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Clinical Utility of Vitamin D Testing

An Evidence-Based Analysis

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The Medical Advisory Secretariat also provides a secretariat function and evidence-based health technology policy analysis for review by the Ontario Health Technology Advisory Committee (OHTAC).

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List of Abbreviations

25(OH)D	25-hydroxyvitamin D
AHRQ	Agency for Healthcare Research and Quality
AI	Adequate intake
AMSTAR	A measurement tool to assess systematic reviews
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Bone mineral density
CI	Confidence interval(s)
CKD	Chronic kidney disease
CPBA	Competitive protein-binding assay
HPLC	High-pressure liquid chromatography
iPTH	Intact parathyroid hormone
IU	International unit
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MAS	Medical Advisory Secretariat
OR	Odds ratio
OHTAC	Ontario Health Technology Advisory Committee
PTH	Parathyroid hormone
RCT	Randomized controlled trial
RIA	Radioimmunoassay
RR	Relative risk
SD	Standard deviation
UVB	Ultraviolet B rays
WHI	Women's Health Initiative

Executive Summary

This report from the Medical Advisory Secretariat (MAS) was intended to evaluate the clinical utility of vitamin D testing in average risk Canadians and in those with kidney disease. As a separate analysis, this report also includes a systematic literature review of the prevalence of vitamin D deficiency in these two subgroups.

This evaluation did not set out to determine the serum vitamin D thresholds that might apply to non-bone health outcomes. For bone health outcomes, no high or moderate quality evidence could be found to support a target serum level above 50 nmol/L. Similarly, no high or moderate quality evidence could be found to support vitamin D's effects in non-bone health outcomes, other than falls.

Vitamin D

Vitamin D is a lipid soluble vitamin that acts as a hormone. It stimulates intestinal calcium absorption and is important in maintaining adequate phosphate levels for bone mineralization, bone growth, and remodelling. It's also believed to be involved in the regulation of cell growth proliferation and apoptosis (programmed cell death), as well as modulation of the immune system and other functions. Alone or in combination with calcium, Vitamin D has also been shown to reduce the risk of fractures in elderly men (≥ 65 years), postmenopausal women, and the risk of falls in community-dwelling seniors. However, in a comprehensive systematic review, inconsistent results were found concerning the effects of vitamin D in conditions such as cancer, all-cause mortality, and cardiovascular disease. In fact, no high or moderate quality evidence could be found concerning the effects of vitamin D in such non-bone health outcomes. Given the uncertainties surrounding the effects of vitamin D in non-bone health related outcomes, it was decided that this evaluation should focus on falls and the effects of vitamin D in bone health and exclusively within average-risk individuals and patients with kidney disease.

Synthesis of vitamin D occurs naturally in the skin through exposure to ultraviolet B (UVB) radiation from sunlight, but it can also be obtained from dietary sources including fortified foods, and supplements. Foods rich in vitamin D include fatty fish, egg yolks, fish liver oil, and some types of mushrooms. Since it is usually difficult to obtain sufficient vitamin D from non-fortified foods, either due to low content or infrequent use, most vitamin D is obtained from fortified foods, exposure to sunlight, and supplements.

Clinical Need: Condition and Target Population

Vitamin D deficiency may lead to rickets in infants and osteomalacia in adults. Factors believed to be associated with vitamin D deficiency include:

- darker skin pigmentation,
- winter season,
- living at higher latitudes,
- skin coverage,
- kidney disease,
- malabsorption syndromes such as Crohn's disease, cystic fibrosis, and
- genetic factors.

Patients with chronic kidney disease (CKD) are at a higher risk of vitamin D deficiency due to either renal losses or decreased synthesis of 1,25-dihydroxyvitamin D.

Health Canada currently recommends that, until the daily recommended intakes (DRI) for vitamin D are updated, Canada's Food Guide (Eating Well with Canada's Food Guide) should be followed with respect

to vitamin D intake. Issued in 2007, the Guide recommends that Canadians consume two cups (500 ml) of fortified milk or fortified soy beverages daily in order to obtain a daily intake of 200 IU. In addition, men and women over the age of 50 should take 400 IU of vitamin D supplements daily. Additional recommendations were made for breastfed infants.

A Canadian survey evaluated the median vitamin D intake derived from diet alone (excluding supplements) among 35,000 Canadians, 10,900 of which were from Ontario. Among Ontarian males ages 9 and up, the median daily dietary vitamin D intake ranged between 196 IU and 272 IU per day. Among females, it varied from 152 IU to 196 IU per day. In boys and girls ages 1 to 3, the median daily dietary vitamin D intake was 248 IU, while among those 4 to 8 years it was 224 IU.

Vitamin D Testing

Two laboratory tests for vitamin D are available, 25-hydroxy vitamin D, referred to as 25(OH)D, and 1,25-dihydroxyvitamin D. Vitamin D status is assessed by measuring the serum 25(OH)D levels, which can be assayed using radioimmunoassays, competitive protein-binding assays (CPBA), high pressure liquid chromatography (HPLC), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). These may yield different results with inter-assay variation reaching up to 25% (at lower serum levels) and intra-assay variation reaching 10%.

The optimal serum concentration of vitamin D has not been established and it may change across different stages of life. Similarly, there is currently no consensus on target serum vitamin D levels. There does, however, appear to be a consensus on the definition of vitamin D deficiency at 25(OH)D < 25 nmol/l, which is based on the risk of diseases such as rickets and osteomalacia. Higher target serum levels have also been proposed based on subclinical endpoints such as parathyroid hormone (PTH). Therefore, in this report, two conservative target serum levels have been adopted, 25 nmol/L (based on the risk of rickets and osteomalacia), and 40 to 50 nmol/L (based on vitamin D's interaction with PTH).

Ontario Context

Volume & Cost

The volume of vitamin D tests done in Ontario has been increasing over the past 5 years with a steep increase of 169,000 tests in 2007 to more than 393,400 tests in 2008. The number of tests continues to rise with the projected number of tests for 2009 exceeding 731,000. According to the Ontario Schedule of Benefits, the billing cost of each test is $51.7 for 25(OH)D (L606, 100 LMS units, $0.517/unit) and $77.6 for 1,25-dihydroxyvitamin D (L605, 150 LMS units, $0.517/unit). Province wide, the total annual cost of vitamin D testing has increased from approximately $1.7M in 2004 to over $21.0M in 2008. The projected annual cost for 2009 is approximately $38.8M.

Evidence-Based Analysis

The objective of this report is to evaluate the clinical utility of vitamin D testing in the average risk population and in those with kidney disease. As a separate analysis, the report also sought to evaluate the prevalence of vitamin D deficiency in Canada. The specific research questions addressed were thus:

1. What is the clinical utility of vitamin D testing in the average risk population and in subjects with kidney disease?
2. What is the prevalence of vitamin D deficiency in the average risk population in Canada?
3. What is the prevalence of vitamin D deficiency in patients with kidney disease in Canada?

Clinical utility was defined as the ability to improve bone health outcomes with the focus on the average risk population (excluding those with osteoporosis) and patients with kidney disease.

Literature Search

A literature search was performed on July 17th, 2009 using OVID MEDLINE, MEDLINE In-Process and Other Non-Indexed Citations, EMBASE, the Cumulative Index to Nursing & Allied Health Literature (CINAHL), the Cochrane Library, and the International Agency for Health Technology Assessment (INAHTA) for studies published from January 1, 1998 until July 17th, 2009. Abstracts were reviewed by a single reviewer and, for those studies meeting the eligibility criteria, full-text articles were obtained. Reference lists were also examined for any additional relevant studies not identified through the search. Articles with unknown eligibility were reviewed with a second clinical epidemiologist, then a group of epidemiologists until consensus was established. The quality of evidence was assessed as high, moderate, low or very low according to GRADE methodology.

Observational studies that evaluated the prevalence of vitamin D deficiency in Canada in the population of interest were included based on the inclusion and exclusion criteria listed below. The baseline values were used in this report in the case of interventional studies that evaluated the effect of vitamin D intake on serum levels. Studies published in grey literature were included if no studies published in the peer-reviewed literature were identified for specific outcomes or subgroups.

Considering that vitamin D status may be affected by factors such as latitude, sun exposure, food fortification, among others, the search focused on prevalence studies published in Canada. In cases where no Canadian prevalence studies were identified, the decision was made to include studies from the United States, given the similar policies in vitamin D food fortification and recommended daily intake.

Inclusion Criteria

- Studies published in English
- Publications that reported the prevalence of vitamin D deficiency in Canada
- Studies that included subjects from the general population or with kidney disease
- Studies in children or adults
- Studies published between January 1998 and July 17th 2009

Exclusion Criteria

- Studies that included subjects defined according to a specific disease other than kidney disease
- Letters, comments, and editorials
- Studies that measured the serum vitamin D levels but did not report the percentage of subjects with serum levels below a given threshold

Outcomes of Interest

- Prevalence of serum vitamin D less than 25 nmol/L
- Prevalence of serum vitamin D less than 40 to 50 nmol/L
- Serum 25-hydroxyvitamin D was the metabolite used to assess vitamin D status. Results from adult and children studies were reported separately. Subgroup analyses according to factors that affect serum vitamin D levels (e.g., seasonal effects, skin pigmentation, and vitamin D intake) were reported if enough information was provided in the studies

Quality of Evidence

The quality of the prevalence studies was based on the method of subject recruitment and sampling, possibility of selection bias, and generalizability to the source population. The overall quality of the trials was examined according to the GRADE Working Group criteria.

Summary of Findings

Fourteen prevalence studies examining Canadian adults and children met the eligibility criteria. With the exception of one longitudinal study, the studies had a cross-sectional design. Two studies were conducted among Canadian adults with renal disease but none studied Canadian children with renal disease (though three such US studies were included). No systematic reviews or health technology assessments that evaluated the prevalence of vitamin D deficiency in Canada were identified. Two studies were published in grey literature, consisting of a Canadian survey designed to measure serum vitamin D levels and a study in infants presented as an abstract at a conference. Also included were the results of vitamin D tests performed in community laboratories in Ontario between October 2008 and September 2009 (provided by the Ontario Association of Medical Laboratories).

