in aBMD was likely due to increments in cortical bone mass. Interestingly, cortical area increased in all groups over time with the largest increase observed in the 1000 IU/d group who consequently showed declines in cortical vBMD. The direction of change in total bone area and areal density in the 1000 IU vitamin D_3/d intervention group suggests that given a longer intervention period, cortical density at the 66% site of the diaphysis of the forearm may improve as well.

Reports of 25(OH)D concentrations and bone health in the older population living in LTC are scarce in the literature. Recent meta-analyses indicate that 70% or more of the pooled data comes from community dwelling women [1, 6, 11, 354, 421-423]. Large prospective studies such as the MrOs study (n=5995; United States) and CHAMP study (n=1705; Australia) were designed to gather more information on bone health in older men living in the community. In a sub-study analysis of the MrOs study group, Cauley et al. [424] revealed that 50% of their cohort had serum 25(OH)D concentrations less than 62.5 nmol/L (n=1606). The Australian men of the CHAMP study reported a serum concentration of 25(OH)D of 55.7±21.9 nmol/L [425] which is similar to the results at the onset of this study of much older men living in LTC. In 2000, a national survey done in England [426] reported that older men in institutions had a mean 25(OH)D concentration of

37.1±1.8 nmol/L (±SE; n=128) whereas women had a mean 25(OH)D concentration of 36.6±1.0 nmol/L (±SE; n=369). More recently, Curtain and al. [427] reported on residents of 31 nursing homes in Australia. The population of 811 older adults (Men: 62%; mean age: 84±9 y) had a mean 25(OH)D concentration of 61.6±26.5 nmol/L and 31% of them had a serum 25(OH)D value below 50 nmol/L. Thirty percent of these institutionalized seniors did not receive vitamin D supplementation which is lower than what was observed in this trial. In The Netherlands, Veleva et al. documented serum 25(OH)D concentrations of 77±30 nmol/L in 71 nursing residents receiving vitamin D supplementation of 800 IU/d for at least 3 months (Men: 35%; mean age: 83±7 y) [265]. This concurs with the mean 25(OH)D concentrations that were maintained by the supplemented groups in this RCT (74.0±16.5 nmol/L and 79.6±17.2 nmol/L for the 500 IU/d group and 1000 IU/d group, respectively). Collectively, these studies demonstrate the maintained physiological capacity to absorb and further metabolize vitamin D in the very old male population and that the capacity to support bone health is promising as well. Conversely, the intake of approximately 300 IU/d of vitamin D by the Placebo group (310 IU/d without the 2000 IU of supplementation at RCT0 and 295 IU/d at RCT24) was insufficient to maintain vitamin D status in this cohort.

Bone mineral density decreases with age [428].Various measures of bone geometry declined over time, without interaction by group, for this cohort of very old men. Only cortical vBMD differentially decreased over time according to group. Combined with the increased cortical area, this suggests that if serum 25(OH)D is maintained on average at 75 nmol/L, further benefits in mineralization would translate into greater vBMD. Nevertheless, aBMD of the 66% site of the diaphysis of the forearm increased in the 1000 IU vitamin D_3/d group. Although vitamin D is well known for its impact on bone health, recent meta-analyses [1-3, 5, 11, 16, 17] disagree on the impact of vitamin D on BMD and calcium is reported as a key co-factor to bone health and

prevention of fractures [1, 6, 17, 429]. In the context of this RCT, dietary intakes were designed to meet the RDA for calcium. However, contrary to our preliminary work [385], the dietary intake assessments revealed that not all of the participants consumed all of the foods served in order to meet the DRIs for calcium (diet + supplement). This could have affected the full potential of the vitamin D supplementation, in both the loading segment of the study and during the RCT.

In LTC facilities, challenging conditions such as advanced aging, decreased nutritional status and difficulty to meet the nutritional needs [65, 67, 68] as well as decreased mobility [430] are not conducive to systematic serial DXA assessment of BMD, although fragility fractures are an important concern [431]. It is for this reason that we utilized two peripheral assessment techniques to measure BMD and tissue composition. Recently, publications have highlighted the importance of male osteoporosis and the impact of testosterone on bone health [50, 432, 433]. It is believed that bone loss is less rapid in men than in women [334, 434] and that loses begin in the sixties with an average rate of 0.5% to 1% per year [435]. Interestingly, based on the pDXA and pQCT assessments, cortical density appears to be relatively constant in this age group, at least over 24 weeks. However, based on the pQCT data, trabecular vBMD declined at a rate of 1.8% over 24 weeks. Normative data for men over 80 y of age is limited [436]. Nonetheless, these data underscore the very low and rapidly declining trabecular BMD in advanced aging men and the pressing need for more data on older men to help manage osteoporosis and prevent fractures.

The minimal changes in cortical vBMD across the present study are supported by stable values over time for multiple markers of mineral metabolism, bone resorption and formation. Biomarkers of bone turnover are envisioned by research teams and clinicians as eventual tools to assess risk of fracture and help explain bone loss to complement measures of BMD [200, 437, 438]. However, they are affected by various factors influencing their variability such as circadian and

seasonal variation, food intake, physical activity and age, sex, vitamin D deficiency and other determinants of bone health [200]. This protocol allowed for control of several of these preanalytical influences: serum collection was done in the early morning and participants were fasted. The participants had generally limited physical activity and were all very old men. The lack of change in bone resorption and formation markers and stable cortical BMD are consistent with the normal PTH concentrations observed across the study. In a younger community-dwelling cohort, Marques et al. [206] looked at serum biomarkers of bone health (49% men; age: 68.2±5.2 y; BMI: 29.2±3.4 kg/m^2) and exercise. The pre-training results on average were 2.4±0.5 nmol/L for OC, 0.123±0.04 nmol/L for CTX, 432±126 pg/mL for osteoprotegerin, 27.2±9.7 pg/mL for RANKL and 0.057±0.146 pg/mL for the RANKL:OPG ratio, with no change reported at the end of the trial. When compared to the results of the present study (Table 5.2), the younger male cohort had lower concentrations of bone formation and much lower bone resorption markers consistent with an overall low rate of bone turnover and perhaps stronger bones. Nevertheless, they could not show a significant relationship between these biomarkers and BMD of the hip or lumbar spine. In advanced aging, higher bone turnover is likely reflective of lower physical activity levels and reduced muscle mass as well as insufficient vitamin D dietary intakes.

Frailty is defined as a state of vulnerability leading to functional deterioration [343]. Sarcopenia, the decline in function with low muscle mass, is a major cause of frailty and vitamin D is believed to have an important impact on this aging syndrome [343-346]. Concentration of 25(OH)D of less than 50 nmol/L was associated with reduced muscle mass [347] and physical function [341] in the older population. Vitamin D supplementation was reported to augment muscle fiber size [348, 349], increase intramyonuclear vitamin D receptors [348, 350] and improved strength and functional capacities [264, 338, 351]. Nevertheless, meta-analyses present conflicting

results when looking at vitamin D and muscle strength. Some show positive effect of vitamin D supplementation on strength [352, 353] or no impact of supplementation [354, 355]. Despite vitamin D concentration above 50 nmol/L and supplementation up to 2000 IU/d of vitamin D_3, this RCT reported decreases in muscle area over time for all groups which concurs with previous reports [372, 439]. However, handgrip strength was maintained only in the group receiving 1000 IU/d. This could mean that vitamin D supplementation help increased the contraction strength within the remaining fibers. Further research is required to explain this finding.

Overall this comprehensive novel study has given new insight into the amount of vitamin D needed in LCT to achieve benefits to musculoskeletal health measures. This RCT is not without limitations. As opposed to community-dwelling cohorts, the participants were continuously monitored for their health and the required services were offered for their activities of daily living. All of the participants had fair skin and limited amount of sunlight exposure. Thus the results may not be generalizable as they are reflective of a very specific segment of the population which is very old men living in a LCT facility. The RCT segment was single blind. It was designed to demonstrate short-term improvements in a dose-response design prior to commencing longer-term studies, but was too short to realize the longer-term benefits to vBMD.