Different threshold levels were used in the studies, thus we reported the percentage of subjects with serum levels of between 25 and 30 nmol/L and between 37.5 and 50 nmol/L. Some studies stratified the results according to factors affecting vitamin D status and two used multivariate models to investigate the effects of these characteristics (including age, season, BMI, vitamin D intake, skin pigmentation, and season) on serum 25(OH)D levels. It's unclear, however, if these studies were adequately powered for these subgroup analyses.

Study participants generally consisted of healthy, community-dwelling subjects and most excluded individuals with conditions or medications that alter vitamin D or bone metabolism, such as kidney or liver disease. Although the studies were conducted in different parts of Canada, fewer were performed in Northern latitudes, i.e. above 53°N, which is equivalent to the city of Edmonton.

Adults

Serum vitamin D levels of < 25 to 30 nmol/L were observed in 0% to 25.5% of the subjects included in five studies; the weighted average was 3.8% (95% CI: 3.0, 4.6). The preliminary results of the Canadian survey showed that approximately 5% of the subjects had serum levels below 29.5 nmol/L. The results of over 600,000 vitamin D tests performed in Ontarian community laboratories between October 2008 and September 2009 showed that 2.6% of adults (> 18 years) had serum levels < 25 nmol/L.

The prevalence of serum vitamin D levels below 37.5-50 nmol/L reported among studies varied widely, ranging from 8% to 73.6% with a weighted average of 22.5%. The preliminary results of the CHMS survey showed that between 10% and 25% of subjects had serum levels below 37 to 48 nmol/L. The results of the vitamin D tests performed in community laboratories showed that 10% to 25% of the individuals had serum levels between 39 and 50 nmol/L.

In an attempt to explain this inter-study variation, the study results were stratified according to factors affecting serum vitamin D levels, as summarized below. These results should be interpreted with caution as none were adjusted for other potential confounders. Adequately powered multivariate analyses would be necessary to determine the contribution of risk factors to lower serum 25(OH)D levels.

Seasonal variation

Three adult studies evaluating serum vitamin D levels in different seasons observed a trend towards a higher prevalence of serum levels < 37.5 to 50 nmol/L during the winter and spring months, specifically 21% to 39%, compared to 8% to 14% in the summer. The weighted average was 23.6% over the winter/spring months and 9.6% over summer. The difference between the seasons was not statistically significant in one study and not reported in the other two studies.

Skin Pigmentation

Four studies observed a trend toward a higher prevalence of serum vitamin D levels < 37.5 to 50 nmol/L in subjects with darker skin pigmentation compared to those with lighter skin pigmentation, with weighted averages of 46.8% among adults with darker skin colour and 15.9% among those with fairer skin.

Vitamin D intake and serum levels

Four adult studies evaluated serum vitamin D levels according to vitamin D intake and showed an overall trend toward a lower prevalence of serum levels < 37.5 to 50 nmol/L with higher levels of vitamin D intake. One study observed a dose-response relationship between higher vitamin D intake from supplements, diet (milk), and sun exposure (results not adjusted for other variables). It was observed that subjects taking 50 to 400 IU or > 400 IU of vitamin D per day had a 6% and 3% prevalence of serum vitamin D level < 40 nmol/L, respectively, versus 29% in subjects not on vitamin D supplementation. Similarly, among subjects drinking one or two glasses of milk per day, the prevalence of serum vitamin D levels < 40 nmol/L was found to be 15%, versus 6% in those who drink more than two glasses of milk per day and 21% among those who do not drink milk. On the other hand, one study observed little variation in serum vitamin D levels during winter according to milk intake, with the proportion of subjects exhibiting vitamin D levels of < 40 nmol/L being 21% among those drinking 0-2 glasses per day, 26% among those drinking > 2 glasses, and 20% among non-milk drinkers.

The overall quality of evidence for the studies conducted among adults was deemed to be low, although it was considered moderate for the subgroups of skin pigmentation and seasonal variation.

Newborn, Children and Adolescents

Five Canadian studies evaluated serum vitamin D levels in newborns, children, and adolescents. In four of these, it was found that between 0 and 36% of children exhibited deficiency across age groups with a weighted average of 6.4%. The results of over 28,000 vitamin D tests performed in children 0 to 18 years old in Ontario laboratories (Oct. 2008 to Sept. 2009) showed that 4.4% had serum levels of < 25 nmol/L.

According to two studies, 32% of infants 24 to 30 months old and 35.3% of newborns had serum vitamin D levels of < 50 nmol/L. Two studies of children 2 to 16 years old reported that 24.5% and 34% had serum vitamin D levels below 37.5 to 40 nmol/L. In both studies, older children exhibited a higher prevalence than younger children, with weighted averages 34.4% and 10.3%, respectively. The overall weighted average of the prevalence of serum vitamin D levels < 37.5 to 50 nmol/L among pediatric studies was 25.8%. The preliminary results of the Canadian survey showed that between 10% and 25% of subjects between 6 and 11 years (N= 435) had serum levels below 50 nmol/L, while for those 12 to 19 years, 25% to 50% exhibited serum vitamin D levels below 50 nmol/L.

The effects of season, skin pigmentation, and vitamin D intake were not explored in Canadian pediatric studies. A Canadian surveillance study did, however, report 104 confirmed cases[1] (2.9 cases per 100,000 children) of vitamin D-deficient rickets among Canadian children age 1 to 18 between 2002 and 2004, 57 (55%) of which from Ontario. The highest incidence occurred among children living in the North, i.e., the Yukon, Northwest Territories, and Nunavut. In 92 (89%) cases, skin pigmentation was categorized as intermediate to dark, 98 (94%) had been breastfed, and 25 (24%) were offspring of immigrants to Canada. There were no cases of rickets in children receiving ≥ 400 IU VD supplementation/day.

Overall, the quality of evidence of the studies of children was considered very low.

[1] Rickets were confirmed by radiographic signs at the wrist or knee by a radiologist. Serum levels of calcium, phosphate, alkaline phosphatase, parathyroid hormone, and 1,25-dihydroxyvitamin D were included if available. Serum 25(OH)D levels had to be below 27.5 nmol/L or > 27.5 nmol/L in absence of isolated dietary calcium-deficient rickets. VD-deficient rickets associated with underlying diseases were excluded.

Kidney Disease

Adults

Two studies evaluated serum vitamin D levels in Canadian adults with kidney disease. The first included 128 patients with chronic kidney disease stages 3 to 5, 38% of which had serum vitamin D levels of < 37.5 nmol/L (measured between April and July). This is higher than what was reported in Canadian studies of the general population during the summer months (i.e. between 8% and 14%). In the second, which examined 419 subjects who had received a renal transplantation (mean time since transplantation: 7.2 ± 6.4 years), the prevalence of serum vitamin D levels < 40 nmol/L was 27.3%. The authors concluded that the prevalence observed in the study population was similar to what is expected in the general population.

Children

No studies evaluating serum vitamin D levels in Canadian pediatric patients with kidney disease could be identified, although three such US studies among children with chronic kidney disease stages 1 to 5 were. The mean age varied between 10.7 and 12.5 years in two studies but was not reported in the third. Across all three studies, the prevalence of serum vitamin D levels below the range of 37.5 to 50 nmol/L varied between 21% and 39%, which is not considerably different from what was observed in studies of healthy Canadian children (24% to 35%).

Overall, the quality of evidence in adults and children with kidney disease was considered very low.

Clinical Utility of Vitamin D Testing

A high quality comprehensive systematic review published in August 2007 evaluated the association between serum vitamin D levels and different bone health outcomes in different age groups. A total of 72 studies were included. The authors observed that there was a trend towards improvement in some bone health outcomes with higher serum vitamin D levels. Nevertheless, precise thresholds for improved bone health outcomes could not be defined across age groups. Further, no new studies on the association were identified during an updated systematic review on vitamin D published in July 2009.

With regards to non-bone health outcomes, there is no high or even moderate quality evidence that supports the effectiveness of vitamin D in outcomes such as cancer, cardiovascular outcomes, and all-cause mortality. Even if there is any residual uncertainty, there is no evidence that testing vitamin D levels encourages adherence to Health Canada's guidelines for vitamin D intake. A normal serum vitamin D threshold required to prevent non-bone health related conditions cannot be resolved until a causal effect or correlation has been demonstrated between vitamin D levels and these conditions. This is as an ongoing research issue around which there is currently too much uncertainty to base any conclusions that would support routine vitamin D testing.

For patients with chronic kidney disease (CKD), there is again no high or moderate quality evidence supporting improved outcomes through the use of calcitriol or vitamin D analogs. In the absence of such data, the authors of the guidelines for CKD patients consider it best practice to maintain serum calcium and phosphate at normal levels, while supplementation with active vitamin D should be considered if serum PTH levels are elevated. As previously stated, the authors of guidelines for CKD patients believe that there is not enough evidence to support routine vitamin D [25(OH)D] testing. According to what is stated in the guidelines, decisions regarding the commencement or discontinuation of treatment with calcitriol or vitamin D analogs should be based on serum PTH, calcium, and phosphate levels.

Limitations associated with the evidence of vitamin D testing include ambiguities in the definition of an 'adequate threshold level' and both inter- and intra- assay variability. The MAS considers both the lack of a consensus on the target serum vitamin D levels and assay limitations directly affect and undermine the clinical utility of testing. The evidence supporting the clinical utility of vitamin D testing is thus considered to be of very low quality.

Daily vitamin D intake, either through diet or supplementation, should follow Health Canada's recommendations for healthy individuals of different age groups. For those with medical conditions such as renal disease, liver disease, and malabsorption syndromes, and for those taking medications that may affect vitamin D absorption/metabolism, physician guidance should be followed with respect to both vitamin D testing and supplementation.