5.6 Conclusion

In summary, this study provides important insight for dietary intake, vitamin D status and response to supplementation as well as musculoskeletal health in Caucasian male octogenarians and nonagenarians living in LTC facilities. First, with a Loading phase and a RCT segment testing maintenance dosages, this protocol provides a novel approach to the assessment of vitamin D status in the older population and demonstrate that supplementation of 500 IU/d of vitamin D_3 was sufficient to maintain status in vitamin D in this very old male cohort. Subsequently, it was reported

that supplementation of 1000 IU/d of vitamin D_3 modestly improved aBMD and maintained handgrip strength. Regardless of the supplemental group, BMD assessed by pDXA was relatively stable whereas trabecular vBMD demonstrated rapid declines in these older men. Despite declines in muscle area, handgrip strength was maintained in those receiving supplementation of 1000 IU/d of vitamin D. The results of this study suggest that simple measures of muscle strength such as handgrip strength would enable assessment of responses to longer-term interventions in LTC designed to reduce the risk of falls and consequent fractures. Further research needs to develop complementary assessment tools for osteoporosis and treatment, prevention of falls and fractures.

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The authors' contributions were as follows—IG and HAW: conceived the study and obtained funding, coordinated and led the data collection, oversaw laboratory analyses, conducted the data analysis, provided technical oversight and input into all aspects of the study and had primary responsibility for the final content, drafted the research manuscript; IG, TH and CV: performed pDXA and pQCT scans and data audits; SA and CB: conducted laboratory analyses; ML: designed and validated the vitamin D food appreciation questionnaire, led interviews and wrote final report; and all authors: contributed to the review and revision of the manuscript and read and approved the final manuscript. All other authors declared no conflicts of interest related to this study.

CHAPTER 6: OVERALL SUMMARY AND CONCLUSIONS

6.1 Main Outcomes and Hypotheses

The main goal of this research was to assess the dietary intake of vitamin D and its impact on vitamin D status and markers of bone metabolism in older men living in a long-term facility (LTC). Study 1 was planned to assess the vitamin D intake, including food and supplement, during the fall and winter seasons and measure the physiological relationship with vitamin D status over the course of several months in very old men. As the literature seldom reports the sources of vitamin D in LTC, study 1 provided detailed dietary data to guide the next intervention and added to the limited body of research in nutrition and geriatric care. Thus, once the dietary habits and the concentration of serum 25-hydroxyvitamin D (25(OH)D) of older men were better documented, it served as solid evidence to design the next steps. Study 2 was then designed to achieve healthy vitamin D status using routine tablet-based supplementation followed by a dose-response study to maintain vitamin D status using a randomized placebo-controlled dose-response trial (RCT) of specially fortified foods. The physiological response to 2000 IU/d of vitamin D_3 in very old men in LTC was assessed in the loading phase of Study 2 using a before after design. A 24 week RCT followed. The RCT aimed at evaluating the capacity of novel and varied foods (muffins, puddings and smoothies) fortified in vitamin D_3, with 2 different levels of fortification, 500 IU and 1000 IU and served daily at breakfast, to maintain the improved 25(OH)D. Identical unfortified food options were served as Placebo. In Study 2, we also used peripheral dual-energy X-ray absorptiometry (pDXA) to measure areal bone mineral density (aBMD), peripheral quantitative computed

tomography (pQCT) to measure volumetric bone mineral density (vBMD) and body composition as well as handgrip strength for muscle function.