Conclusions

1. Studies indicate that vitamin D, alone or in combination with calcium, may decrease the risk of fractures and falls among older adults.
2. There is no high or moderate quality evidence to support the effectiveness of vitamin D in other outcomes such as cancer, cardiovascular outcomes, and all-cause mortality.
3. Studies suggest that the prevalence of vitamin D deficiency in Canadian adults and children is relatively low (approximately 5%), and between 10% and 25% have serum levels below 40 to 50 nmol/L (based on very low to low grade evidence).
4. Given the limitations associated with serum vitamin D measurement, ambiguities in the definition of a 'target serum level', and the availability of clear guidelines on vitamin D supplementation from Health Canada, vitamin D testing is not warranted for the average risk population.
5. Health Canada has issued recommendations regarding the adequate daily intake of vitamin D, but current studies suggest that the mean dietary intake is below these recommendations. Accordingly, Health Canada's guidelines and recommendations should be promoted.
6. Based on a moderate level of evidence, individuals with darker skin pigmentation appear to have a higher risk of low serum vitamin D levels than those with lighter skin pigmentation and therefore may need to be specially targeted with respect to optimum vitamin D intake. The cause-effect of this association is currently unclear.
7. Individuals with medical conditions such as renal and liver disease, osteoporosis, and malabsorption syndromes, as well as those taking medications that may affect vitamin D absorption/metabolism, should follow their physician's guidance concerning both vitamin D testing and supplementation.

Background

The Medical Advisory Secretariat (MAS) evaluation was intended to evaluate the clinical utility of vitamin D testing in average risk Canadians and in those with kidney disease. As a separate analysis, the MAS evaluation also included a systematic literature review of the prevalence of vitamin D deficiency in these two subgroups.

The MAS evaluation did not set out to determine the serum vitamin D thresholds which would affect the risk of non-bone health outcomes. For bone health outcomes, no high quality or even moderate quality evidence could be found to support a target serum level above 50 nmol/L. Similarly, no high or moderate quality evidence was found to support vitamin D's effects in non-bone health outcomes, other than falls could be found.

Vitamin D

Vitamin D is a lipid soluble vitamin that acts as a hormone. (1) It's synthesized in the skin through exposure to ultraviolet B (UVB) radiation from sunlight (2) and may also be obtained from dietary sources and supplements. (3;4) There are two forms of vitamin D: vitamin D_2, which is derived from plants, and vitamin D_3, which is the main form obtained from animal sources and exposure to sunlight. (5) Exposure to UVB converts 7-dehydrocholesterol present in the skin into previtamin D_3, which is then converted into vitamin D_3. (6) Foods that naturally contain vitamin D include fatty fish (salmon, tuna, sardines etc.), egg yolks, fish liver oil, and certain types of mushrooms (see Table 1). (6;7) It is usually difficult, however, to obtain sufficient vitamin D from non-fortified foods either due to their low content (8) or infrequent use (9), thus most vitamin D is obtained from fortified foods and/or exposure to sunlight. The use of vitamin D supplements may also be necessary in some cases (5;7) Other factors such as living at high latitude, cloud cover, darker skin pigmentation, and the use of sunscreen can also affect UVB exposure and influence the amount of vitamin D produced through the skin. (5)

Regulations regarding the fortification of foods with vitamin D vary between countries. In Canada, which has similar regulations to the United States, vitamin D fortification of milk (including evaporated and powdered milk), soy milk, and margarine is mandatory. (7) One serving (250 ml) of milk contains approximately 44% of the 200 IU adequate daily intake of vitamin D. (7) Vitamin D fortification is also permitted for orange juice, meal replacements, nutritional supplements, and formulated liquid diet. (7;9)

Vitamin D Metabolism and Physiology

Vitamin D from diet, supplements, or sunlight exposure first undergoes hydroxylation in the liver, producing 25-hydroxyvitamin D. A second hydroxylation occurs in the kidneys and produces 1,25-dihydroxyvitamin D, which is the active form of vitamin D (5). This step can also occur extrarenally. (2;10) Renal synthesis of the active vitamin D form, 1,25-dihydroxyvitamin D is regulated by plasma parathyroid hormone, serum calcium and phosphorus levels. (11)

Vitamin D stimulates intestinal calcium and phosphate absorption and is important in maintaining adequate calcium levels for bone mineralization, bone growth and remodelling, and to prevent hypocalcemic tetany. (1;12) Serum parathyroid hormone (PTH) has an inverse correlation with absorbed calcium. (13) By decreasing calcium absorptive efficiency, vitamin D deficiency can indirectly result in increased serum PTH (13), which may lead to the mobilization of calcium from the bone. (14)

Vitamin D is also believed to be involved in the regulation of cell growth and metabolism (15), modulation of immune function, and inflammation reduction. (5) Other tissues such as the prostate, colon, and breast can convert 25-hydroxyvitamin D into1,25-dihydroxyvitamin D, which has the potential to partially modulate genes involved in cell growth, differentiation, and apoptosis (programmed cell death). (5;16) This may be a possible mechanism for the effect of vitamin D in cancer. (4)

Table 1: Estimated Amount of Vitamin D Present in Some Foods

Type of Food	Estimated Vitamin D Content (Approximate Content)
Naturally present in food	
Salmon (fresh, farmed), 3.5 oz	100-250 IU vitamin D_3 or D_2 (farmed) 600 – 1,000 IU vitamin D_3 (wild)
Mackerel, (canned), 3.5 oz	250 IU vitamin D_3
Cod liver oil, 1 teaspoon	400-1,000 IU vitamin D_3
Tuna (canned), 3.6 oz	230 IU vitamin D_3
Shiitake mushrooms (fresh), 3.5 oz	100 IU vitamin D_2 (fresh) 1,600 IU vitamin D_2 (sun-dried)
Egg yolk (1 unit)	20 IU vitamin D_3 or D_2
Vitamin D-fortified foods (Canada)	
Cow's milk, 250 ml	88 IU
Plant-based beverages, 250 ml	80 IU
Margarine, 1 teaspoon	25 IU

oz refers to ounce; IU international unit .

Source: Natural content of food: Holick. (6); vitamin D-fortified foods (Canada): Health Canada. (17)

Vitamin D Nomenclature

The term 'vitamin D' encompasses both vitamin D_2, also known as ergocalciferol, and vitamin D_3, known as cholecalciferol, either of which may be used in supplements. (5;6) Synthetic vitamin D analogs are also available (doxercalciferol, paricalcitol, and alfacalcidiol) and can be used in the treatment of patients with kidney disease. (18)

Table 2: Vitamin D Forms and Metabolites

Vitamin D type	Description
Vitamin D_2	Also known as ergocalciferol. It is present in plants (e.g., mushrooms).
Vitamin D_3	Also known as cholecalciferol. Animal origin (such as some fishes) or produced by cutaneous synthesis.
Calcidiol	Also known as 25-hydroxyvitamin D or 25(OH)D Vitamin D metabolite produced by hydroxylation of vitamin D_2 or D_3 in the liver.
Calcitriol	1,25-dihydroxyvitamin D Hormonal form of vitamin D. Active metabolite produced by hydroxylation of calcidiol in the kidneys. Hydroxylation can also occur in other tissues.

Source: Johnson and Kimlin. (19)

Vitamin D Testing

Vitamin D metabolites are used to assess serum vitamin D level and metabolism. Specifically, 25-hydroxy vitamin D, referred to as 25(OH)D, and 1,25-dihydroxyvitamin D. (20) The 25(OH)D metabolite (5;20) has an estimated half life of approximately 2 to 3 weeks, (5;21) and provides a measure of the vitamin D originating from both dietary/supplement sources and from cutaneous production. (5) Vitamin D stored in other body tissues are, however, not reflected in the serum 25(OH)D levels. (5) Serum levels of the active vitamin D metabolite, 1,25-dihydroxyvitamin D, may not accurately indicate the individual's vitamin D status due to its short half-life (15 hours). Since it's closely regulated by parathyroid hormone and the intake of calcium and phosphate, serum levels of 1,25-dihydroxyvitamin D (3;5;7) may appear normal in individuals with vitamin D deficiency. (3)

Different assays for measuring 25(OH)D are available including radioimmunoassays, competitive protein-binding assays (CPBA), high pressure liquid chromatography (HPLC), and liquid chromatography-tandem mass spectrometry (LC-MS/MS). (22) Notably, radioimmunoassays measure total vitamin D levels ($D_2 + D_3$), while liquid chromatography and tandem mass spectroscopy report D_2 and D_3 separately. (6) Different assays may yield different results, with inter-assay variation reaching up to 25% at low serum 25(OH)D levels (15 nmol/L). (20) The intra-assay variation can also reach up to 10% and there may be considerable variation between laboratories, even when quality control and standardization programs are in place. (20) These issues may need to be taken into account when following an individual over time. (20) Part of the variation may be explained by the fact that 25(OH)D assays have different affinities for vitamin D_2 and D_3. (19;20) This may be lead to lower measured levels of serum vitamin D in regions where vitamin D_2 is predominantly used in supplementation or food fortification, depending on the vitamin D assay used. (19;20)

Risk Factors for Vitamin D Deficiency

There are concerns about the prevalence of vitamin D deficiency in healthy individuals in Canada, the United States, and other countries. (23) Vitamin D deficiency is a known contributing factor in nutritional rickets (24), a condition that has reportedly resurged in developed countries in recent years (25;26), with most of the affected being immigrants from Asia, Africa, and the Middle East. Other risk factors include darker skin pigmentation and breastfeeding without vitamin D supplementation (see Table 3). (26;27) Newborn children also depend primarily on the maternal supply of vitamin D (15) and vitamin D deficiency in mothers may result in newborn hypocalcaemia and rickets. (15)

A number of other factors also affect the body's ability to produce vitamin D or absorb it from the diet. Melanin, for example, which is present in greater amounts in individuals with darker skin, acts as a sunscreen, filtering UVB radiation and reducing the cutaneous production of vitamin D. (19)
At higher latitudes, the inclination of the sun during the winter months can also prevent vitamin D synthesis. (1) One study showed that at a latitude of 52°N (equivalent to Edmonton, Alberta) vitamin D_3 production through sunlight exposure is practically nil between October and April, or November and February for a 42°N latitude (equivalent to Boston). (15)

The symptoms associated with vitamin D deficiency, such as bone pain and muscle weakness, may be difficult to notice. (5) But prolonged vitamin D deficiency can lead to brittle bones, rickets in children and osteomalacia in adults. (5) Rickets is caused by inadequate bone mineralization leading to soft bones and skeletal deformities, while osteomalacia is characterized by weak bones and muscles. (5)

Table 3: Risk Factors for Vitamin D Deficiency

Risk factors for vitamin D deficiency
Factors related to sunlight exposure
▪ Darker skin pigmentation ▪ Season (winter) ▪ Higher latitude ▪ Skin coverage for instance the use of veil ▪ Cloud cover ▪ Use of sunscreen
Factors related to dietary intake
▪ Exclusive breastfeeding (risk for the infant)
Factors related to age and disease conditions
▪ Obesity ▪ Older age worsened by immobility and aging kidneys ▪ Kidney disease ▪ Malabsorption syndromes/other conditions : Crohn's disease, cystic fibrosis, severe liver disease ▪ Drug interactions: anticonvulsants, cimetidine, thiazides, corticosteroids ▪ Drugs that decrease absorption: mineral oil, laxatives orlistat, cholestiramine etc. ▪ Genetics: Indo-Asians

Sources: Schwalfenberg (23), United States Office of Dietary Supplements (5)

Vitamin D and Kidney Disease

Patients with chronic kidney disease (CKD) are at a higher risk of vitamin D deficiency due to either renal losses or decreased synthesis of 1,25-dihydroxyvitamin D. (28) The condition is defined by the presence of kidney damage for more than 3 months (29) and is divided into 5 stages (stages 1 to 5) according to the glomerular filtration rate (GFR), i.e., the greater the kidney damage, the higher the stage[2]. (29) Patients with chronic kidney disease present with abnormalities of bone metabolism and mineralization that start to appear early in the disease process. (29;30) Pharmacological treatments used to improve bone and mineral metabolism include vitamin D compounds, calcium supplements, non-calcium-containing phosphate binders, and calcimimetics (30). The vitamin D compounds used for such cases are alfacalcidiol [1 α-hydroxyvitamin D_3, 25(OH)D] and calcitriol, which reduce serum PTH levels and raise serum calcium and phosphorus levels. (30) More recently, newer vitamin D analogues (oxacalcitriol, doxercalciferol, among others) have been proposed to treat the condition. (30)

The 2008 guidelines from the Canadian Society of Nephrology state that there is no randomized controlled trial (RCT) data demonstrating the improvement of outcomes such as a reduction of fractures and survival with improved mineral metabolism control among kidney patients. (29) In the absence of such data, the authors consider it best practice to maintain serum calcium and phosphate at normal levels and supplementation with active vitamin D (1,25-dihydroxyvitamin D, calcitriol) should be used if the

[2] CKD stage 1 is defined by the United States National Kidney Foundation as kidney damage with normal or increased GFR (≥90 ml/min/1.73m^2), stage 2 is defined as kidney damage with mild decreased GFR (60-89 ml/min/1.73m^2). (29) Stage 5 is defined as kidney failure (GFR < 15 ml/min/1.73m^2) or dialysis. (29)

serum parathyroid hormone level is elevated (based on expert opinion). The authors also believe that there is not enough evidence to support routine vitamin D testing. (29) According to the guidelines, when serum PTH levels are above 53 pmol/L, vitamin D analogs should be considered (based on expert opinion). (29) If hypocalcaemia or hypophosphatemia occurs, or if serum PTH levels fall below 10.6 pmol/L, therapy with vitamin D analogues should be discontinued. (29) The guidelines also state that the use of vitamin D analogues should be monitored by a specialist with experience using these agents. (29)

Separate guidelines issued by the United States Kidney Disease Outcomes Quality Initiative (KDOQI) on bone metabolism in CKD recommend that a vitamin D test be performed if PTH is above the target range in adults with CKD stages 3 to 5 (31) and children with CKD stages 2 to 4. (32) A target vitamin D level of 75 nmol/L is mentioned for adults with CKD stages 3 and 4 (31) and children with CKD states 2 to 4, (32), however, this is again based on expert opinion only. (31;32) Vitamin D treatment should be guided by PTH, phosphorus, and calcium levels for both adults with CKD 3 to 5 (31) and children with CKD 2 to 5. (32)

The 2009 United States Kidney Improving Global Outcomes (KDIGO) guidelines stated that there are as yet no RCTs conducted among CKD patients to evaluate the effects of vitamin D, its analogs, or calcitriol on clinical outcomes such as fractures and mortality. Further, the results of observational studies of this patient group have been inconclusive. (18) The guidelines do recommend that phosphate, calcium, and vitamin D levels be evaluated in patients with CKD stages 3 to 5 who are not on dialysis and that have an intact PTH level above the upper normal limit for the assay. (18) The authors suggest that, depending on the serum PTH, calcium, and phosphate levels, treatment with calcitriol or vitamin D analogs be started or discontinued. (18) The authors also suggest that serum 25(OH)D levels might be measured in patients with CKD levels 3 to 5D (weak level of recommendation, low quality of evidence). (18) The authors do recognize, however, that there is a lack of consensus on target serum vitamin D levels. (18) Given this and assay limitations, the decision of whether or not vitamin D testing is necessary and when and how often it should be done, should be individualized. (18) The authors also point out that, because of this uncertainty, the impact of performing vitamin D tests on healthcare resources should be considered. (18) This is congruent with the Canadian Society of Nephrology guidelines. (29)

Vitamin D Toxicity

Vitamin D toxicity is believed to be uncommon. (3) Its clinical signs are non-specific but include nausea, vomiting, loss of appetite, constipation, weakness, and weight loss. (5) Excessive levels of vitamin D may lead to hypocalcaemia, confusion, arrhythmias, and calcification of the soft tissues and kidneys. (5) Excessive vitamin D originating from cutaneous synthesis does not result in intoxication. (6)

A systematic review published in August 2007 by the Agency for Health Research and Quality (AHRQ) evaluated the safety of vitamin D supplementation. (33) The evaluation included 22 randomized controlled trials (RCTs) in adults and children, but most were of older adults (postmenopausal women and elderly men) and subjects with a history of hypocalcaemia and kidney stones were excluded. (33) There was a non-statistically significant increase in the risk of hypocalcaemia and hypercalciuria in subjects receiving vitamin D ± calcium compared to placebo. Only one study included in the systematic review showed an increased risk of kidney stones in women taking vitamin D combined with calcium. The study consisted of a large RCT comprised of 36,282 postmenopausal women with a mean follow-up of 7 years. (33) In the study, women were randomized to 400 IU vitamin D + 1,000 mg calcium or placebo per day, with both groups being allowed to take up to 600 IU of vitamin D (increased to 1,000 IU during the study) and 1,000 mg of calcium, in addition to the study drugs. There was an increased risk of kidney stones in some women receiving a combination of calcium and vitamin D (2.6%) compared to those in the vitamin D and placebo groups (2.3%). (34) In a letter to the editor, it was suggested that kidney stones may have been avoided if calcium citrate had been used instead of calcium carbonate. (35)

The authors of the systematic review concluded that there is fair[3] evidence that vitamin D above current recommended intake levels, alone or in combination with calcium, was well tolerated. (33) Limitations pointed out by the authors included heterogeneities in study length (2 months to 7 years) and treatment doses (100 IU to 4,000 IU of vitamin D_3 with varying treatment duration), inadequate quality of reporting safety outcomes, short duration of exposure, and insufficient power to detect safety outcomes in most studies. (33) Most of the studies identified in the review used vitamin D3 doses of 400 IU to 800 IU and the studies that used doses of 1,000 IU or higher had follow-up times of less than one year.

Effects of Vitamin D on Clinical Outcomes

Fractures

The results of a 2009 systematic review and meta-analysis of postmenopausal women and men over 65 years of age with involutional or post-menopausal osteoporosis found a statistically significant reduction in the risk of hip fractures among those treated with a combination of vitamin D and calcium, versus placebo or no intervention (RR 0.84; 95% CI: 0.73 , 0.96). (36) Eight RCTs comprising 46,658 subjects were included in this analysis. (36) The authors of the review concluded that there was an indication that vitamin D_3 doses between 400 IU and 800 IU plus 1,000 mg of calcium daily result in a decreased risk of hip fractures but not non-vertebral fractures. (36) These results were corroborated by three other meta-analyses in postmenopausal women and elderly men. (33;37;38) The authors of the first study concluded that vitamin D reduces the risk of hip and non-vertebral fractures at doses of 482 IU to 770 IU/day (38), while the authors of the two others found that doses of 700 IU to 800 IU/day (33;37) could achieve the same result (this was examined in combination with 500 mg – 1,200 mg of calcium in one). (33)

Heterogeneity among the studies was investigated in the four meta-analyses, examining factors such as vitamin D dose, age, fracture site, patient population (community-dwelling or institutionalized), and use of vitamin D alone or with calcium. (33;36;38;39) In two meta-analyses it was observed that the effects of vitamin D ± calcium on hip fractures was statistically significant in institutionalized patients but not in community-dwelling patients with vitamin D doses ranging from 400 IU to 800 IU/day. (33;36) One analysis had contrasting findings, i.e., the reduction in the risk of hip fractures was statistically significant in community-dwelling but not in institutionalized patients in studies using vitamin D doses above 400 IU. (38)

Two meta-analyses did not find a statistically significant effect of vitamin D alone on hip or non-vertebral fractures. (33;36) The authors of one in postmenopausal women and elderly men concluded that there was robust evidence that vitamin D alone, either by annual injection, periodic bolus oral dosage, or daily oral dosage, is unlikely to prevent fractures at doses below 1,100 IU /day, (RR 1.01; 95% CI 0.93, 1.09 over 10 studies, N=25,016). (36)

An attempt was made in one of the meta-analyses to evaluate the effect of vitamin D in the prevention of osteoporosis in younger women (19-49 years old) but no RCTs conducted among this age group could be identified. (33)

Falls

A report from MAS published in 2008 concluded that there was moderate to high quality evidence that vitamin D alone or in combination with calcium reduces the risk of falls among community-dwelling seniors by improving muscle function and strength. (40)

3 Cranney et al. (2007) defined 'fair evidence' as: "evidence sufficient to establish an association but was limited by consistency of results, quantity [of studies], or [study] quality (i.e., no studies graded as good)."