Study 1 confirmed that insufficient dietary intake of vitamin D from food and meal supplements remains a pressing problem in LTC. The mean vitamin D intake was just over 430 IU per day. This was below both the 600 IU/d recommended Adequate Intake (AI) at the time of the study and the new Institute of Medicine (IOM) Recommended Dietary Allowances (RDA) of 800 IU/d for this population. Only one participant met the 600 IU of vitamin D with food alone. The consumed dietary vitamin D was approximately 30% lower than what was actually sent on their tray, revealing that the appetite of these seniors needs to be considered seriously when designing an intervention with this population. The four main sources of vitamin D were enriched homemade milk, meal supplements, powdered milk enriched cream soups and margarine in preparation and sauces. Although the mean serum 25(OH)D was above 50 nmol/L in these older men, values of less than 37.4 nmol/L were observed in more than 20% of the population. This was present despite the fact that medical, clinical and nutritional supervision was available in the institution. The biomarkers of bone metabolism were stable over the fall to winter period. However, a seasonal variation was observed with higher 25(OH)D concentration values in early fall and lower 25(OH)D concentration values in the winter. Only 33% of the participants had a vitamin D prescription. Minimally, this was not respecting Canada's Food Guide recommendation of 400 IU/d for adults over the age of 50 y. This confirmed the hypothesis that the supervision LTC facility milieu would not provide additional protection against poor vitamin D intake and status.

At the onset of Study 2, we noted that 40 % of the participants were receiving vitamin D tablet supplementation. Serum values of 25(OH)D obtained at screening averaged 56.1±13.8 nmol/L. Yet, serum concentrations of 25(OH)D of less than 50 nmol/L were reported in 33% of the participants. As the trial commenced, all supplementation was ceased and the 8-wk loading dose of 2000 IU/d vitamin D supplementation in the form of a tablet was started. The loading period was successful in raising 25(OH)D to an average value of over 65 nmol/L in men over 88 y of age. Although compliance was ensured by dispensing the supplement daily, as part of the morning medication round, a variable response to the intervention was observed. At the beginning of the RCT segment of the study, 5 participants had a vitamin D concentration of less than 50 nmol/L (Placebo group: n=1; 500 IU/d group: n= 3 and 1000 IU/d group: n=1). Only 8.6% of the participants reached the 75 nmol/L and this refuted the proposed hypothesis that 100% of participants would reach that mark. At week 24 of the RCT, 6 participants in the Placebo group and 2 participants in the 500 IU/d group had a 25(OH)D concentration of less than 50 nmol/L. None of the participants in the 1000 IU/d group had a 25(OH)D concentration value of less than 50 nmol/L. Contrary to our hypothesis for the RCT, both the 500 IU/d and 1000 IU/d fortified foods maintained 25(OH)D over the course of the 24-wk and some continued to rise resulting in an average of 75 to 80 nmol/L of 25(OH)D. These results confirm that large dosages are not required for the maintenance of adequate status. Nearly 80% of the participants were interested in consuming vitamin D fortified foods and 81.4% explicitly said they would prefer the food carriers over taking a tablet supplement. When considering quality of life in this population, providing fortified foods, rather than one more pill, would be an enjoyable

option. Despite, the intake of 2000 IU/d for 8 weeks and 500 IU/d or 1000 IU/d for 24 weeks, biomarkers of bone metabolism did not change over time in any of the groups. Parathyroid hormone (PTH), phosphate, calcium and ionized calcium remained in the normal range throughout Study 2 confirming safety. The aBMD increased and the handgrip strength also improved in the 1000 IU/d group. The pQCT revealed that total area at the diaphysis site (66% site) increased in the 1000 IU/d group. But, the 24 weeks of supplementation was not sufficient to impact the cortical vBMD and thus vBMD decreased in the 1000 IU/d group.

Although variability in dose-response was observed, it appears that the supplementation of vitamin D to the level of 600 IU per day as recommended by the IOM is sufficient to maintain vitamin D status at or above 40 nmol/L. In addition, reaching the RDA of 800 IU/d readily supported maintenance of 25(OH)D over 50 nmol/L. Nevertheless, the adequate circulating 25(OH)D concentration required to improve bone health in seniors was not fully addressed in this thesis. The research generated shows promising results that will need to be tested in longer trials. The new Canadian guidelines for prevention of fractures in LTC [382], including vitamin D supplementation, will need to be realized through continued effective knowledge transfer activities as vitamin D intake from dietary sources is limited in this population living in LTC facilities. Training modules and webcasts are available for professionals at Osteoporosis Canada (http://www.osteoporosis.ca/health-care-professionals/).

6.2 Vitamin D and Bone Health in Seniors

As reported by the IOM in 2011 [17, 220], a care gap exists in the knowledge base of vitamin D intake and status in older men, in the community and in LTC. This stems from the fact that bone health was traditionally studied in the context of menopause. The Canadian Community Health Survey (CCHS) [224] reported that community dwelling older men over the age of 70 in Canada consumed 252 ±1.6 IU/d of vitamin D. The Canadian Multicentre Osteoporosis Study (CaMos) research group reported dietary intakes of vitamin D of 436±28 IU/d for men of 71 y or more, when food and supplements were considered [229]. A few studies [63, 64, 66] looked at dietary intake of vitamin D in Canadian LTC facilities. The dietary intake of vitamin D ranged from 254 to 295 IU/d. Others looked at how the planning of the menu could influence the provision of foods containing vitamin D [65, 67, 68] and revealed they could not meet the IOM recommendations for vitamin D. All the studies reported inadequate menu content and low intake of vitamin D. Study 1 confirmed that these concerns continue to be relevant to LTC. This situation prevails despite the presence of clinical staff and supervision in LTC facilities. Insufficient vitamin D intake will reduce absorption of calcium, resorption of bone, reduced BMD and possibly limit muscle function leading to osteoporosis, falls and fractures. Furthermore, it can also lead to osteomalacia and muscular pain which can impede mobility and reduced quality of life.

Vitamin D is inexpensive and an easily accessible supplement. However, it is one more ‘pill’ that is added to the common polypharmacy that is often the burden of seniors [402, 406, 440]. The use of a small portion (30 mL) of pudding or smoothie and mini-muffins of 30 g provided a small addition to breakfast and did not alter the dietary intake

of the participants. The vast selection available allowed for the respect of personal preferences and flexibility required to provide foods to clients presenting with dysphagia. The food was appreciated and well consumed. Nutritionists, pharmacists and physicians need to improve vitamin D status in the older population by adequate assessment of intake and adequate supplementation. The option of fortifying foods that are appreciated by seniors and accessible by LTC facility administrators would represent a viable and pleasant alternative.

Study 2 was designed before the publication of the 2011 IOM report [17] on vitamin D and calcium and before the publication of the 2015 recommendations of Osteoporosis Canada regarding fractures in LTC [220]. The loading dose 2000 IU/d of vitamin D_3 used was equivalent to the Tolerable Upper Intake Level (UL) proposed by the IOM in 2007 for vitamin D. During the 8 weeks of supplementation, a dosage of 2000 IU/d of vitamin D was well tolerated. All biomarkers of bone metabolism were stable and within their normal ranges. However, it did not raise 25(OH)D above the 75 nmol/L for all of the participants as anticipated. Only 8.6% of these older men reached a level over 75 nmol/L and 8.3% did not surpass the 50 nmol/L concentration. Perhaps this was due to the 8 weeks duration as discussed in chapter 5 or due to genetic variation. Genetic profiling was not assessed at screening and might explain part of the variability in response. The vitamin D response index [441] is a new concept that is now suggested in explaining the variability in vitamin D dose-response observed in the literature. This is based on the fact that vitamin D activates the transcription factor vitamin D receptor via 1α,25-dihydroxyvitamin D. This has a direct effect on the epigenome and transcriptome of many tissues and cell types. Individuals can be identified as high, mid and low

responders to vitamin D by measuring vitamin D sensitive molecular parameters, such as mobile immune cells from blood or the level of proteins or metabolites in serum. This could bring a new explanation to the variability in response seen in this research. Lastly, this limited increase in 25(OH)D for some participants could also be due to physiological mechanisms yet to be determined in older men.