Non-Bone Health Outcomes

A comprehensive systematic review published in July 2009 by the AHRQ evaluated the effects of vitamin D, calcium alone, and a combination of the two in different bone (refer to section "Vitamin D Target Serum Level) and non-bone health outcomes. (11) The overall quality of the systematic review (11) was considered high (rated 10 out of 11 based on the AMSTAR criteria). (41) The systematic literature search extended from 1969 to April 2009. (11) Observational or interventional studies published in English in a generally healthy population were included (i.e., studies with ≤ 20% of subjects with any disease at baseline), with the exception of older adults, for which only those studies where the entire patient population presented with a particular disease were excluded. (11) Studies that evaluated the effects of vitamin D either through serum markers, specifically, 25(OH)D or 1,25-dihydroxyvitamin D, or known vitamin D doses were included. (11) Studies that based vitamin D doses on dietary intake from different types of food were excluded due to the possible imprecision in the estimation of the food vitamin D content. (11) Vitamin D combinations other than with calcium, such as multivitamins, were excluded unless the independent effects of vitamin D could be determined. (11)

In total, over 165 interventional and observational studies were included. (11) No studies of outcomes such as multiple sclerosis, type 1 diabetes, Crohn's disease among others met the quality and inclusion criteria for the review. Eligible studies were identified in non-bone health outcomes such as cancer, all-cause mortality, cardiovascular disease, growth, body weight, blood pressure, and autoimmune and infectious diseases. In most of these studies, the evaluation of non-bone health outcomes such as different types of cancer, cardiovascular disease, and all-cause mortality was based on studies originally designed to evaluate bone health outcomes. The authors concluded that the evidence available did not permit firm conclusions to be drawn on the effects of vitamin D for the latter. They also found considerable heterogeneity among the studies including inconsistent results and limitations in study design that precluded firm conclusions to be drawn for non-skeletal health outcomes. A dose-response meta-analysis of the effects of serum 25(OH)D levels and health outcomes could not be carried out because of limited and inconsistent data. (11) Moreover, most of the cohort studies that evaluated a dose-response of serum 25(OH)D levels were based on studies originally designed to evaluate bone health outcomes in white postmenopausal women, limiting their generalizability to other age groups and ethnicities. (11) The authors also commented that the observational studies included were designed to be hypothesis generating rather than to confirm the association between vitamin D ± calcium and different health outcomes as they were originally designed to study other outcomes. (11)

Other possible limitations in the evidence brought up by the authors were:

- Potential selection bias from the exclusion of large number of participants from the original cohort (up to 60-70% in some cases due to the unavailability of blood samples or questionnaires)
- Potential outcome misclassification (e.g., the identification of cancer cases without histopathological confirmation)
- Possibility of unmeasured or residual confounding, especially given the relatively small to moderate effect sizes (odds ratios below 2.0)
- Unclear statistical power to detect an association (i.e., even though some of the studies were very large it is possible that they were underpowered to detect the true effect sizes)
- In some observational studies, the time lag between the measurement of serum vitamin D and the diagnosis of cancer type varied between 1 and 16 years, while potential confounders such as family history were not consistently reported
- Issues such as the use of different assays to measure serum vitamin D and problems with processing of the blood sample may also have affected the validity of study results.

A 2007 conference with participation from the United States National Cancer Institute, Institute of Medicine, and National Institute of Health was held in order to discuss the evidence of the association between vitamin D and cancer. (42) The conference group stated that more information is required to understand how genetic factors, obesity, and dietary components affect serum vitamin D levels. (42) The publication authors also believed that further research is needed in order to determine the vitamin D dose, duration, and period of life during which exposure would be more relevant for a reduction in cancer risk, as well as to determine the long-term safety of high doses of vitamin D. (42) It was pointed out that a better understanding of exposure biomarkers, such as dietary and supplemental vitamin D intake, instead of serum 25(OH)D levels is necessary. (42) The limitations of using serum 25(OH)D as a marker of exposure were covered and included assay variation and the fact that measuring the serum 25(OH)D level once does not appropriately reflect long-term exposure due to seasonal variation. (42) With regard to the effects of vitamin D and cancer, a fact sheet from the United States National Cancer Institute from September 2009 (43) pointed out that new RCTs are necessary in order to understand the effects of vitamin D on cancer and other health outcomes.

For the purposes of this report, it was decided that, until the issues concerning the effects of vitamin D alone or combination with calcium, are resolved, our research would focus on the effects of vitamin D on bone health. However, for the sake of completion, studies identified through the AHRQ systematic literature review (11) that evaluated the effects of vitamin D ± calcium in cancer incidence or cancer mortality are summarized below. Studies of other non-bone health outcomes evaluated, i.e., site-specific cancers (colorectal, breast, prostate, and pancreatic), cardiovascular outcomes, and all-cause mortality are described in Appendix 1. Quality assessment of the evidence performed by MAS according to GRADE Working Group criteria (44) concluded that there is no high or even moderate quality evidence to support an association between vitamin D and these non-bone health outcomes (Appendix 1). Even if there is any residual uncertainty, there is no evidence that testing vitamin D levels encourages adherence to Health Canada's guidelines for vitamin D intake. The normal threshold for vitamin D levels to prevent non-bone health related conditions cannot be resolved until a causal effect or correlation has been demonstrated between vitamin D and these health conditions. This is as an ongoing research issue around which there is currently too much uncertainty to base any conclusions that would support routine vitamin D testing.

Vitamin D ± Calcium in Cancer Incidence and Mortality

Vitamin D, by its effect on cell proliferation, differentiation, and apoptosis (programmed cell death), may affect the risk of cancer. (45) In total, three RCTs (45-47) and two cohort studies (48;49) that evaluated the effects of vitamin D in overall cancer risk were included in the AHRQ systematic review AHRQ. (11) The RCTs evaluated the effects of vitamin D ± calcium on cancer incidence or cancer mortality risk with a mean follow-up of 4 to 7 years. Their results, however, were inconsistent (as detailed below).

RCTs

Lappe et al. conducted a 4-year RCT (published in 2007) designed to compare the effects of vitamin D_3 (1,000 IU/day) plus calcium (1,400-1,500 mg/day), to a placebo and the same dose of calcium alone on the risk of fractures. Treatment effects on the risk of any type of cancer was a secondary endpoint. (45) The study included 1,179 healthy postmenopausal women (> 55 years old) without any known cancer, chronic kidney disease, or metabolic bone disease. (50) Outcomes were self-reported and confirmed through medical records. (45) All women were white with a mean age of 66.7 ± 7.3 years and a mean baseline 25(OH)D level of 71.8 ± 20.3 nmol/L. (45) Baseline characteristics by study group were not provided and the authors did not mention if they were comparable, especially with regards to cancer risk factors. An intention-to-treat analysis with logistic regression was used. (45) Cox proportional hazards analysis was not used as, according to the authors, the assumption of a constant hazard ratio was not satisfied by their data. The authors did not, however, provide additional information on how the violation occurred. (45)

A total of 1,024 of 1,180 (86.8%) subjects completed the study with treatment adherence being 85.7% in the vitamin D + calcium group and 74.4% in the calcium group. (45) Cancer was diagnosed in 20 of 288 (6.9%) patients in the placebo group, 17 of 445 (3.8%) in the calcium group, and 13 of 446 (2.9%) in the vitamin D + calcium group over 4 years of follow-up. (45) There was a 60% decrease in cancer risk with vitamin D + calcium compared to placebo [unadjusted RR 0.402 (95% CI: 0.20, 0.82)] and a trend to risk reduction with calcium compared with placebo [RR 0.532 (95% CI: 0.27, 1.03)]. (45) Excluding the cancer cases diagnosed during the first year, the RR for vitamin D + calcium was 0.232 (95% CI 0.09, 0.60) and 0.587 (95% CI: 0.29 , 1.21) for calcium alone. (45)

Limitations of the study included the use of logistic regression to analyze time-to-event data, which may have lead to bias since losses-to-follow-up and censoring was not taken into account. In addition, demographic characteristics by study group were not provided and the authors did not provide comment on whether the two groups were comparable. These factors were later brought up in letters to the editor. (51;52) One letter also brought up the fact that the cancer rate observed in the placebo group (6.9%) was higher than the population estimate (4.9%) in the Nebraska Cancer Registry where the study was conducted and this was not the case in the treated groups. (53) This may have resulted in an overestimate of the effects of vitamin D + calcium combination. (53) Other factors brought up in the letters to the editor were that the results may have occurred by chance due to the small number of cases reported and possible demographic differences between study groups, which were not provided in the publication. (53).

The second RCT was part of the Women's Health Initiative (WHI) study, which compared the effects of vitamin D (400 IU/day) + calcium (1,000 mg/day) to placebo on the risk of hip fractures (primary outcome), colorectal cancer (secondary outcome), and other types of cancer. (46) The outcomes were self-reported and confirmed in the subjects' medical records. (46) Women in both study groups were allowed to take up to 600 IU/day of vitamin D (later increased to 1,000 IU) and up to 1,000 mg of calcium/day in addition to the study drug. (46) The women included in the vitamin D + calcium study had been participating for a year in a component of the WHI trial in which women were randomized to either:

1) dietary interventions through a low-fat diet high in fruits and vegetables,

2) postmenopausal hormone therapy,

3) a combination of the two, or

4) placebo and usual diet. (54)

The effect of vitamin D + calcium on overall cancer and colorectal cancer was evaluated based on an intention-to-treat time-to-event analysis using a Cox proportional hazards model. (46)

A total of 36,282 postmenopausal women, 50 to 79 years old, without a history of hypocalcaemia or renal calculi, not using corticosteroids or > 600 IU/day of vitamin D, were included (18,176 in the vitamin D plus calcium group and 18,106 in the placebo group). (46) Of these, 30,153 (83%) were of white ethnicity and the two study groups were comparable with regards to demographic characteristics. The results were stratified by age, colorectal cancer history, and hormone therapy/dietary modification study group assignment. The mean follow-up was 7 ± 1.4 years. (46) Treatment compliance was low in both groups, at approximately 60%. (46) There was no statistically significant difference in the risk of overall cancer with vitamin D + calcium compared to placebo [HR: 0.98 (95% CI: 0.91, 1.05)]. There were 1,634 cases (1.28%/year) in the combination group and 1,655 (1.30%/year) in the placebo group. (46) Despite being a large study, the fact that vitamin D and calcium intake in addition to the study drug was allowed, as well as a relatively low compliance rate, may have contributed to the lack of a statistically significant difference between the study groups.