During the RCT segment of Study 2, a dose-response was observed and participants receiving the Placebo foods reverted back to their RCT -8 wk value within 2 months. Both dosages, 500 IU/d and 1000 IU/d, maintained 25(OH)D status. However, when looking at individual results, 2 participants of the 500 IU/d group had decline to serum concentration of 25(OH)D below 50 nmol/L. This suggests that a supplement of 1000 IU/d of vitamin D_3 might be a more effective dose to maintain 25(OH)D status in older men. However in this very old cohort, one participant reach a serum concentration of 25(OH)D above 125 nmol/L. Other sources of variability could have been sunlight exposure. As Study 2 took place from fall to early summer and sunlight exposure was limited due to poor mobility and clothing, it is believed that endogenous production of vitamin D was minimal and did not influence the serum 25(OH)D concentrations reported.

Measuring BMD in very old people presents a number of challenges. Several of the men were wheel-chair bound or necessitated help in mobilizing (walker or cane). Therefore, transfer to the bed of traditional densitometry equipment or positioning for adequate scanning would be nearly impossible. Also, access to the equipment is not standard in LTC facilities. Using the pDXA for the wrist and the pQCT for the non-dominant forearm allowed for imaging without having to transfer the participants to a

different facility and did not require important transfers onto the bed of a DXA. A number of limitations remained. Careful positioning was required, considering the decreased flexibility in these participants. Fatigue and difficulty in following commands in frail and very old individuals were also factors to consider during the scanning period, mostly for the pQCT assessments. Finally, uncontrollable tremors were observed for 2 participants. This resulted in diminished image quality and required the exclusion of these scans from the pool of acceptable data.

The aDXA of the wrist detected an increase in BMD for the group of 1000 IU/d, after controlling for age. The vBMD as well as trabecular BMD were decreased at the 4% site (epiphysis) and vBMD at the 66% site (diaphysis) in all the groups under study. However in the 1000 IU/d group, the pQCT values confirmed an increase in total diaphysis cross-sectional area, from 177±27 mm^3 to 187±32 mm^3 ($p< .0001$) at the 66% site. It is possible that a longer period would have been required to adequately mineralize the bones in such an old population. Furthermore, calcium intake was not optimized during the course of the study. Future research would benefit from encouraging calcium intakes at the RDA levels while supplementing vitamin D.

The results of pQCT also provided information on muscle composition. Muscle area was decreased in all groups at the end of the RCT. Muscle density was not affected by any of the vitamin D supplementation regimens. The handgrip measure significantly increased in the 1000 IU/d group, after controlling for age and Disease Activity Score (DAS) 28 scores. The DAS28 evaluation for rheumatoid arthritis was measured using erythrocyte sedimentation rate. This score also reflects tenderness or pain felt in 28 joints, mainly situated in the upper body (fingers, wrists, elbows, shoulders and knees), and

which was believed to impact handgrip strength measurements. With an increase in handgrip strength and a decrease in muscle density, we could suspect that vitamin D positively influenced the muscle strength generated. These results concur with a recent meta-analysis [352] that concluded that supplementation of vitamin D provided a small, but positive effect of on global muscle strength. The results are unique in that prior to this work only 2 Canadian LTC studies included men of this age group. The dynamometer assessment detected this change. Better muscle function could translate into less falls and less fractures.

6.3 Vitamin D and Sarcopenia

In LTC facilities, a number of factors could influence bone health, falls and fractures in frail individuals. Sarcopenia, the decline in muscle mass, is now recognized as an important contributor to frailty. Adequate nutrition, vitamin D status and exercise were identified as elements to monitor in frailty [343]. Furthermore, vitamin D status was associated to decrease of physical function in older men [341]. Although, vitamin D supplementation was not associated to increase muscle mass, supplementing vitamin D in depleted older women increased muscle strength in past research [336, 348, 351, 353]. More research is required to monitor change in muscle mass and muscle strength in correlation to vitamin D intake and supplementation, with and without exercise, over an extended period of time. LTC facilities could provide the adequate environment to conduct such studies as several professionals could be implicated (physicians, nutritionists, nurses, occupation therapist and physiotherapists). Improved vitamin D status and physical capacities would possibly reduce the falls and prevent fragility fractures.