The third RCT evaluated the effect of vitamin D_3 (100,000 IU every 4 months) compared to placebo on the risk fractures and overall mortality in 2,686 men and women, 65 to 85 years old, selected from the Doctors Study Register in the UK. (47) Cancer incidence was a secondary outcome and all outcomes

were self reported (unclear if diagnosis was confirmed). An intention-to-treat analysis using age-adjusted Cox regression was used. The two groups were comparable with regards to demographic characteristics with a mean participant age of 74 ± 4.6 years. The mean calcium intake at 4 years of 742 mg/day did not differ between the study groups. The compliance rate was approximately 76% and did not differ between the study groups. There were 188 (14%) cancer cases reported in the treatment group and 173 (13%) in the control group. After a follow-up of 5 years, there were no statistically significant differences between the study groups with regards to both incidence of cancer [RR 1.09 (95% CI: 0.86, 1.36)] and overall cancer mortality [RR 0.86 (98% CI: 0.61, 1.20)].

Table 5 summarizes the results of these three RCTs.

Cohort studies

The two cohort studies identified in the AHRQ systematic review used participants of the Third National Health and Nutrition Examination Survey (NHANES III), which included a national sample of non-institutionalized subjects in the United States. (48;49) Both evaluated the association between baseline serum 25(OH)D and cancer mortality over a mean follow-up of 8.7 years. (48;49) with baseline evaluation performed between 1994 and 1998 and follow-up ending on December 31st 2000. (48;49) Cancer mortality was determined by matching participants with the National Death Index with cancer mortality based on ICD-10 codes. (48;49)

The first study (Freedman et al., 2007) included 16,818 men and women over the age of 17. (48) Cox proportional hazards analysis adjusted for age, ethnicity and smoking was used. (48) Baseline serum 25(OH)D was divided into quintiles and the rate of cancer mortality over 8 years of follow-up was compared among the quintiles. (48) The mean age varied between 40 and 45 years depending on the baseline 25(OH)D. (48) In total there were 536 deaths due to cancer but no association between baseline 25(OH)D and cancer mortality over the 8-years of follow-up (Table 5). (48)

The second study (Melamed et al. 2008) evaluated the association between baseline 25(OH)D and overall mortality with cancer mortality being one of the secondary endpoints along with mortality due to cardiovascular disease, infectious disease, or external causes. (49) Participants included men and women over the age of 20 who were given both a baseline 25(OH)D measurement and a physical examination. (49) The baseline serum 25(OH)D was divided into quartiles and the rate of cancer mortality over 8 years of follow-up was compared among quartiles. (49) Poisson regression analysis adjusted for several potential confounders (Table 5) was used with censoring at the time of death for other causes. (49) A total of 13,331 participants were included with a mean age of 42 to 46 years, depending on the baseline 25(OH)D quartile. (49) In total, 424 deaths due to cancer occurred but no association was found between baseline 25(OH)D levels and cancer mortality over the 8-years of follow-up. (49)

In both of these cohort studies, the effects of vitamin D intake on cancer mortality was not directly evaluated. Instead, the rate of cancer mortality over 8 years of follow-up was compared to the serum 25(OH)D level measured at baseline, without accounting for changes in exposure over this period. Moreover, there was limited adjustment for potential confounders in the analysis. Although both studies reached similar conclusions, slight differences were present despite their having drawn from the same population. This may be due to imprecision, different statistical methodologies, and adjustments for different covariates and illustrates the importance of accounting for all the factors that may affect serum vitamin D levels (e.g. adjustment for calcium intake was not done in these studies). As pointed out by Speers and Brown, different factors may affect serum vitamin D levels including sunlight exposure, BMI, vitamin D metabolism, physical activity, and genetic factors. (55) Lastly, the baseline 25(OH)D quartiles/quintiles were defined by slightly different serum 25(OH)D levels, which makes it difficult to interpret the results of the two studies.

Table 4: Summary of Findings from AHRQ Report (Cancer Incidence)

Study	Study Characteristics	Study population	Exposure assessment	Outcome ascertainment	Statistical Analysis	Study Results	Summary of conclusions
Lappe.(45) (2007) United States N=1,180	RCT f-up: 4 yr	Age> 55 yrs Postmenopausal women Mean age: 66.7 ±7 yrs Demographic information per group not provided	▪ VD3 1,000 IU + Ca 1,400-1,500 mg/day (n=446) ▪ Ca 1,400-1,500 mg/day (n=445) ▪ placebo (n=288)	▪ Incidence of any cancer ▪ Secondary endpoint ▪ Self-reported (confirmed in medical record)	▪ ITT, Logistic regression used instead of Cox proportional hazards. ▪ Unadjusted.	VD+ Ca vs. placebo RR: 0.402 (0.2, 82) Ca vs. placebo RR 0.532 (0.27,1.03) Excluding cancers developed in yr 1 VD+ Ca RR 0.232 (0.09 , 0.6) Completed the study: 86.8% Compliance: 85.7% (≥80% doses)	▪ Association between use of VD + Ca and reduced risk of cancer. ▪ Logistic regression does not take into account censoring and may affect the validity of the results
Wactawski-Wende (46) (2006) United States N=36,282	RCT F-up: 1-12 yrs (mean 7 yrs)	Age: 50-79 yrs Postmenopausal women Age 50-69 yrs: 29,930 (82.5%) Demographic characteristics appear to be comparable between groups.	▪ VD 400 IU + Ca 1 g vs. ▪ Placebo (Pl) ▪ Use of up to 600 IU (1,000 IU later) and 1,000 mg Ca/day in addition to study drugs allowed in both groups	▪ Incidence of any cancer ▪ Secondary endpoint ▪ Self-reported (confirmed in medical record)	▪ ITT, Cox proportional hazards. ▪ Matched: age, study centre, ethnicity ▪ Results stratified by age, colorectal cancer history, and treatment assignment in the HRT/dietary modification component of the study.	Any cancer HR: 0.98 (0.91 , 1.05) VD vs. Pl Compliance: ~ 60%	▪ No association between VD + Ca and cancer vs. placebo. ▪ Use of VD and/or Ca in addition to study drugs may have biased the results towards the null.
Trivedi (47)(2003) UK N= 2,686	RCT F-up: 5 yrs	Age 65-85 yrs Men and women Excludes history of renal stones, sarcoidosis, or malignancy. Demographic characteristics appear to be comparable between groups.	▪ VD3 100,000 IU every 4 months ▪ Placebo (Pl)	▪ Incidence of any cancer ▪ Cancer mortality ▪ Self-reported	▪ ITT ▪ Cox proportional hazards. ▪ Adjusted for age.	Any cancer RR 1.09 (95% CI: 0.86, 1.36) Overall cancer mortality RR 0.86 (98% CI: 0.61, 1.20) Completed the study: 76.5% Compliance rate: 76% (≥80% doses), Similar between groups.	▪ No association between VD + Ca and cancer vs. placebo

Ca calcium; f-up follow-up; HRT hormone therapy; ITT intention-to-treat; VD vitamin D; yr year

Table 5: Summary of Studies Evaluating the Effects of Vitamin D on Mortality from any Cancer

Study	Study Characteristics	Study population	Exposure assessment	Outcome ascertainment	Statistical Analysis	Study Results (By 25(OH)D nmol/L)	Summary of conclusions
Freedman (48)(2007) NHANES III United States N= 16,818	▪ Cohort study ▪ F-up: 8.8 yrs	▪ Mean age: 40-45 yrs (> 17 yrs) ▪ Men and women	▪ Baseline 25(OH)D ▪ Collected during different seasons	▪ Overall cancer mortality ▪ Secondary endpoint ▪ Probabilistic linkage to National Death Index (ICD-10)	▪ Cox proportional hazards regression. ▪ Adjusted for ethnicity and smoking. ▪ Not adjusted for BMI, education despite association with baseline 25(OH)D	Cancer mortality HR (95% CI) by 25(OH)D quintile (nmol/L) < 50 nmol/L: reference 50-62.5: 1.22 (0.91, 1.64) 62.5-80: 1.02 (0.69, 1.50) 80-100: 1.00 (0.71, 1.40) 100 to < 120: 0.92 (0.58, 1.46) ≥ 120: 1.49 (0.85, 2.64) p for linear trend: 0.6	▪ No association between baseline 25(OH)D and cancer mortality. ▪ White men with lower BMI, and higher education had higher baseline 25(OH)D - not adjusted for. ▪ No adjustment for important confounders*, maybe because of lack of power.
Melamed (49) (2008) NHANES III United States N= 13,331	▪ Cohort study ▪ F-up: 8.8 yrs	▪ Mean age: 42-46 yrs (> 20 yrs) ▪ Men and women	▪ Baseline 25(OH)D ▪ Collected during different seasons	▪ Overall cancer mortality ▪ Secondary endpoint ▪ Probabilistic linkage to National Death Index (ICD-10)	▪ Poisson regression. ▪ Adjusted for age, ethnicity, smoking, season of blood draw, BMI, CRP, physical activity, VD supplementation, socioeconomic status, etc. ▪ Censoring for deaths due to other causes.	Cancer mortality HR (95% CI) by 25(OH)D quartile (nmol/L) > 80 nmol/L: reference 61-80: 0.80 (0.54, 1.19) 44.5-61: 1.08: 0.80, 1.46) < 44.5: 0.91 (0.63, 1.31) p for linear trend: NR	▪ No association between baseline 25(OH)D and cancer mortality.

BMI, medical conditions, season of blood sample, Ca intake, education.