6.4 Public Health Implications

Costs associated with fractures are an important burden to the Canadian health care system. A recent analysis revealed that the overall costs of osteoporosis, including acute care hospitalisation due to fractures, LTC services and rehabilitation, were over $4.6 billion [442] for the fiscal year of 2010-2011. The cost of LTC alone was $31 million. In Canada, 7.1% of all seniors over the age of 65 resided in nursing homes, residences for seniors, chronic care hospitals or LTC facilities in 2011. Nearly 30% of these seniors are 85 y and over [48]. Similarly, in the US in 2014, an estimated 1,369,700 Americans were residing in nursing homes and 41.6% were 85 y and over [236]. Bone health is a major concern in LTC facilities. The risk of hip fractures is 2 to 4 times higher in LTC facilities than in the community [237]. Improving the prevention of fractures is essential to reduce this economic burden. Vitamin D_3 is regarded as important for bone health and healthy aging [214, 220, 381] and specific guidelines for bone health for LTC residents are emerging [219, 382]. However, meta-analyses providing conflicting results regarding the impact of vitamin D in prevention of fractures and bone health [1-3, 5, 6, 10, 11, 16, 19, 49, 222, 381]. As the LTC clientele is frail, fracture prevention must be considered in conjunction with the development of sarcopenia, the control of environmental factors and polypharmacy, which all can increase the risk of falls. This thesis research demonstrated that only 40% of the LTC population studied was receiving vitamin D supplementation. The second study showed that vitamin D status can be improved by vitamin D_3 fortified foods and, that 500 IU/d or 1000 IU/d of supplementation could maintain 25(OH)D status. However, BMD could not be improved in all supplemented participants after a 24 week RCT period. The 1000 IU/d

supplementation group maintained muscle strength, as shown by the handgrip measurement, which can signify that vitamin D would impact musculoskeletal health. To decrease the costs associated to osteoporosis, all efforts should be put in place to reinforce the supplementation recommendations in LTC. Improvement to adequate supplementation, such as regular medication reviews and informative documentation, were suggested to professionals [443].

6.5 Future Directions

This thesis provides important new knowledge base regarding vitamin D intake and status in older men living in LTC. An important research gap, under-representation of older men in investigations on bone health, was address in these studies. Future studies should continue to focus on older men. As LTC facilities are providing a privileged research microcosm, future studies should be designed for a longer period of time and include a more comprehensive approach including exercise and supplementation. As heterogeneity of the population is increased in aging, due to disease and prescription drugs, genetic profiling for the more common allele could also be an asset in teasing out the non responders to vitamin D supplementation and potentially change the reported results. Developing more fortified foods that would provide vitamin D and calcium to increase the potential of calcification could help in successfully achieving bone health and reducing fractures.

6.6 Conclusions

This thesis confirms the limited dietary sources of vitamin D on LTC menus and that intake of vitamin D from foods is inadequate as observed elsewhere in in Canada. Systematic tablet supplementation was not provided in 60% of the seniors in this LTC.

A loading dose of 2000 IU/d for a period of 8 weeks safely raised 25(OH)D concentrations to an average of 65 nmol/L, but not to the 75 nmol/L proposed by the Endocrine Society [58]. The RCT segment demonstrated that a supplement of 500-1000 IU/d was sufficient to maintain the status which concurs with the IOM recommendations. A selection of vitamin D_3 fortified foods was well appreciated by the older men included in this study.

This body of work contributes to the knowledge regarding vitamin D in older men and provides professionals and administrators important elements to the vitamin D intake and status question in the older men living in LTC. Further investigation of longer duration will help confirm these results across the geriatric care spectrum, from the community to the LTC.

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