Vitamin D Target Serum Level

According to the Institute of Medicine, Food and Nutrition Board, an optimal serum concentration of vitamin D has not been established and this value may vary across different stages of life. (5) While some authors believe that target serum levels should be above 50 nmol/L (1;6;12;20;21;28), others believe that it should be above 75 nmol/L. (2;5;6;9;16;21) Vitamin D deficiency is defined as a serum level below 25 nmol/L (3;6;19;20;56), based primarily on the risk of rickets and osteomalacia (25). Severe deficiency has also been defined as a serum level below 12.5 nmol/L. (1;2;12) The Institute of Medicine also states that vitamin D serum levels below 25-27.5 nmol/L may lead to rickets in infants and children and osteomalacia in adults, while serum levels ≥ 37.5 nmol/L are generally considered adequate for both bone and overall health in healthy persons. (5) The latter limit seems to be based on a cross-sectional study of 98 Caucasian postmenopausal women in which it was found that women with serum 25(OH)D < 38 nmol/L exhibited reduced vertebral bone density. (57)

In the Netherlands, a 2000 Dietary Reference Values Advisory Report indicated that serum 25(OH)D levels above 30 nmol/L in adults and children is adequate, based on a lack of evidence that a higher threshold would improve bone mineral density or decrease the risk of fractures. (12) Subsequently, in a 2008 report, The National Health Council of the Netherlands conducted a review of the literature on the clinical and safety of vitamin D and recommended that the minimum serum 25(OH)D level should be raised to 50 nmol/L in women > 50 years and men >70 years, since bone mineral density (BMD) decline starts to accentuate at this age. (12) The report does not recommend an increase in the minimum serum level of 30 nmol/L for other age groups, as this was deemed sufficient to prevent rickets and it's unknown if this would affect fracture risk later in life. It is pointed out in the report that these conclusions were not based on adequate studies and most were conducted among Caucasian women (specific references not provided). The authors also state that the evidence for effects of vitamin in other non-skeletal outcomes was inconsistent. They believe that raising the threshold to 75 to 100 nmol/L, as has been suggested by other authors, may require vitamin D supplementation. (12) They stated, however, that these doses have not been adequately studied and may be higher than the upper tolerable level of 2,000 IU/day in at least part of the population.

Targets Based on Interactions with PTH

Attempts to establish an optimal level of serum vitamin D based on its interaction with PTH over five studies yielded different results varying from 30 nmol/L to 99 nmol/L, the highest estimates originating from cross-sectional studies. (58) Most of these studies were conducted in Caucasian elderly men or postmenopausal women. The results of these and other studies are described in detail below.

- Dawson-Hughes et al. (1997) evaluated the association between serum 25(OH)D and PTH in 391 elderly men and women (mean age 71 ± 4.5 years). (59) Serum levels of the metabolites were measured at the time of enrolment and subjects were enrolled in different seasons. The mean calcium intake was 732 ± 356 mg/day and the mean vitamin D3 intake was approximately 190 ± 108 IU/day. Through unadjusted non-linear regression analysis, it was estimated that PTH reached a plateau at a mean serum 25(OH)D level of 110 nmol/L, but this exhibited a wide 95% CI of 60 nmol/L to 168 nmol/L. (59) The authors found that calcium intake has no affect on the association between 25(OH)D and PTH. (59)
- A cross-sectional study by Krall et al. (1989) evaluated the effects of 25(OH)D and serum PTH levels across different seasons. (60) A total of 333 healthy, white, postmenopausal women with low calcium intake (<650 mg/day) were included with a mean age of 58 years (range: 43-71) and a mean total vitamin D intake of 112 (0-1,687) IU/day. An inverse correlation was found between serum 25(OH)D levels and PTH (Pearson correlation, r:-0.26, p<0.001) adjusted for vitamin D and calcium intake. (60) The correlation was stronger in women whose serum level was measured during the spring (r: 0.65, p<0.001) when serum 25(OH)D is expected to be at its lowest compared to the August to October period (r: 0.13, p>0.10) when it's expected to be at its highest. According to the authors, given the wide scattering of the points, it was not possible to predict with confidence the serum 25(OH)D threshold above which serum PTH reaches a plateau. They concluded that a vitamin D intake > 220 IU/day was

sufficient to maintain a constant PTH level throughout the year. (60) It was estimated that the serum 25(OH)D would be 95 nmol/L in women with vitamin D intake > 220 IU/day, however, this does not appear to be based on actual serum levels measured since, according to the authors, it was calculated using "calibration methods". (60) Additionally, it was stated that the data does not provide evidence that the effect would persist in subjects with higher calcium intake.

- Chapuy et al. (1997) conducted a large cross-sectional study to evaluate the effect of serum 25(OH)D on serum intact PTH (iPTH) in 1,579 healthy men and women. (61) The mean age of participants was 50 years and the mean vitamin D intake was 136 ± 304 IU/day as measured by a food frequency questionnaire with a 24-hour recall. Mean calcium intake was 843 ± 481/day, which is below the recommended level of 1,000 mg/day. Serum 25(OH)D was measured between November and April when serum levels are expected to be at its lowest. A statistically significant inverse correlation was found between iPTH and 25(OH)D levels in an analysis of variance (ANOVA) adjusted for sex and date of blood collection. Using non-linear weighted least squares regression analysis, the authors concluded that iPTH reached a plateau at 36 pg/ml when serum 25(OH)D was 78 nmol/L. (61) The authors also reported that iPTH started to increase when serum 25(OH)D dropped below 78 nmol/L but the upper limit of the normal range for iPTH was only reached when serum 25(OH)D was 11.3 nmol/L. Thus it appears that iPTH was within normal ranges when serum 25(OH)D was between > 11.3 nmol/L and 78 nmol/L. According to the graph provided in the publication (Figure 1), it seems that there is little change in serum PTH with 25(OH)D ≥ 50 nmol/L, which has been corroborated in other studies that noted that the increase in serum PTH was minimal within a 25(OH)D range between 50 and 75 nmol/L, suggesting that 50 nmol/L may be a more appropriate threshold than 75 nmol/L. (62) Lastly, according to the authors, "a low serum 25(OH)D does not always lead to an increase in serum PTH", no additional comments were provided on this statement.

Figure 1: Relationship between Intact Parathyroid Hormone (IPTH) and Serum Vitamin D

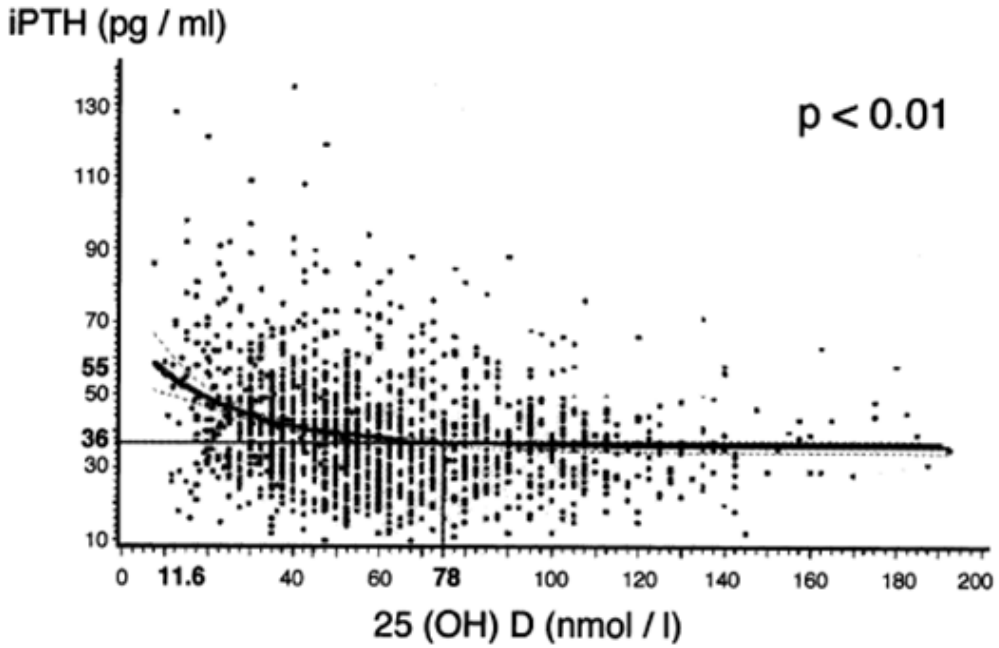


Fig. 1. Relationship between serum intact parathyroid hormone (iPTH) and 25-hydroxyvitamin D (25(OH)D) values in the whole population studied. For a 25(OH)D concentration higher than 78 nmol/l (31 ng/ml), there is a plateau level at 36 pg/ml for iPTH. When 25(OH)D values are lower than 78 nmol/l (31 ng/ml), the serum iPTH values begin to increase.

Source: Springer/Osteoporosis International 1997, 7(5):439-443. Prevalence of Vitamin D Insufficiency in an Adult Normal Population. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S et al. Figure 1. With kind permission from Springer Science and Business Media.

- Malabanan et al. (1998) conducted a cohort study that included 35 adults (mean age: 67 years, 49-83 years) with baseline 25(OH)D levels between 25 nmol/L and 62.5 nmol/L. (63) They were prescribed 50,000 IU of vitamin D2 per week and 1,200 to 1,500 mg calcium per day for 8 weeks. Serum iPTH was measured in these subjects before and after treatment with vitamin D plus calcium. After treatment, PTH decreased by 35% in 11 subjects with a pretreatment 25(OH)D of 27.5 to 39.9 nmol/L ($p<0.02$). In 17 subjects with pretreatment 25(OH)D levels between 40 and 49.9 nmol/L, iPTH decreased by 26% ($p<0.001$). No statistically significant changes in iPTH were observed in seven subjects with pretreatment 25(OH)D levels between 50 and 60 nmol/L, despite a 66% increase in serum 25(OH)D after treatment. The authors concluded that adults > 49 years may require serum 25(OH)D levels > 50 nmol/L in order to achieve optimal serum iPTH levels and that iPTH tended to increase when 25(OH)D levels were below 50 nmol/L.
- Thomas et al. (1998) carried out a cross-sectional study that included 290 men and women, ages 18-95 (mean: 62 years), who were mostly white (79%), and 54 (21%) of which taking multivitamins. (64) The study evaluated the effects of serum 25(OH)D on serum PTH. Serum 25(OH)D was measured in March (when it's expected to be at its lowest) in 150 subjects and September (expected to be the highest) in 140 subjects. The authors concluded that PTH levels rose sharply when 25(OH)D fell below 37.5 nmol/L. It is not clear if age was adjusted for in the analysis given the wide range of the subjects included.
- Lips et al. (1998) conducted an RCT that included 109 elderly men and women (mean age: 81-84 years), living either in nursing homes or in aged people's homes, to evaluate the effect of serum vitamin D levels on serum iPTH. (65) The subjects were randomized into three groups, 1) vitamin D3 400 IU/day, 2) vitamin D3 800 IU/day, or 3) control group; treatment in all groups lasted for 1 year. In the two treated groups, the mean baseline 25(OH)D was 23.6 nmol/L, which increased to above 40 nmol/L. There was a 15% decrease in serum PTH levels in the two treated groups at 3 months, which was maintained until the end of treatment. An inverse correlation between serum calcium and serum PTH was also demonstrated after 3 months of treatment (Pearson r:-0.25, $p=0.01$).
- Vieth et al. (2003) carried out a cross-sectional study of 1,741 euthyroid Canadians receiving treatment for thyroid conditions evaluated the association between serum vitamin D and serum PTH . (66) An estimate of calcium intake was not provided and calcium intake was not accounted for in the analysis. The graphs showed in this publication seem to corroborate the fact that once serum vitamin D levels are above the bracket of 40 to 50 nmol/L, there is little to no further drop in serum PTH.

Based on the results of the studies, we believe that a bracket of 40 to 50 nmol/L would be an appropriate threshold for serum 25(OH)D, a line that was also used in a publication by Lips et al. (62) Since this evaluation focuses on average risk individuals (excluding osteoporosis patients), fracture risk was not used as an endpoint to evaluate the serum vitamin D level.

Targets Based on Bone Health Outcomes

A systematic review published in August 2007 by AHRQ evaluated the association between serum vitamin D levels and different bone health outcomes in different age groups. (33) The overall quality of the systematic review was considered high, rating 10 out of 11 on the AMSTAR criteria (a measurement tool to assess systematic reviews). (41) Studies evaluating the effects of vitamin D_2 or D_3 supplementation, with or without calcium, compared to either placebo or a lower vitamin D dose were included. The literature search extended from 1966 until the second quarter of 2006. A total of 72 studies evaluating bone health outcomes across different age groups met the eligibility criteria. Most of the studies were conducted among white postmenopausal women and older men. (33;67)

In summary, although a precise target serum vitamin D level across age groups could not be established there was a trend towards improvement in some bone health outcomes with higher serum vitamin D levels. (33;67) Exceptions included fractures and performance measures, for which the evidence of an association between serum vitamin D levels and health outcomes was inconsistent[4]. (33)

On the other hand, the authors concluded that there was fair evidence[5] that higher serum 25(OH)D levels may lead to improved outcomes in falls among the institutionalized elderly, and bone health outcomes such as rickets (infants), PTH levels (infants, older children, and pregnant women), bone mineral density (BMD)/ bone mineral content (BMC) (older children 6-17 years, postmenopausal women and older men). (33) Additional information on some of these outcomes is illustrated below.

- In children with rickets the mean serum 25(OH)D at baseline or diagnosis was below 27.5 nmol/L in affected children in six studies (one RCT, five observational). (33) Five case-control studies observed a mean or median 25(OH)D between 30 and 50 nmol/L in children with rickets. (33) There was fair evidence of an association between low serum 25(OH)D and rickets, although it wasn't possible to set the threshold above which rickets will not occur. The authors point out that most of these studies were conducted in developing countries where dietary calcium intake may be low (33), which may exacerbate the development of vitamin D-deficiency rickets (24) and limit the generalizability of the results to North America. (33)
- In four out of five studies identified (1 RCT, 2 out of 3 cohorts and 1 case control study) there was an association between serum 25(OH)D and risk of falls among postmenopausal women and elderly men. The association was not maintained, however, after adjustment for age and illness severity in one cohort study and PTH in a case-control study. One cohort study suggested a higher risk for falls with serum levels < 39 nmol/L and PTH < 66 pg/ml (hazard ratio: 1.65, 95% CI: 1.10, 2.46). (33) The authors concluded that there was fair evidence of association between lower serum 25(OH)D and increased risk of falls in institutionalized elderly, with one study suggesting a specific serum concentration of 39 nmol/L, below which the risk of falls is increased. (33)

No overall serum vitamin D threshold level could be established across age groups for improved bone health outcomes due to inconsistencies in the evidence. (33;67) According to the authors the conclusions were limited by study quantity and quality and/or inconsistencies in study results. (33) Limitations raised in the studies included failure to control for potential confounders, the fact that the association between serum vitamin D levels and bone health outcomes was a secondary endpoint, and limitations with the 25(OH)D assays, which may have affected study results. (33) A more recent systematic review of vitamin D published in July 2009 did not identify any further studies on the association between bone health outcomes and serum vitamin D levels (literature search update: April 2009). (11)

Targets Based on Non-Bone Health Outcomes

Widely varying optimal serum vitamin D levels based on outcomes other than bone disease have been proposed, from as little as 27.5 nmol/L to 100 nmol/L. (9) The use of different outcomes to determine optimal serum 25(OH)D levels and the issues with different laboratory assays mentioned above may partially explain these differences. (5) Some authors propose that serum 25(OH)D level should be above 75 nmol/L based on different disease outcomes (other than bone health), but according to the United States Office of Dietary Supplements (2009), this is not based on sufficient evidence. (5) Quality assessment of the evidence on non-bone health outcomes performed by MAS according to GRADE Working Group criteria (44) concluded that there is no moderate or high quality evidence to support an association between vitamin D and non-bone health outcomes (see Appendix 1).

[4] Inconsistent evidence: impossibility to draw conclusions regarding an association due to inconsistencies in results across studies.

[5] Fair evidence: sufficient evidence to establish an association; however this was limited by inconsistent results, small numbers of studies, and/or the absence of good quality studies.

The 2003 K/DOQI guidelines on bone metabolism and disease in CKD did suggest a target serum vitamin D level of 75 nmol/L in CKD patients stages 3 and 4 (31) and children with CKD stages 2 to 4 (2005) (based on expert opinion). (32) Both the 2008 guidelines from the Canadian Society of Nephrology for the management of kidney disease and the 2009 KDIGO on CKD mineral and bone disorder concluded that there is no evidence from RCTs supporting a reduction of fractures or mortality with treatment with calcitriol or vitamin D analogs in CKD patients. (29) In 2009, the authors of KDIGO guidelines stated that there is a lack of consensus on the target serum 25(OH)D level. (18)

Summary of Targets

In conclusion, there seems to be consensus on the definition of vitamin D deficiency at 25(OH)D < 25 nmol/l, based on diseases such as rickets and osteomalacia. Higher target serum levels have been proposed for subclinical endpoints such as PTH level, but considerable ambiguity remains as to what the appropriate target serum vitamin D level should be. Moreover, most of the studies were conducted in postmenopausal women and elderly men, which may not be generalizable to other age groups.

The normal threshold for vitamin D levels to prevent non-bone health related conditions cannot be resolved until a causal effect or correlation has been demonstrated between vitamin D levels and these health conditions and an appropriate normal threshold identified. This is an ongoing research issue around which there is presently too much uncertainty to form any conclusions that would support routine vitamin D testing. Therefore, in this report, two conservative target serum levels were adopted, 25 nmol/L (based on the risk of rickets and osteomalacia) and the bracket of 40 to 50 nmol/L (based on interactions with PTH).

Vitamin D Daily Adequate Intake Recommendations (Canada)

Health Canada is currently reviewing more recent data on the safety and effectiveness of vitamin D in order to decide if current recommended levels need to be updated. (68) Health Canada recommends that until the daily recommended intakes (DRI) for vitamin D are updated, the 2007 Canada's Food Guide ("Eating Well with Canada's Food Guide") (69) be followed for vitamin D intake. (68) Canada's Food Guide recommends that Canadians over the age of 2, including pregnant and lactating women, should have 2 cups (500 ml) of fortified milk or fortified soy beverages daily in order to obtain an adequate daily intake of vitamin D (200 IU). (68;69) In addition, men and women over the age of 50 should take 400 IU of vitamin D supplementation daily. (69) This was based on the 1997 Health Canada recommendations for the daily adequate intake of vitamin D for Canadians of different age groups (Table 6), (70) which are based on evidence on health outcomes and safety originating from a systematic literature review. (71)

The term 'adequate intake' (AI) is used when not enough scientific evidence is available to define the recommended daily intake[6]. (17;68) For vitamin D, the AI is the "daily intake that is sufficient to maintain bone health and normal calcium metabolism in healthy people". (5) Table 6 displays the upper daily tolerable level for different age groups, defined as "… the highest continuing daily intake of a nutrient that is likely to pose no risks of adverse health effects for almost all individuals" (17), i.e., the most sensitive members of the healthy population. (72)

During the first 6 months of life, infants depend initially on the maternal vitamin D status during pregnancy and then on dietary intake or sunlight exposure. (17) While recognizing that breast milk is the optimal food for infants, because the amount of vitamin D in human milk is low (1-10 IU/250 ml), exclusively breastfed infants who are not exposed to sunlight may not obtain sufficient amount of vitamin D. (17) For this reason, Health Canada issued a recommendation in 2004 that all breastfed full term infants should receive 400 IU

[6] According to Health Canada: "recommended average daily nutrient intake level based on the experimental data or determined by estimating the amount of a nutrient eaten by a group of healthy people. An AI is set when there is not sufficient scientific evidence available to determine an Estimated Average Requirement and calculate a Recommended Daily Allowance." (17)

of vitamin D supplementation daily from birth until this amount can be obtained through their diet or until they reach 12 months, in order to reduce the risk of rickets. (17) A population-based approach to vitamin D supplementation is favoured since in their opinion, testing all mothers and infants for their vitamin D status would be impractical and costly. (17) The recommendations also stated that non-breastfed infants do not need additional supplementation as formula is already fortified with vitamin D (100 IU/250 ml). (17) After one year, infants should receive 200 IU of vitamin D per day, which is the amount obtained through 2 cups of fortified milk or fortified soy beverage. (68)

For individuals with osteoporosis, the 2006 Canadian Consensus Conference on Osteoporosis recommends 800 IU/day of vitamin D_3 and 1,000 mg/day of calcium as an adjunct therapy to the main pharmacological interventions for osteoporosis. (73) This is consistent with the results of four meta-analyses previously mentioned on the effects of vitamin D doses between 400 IU and 800 IU/day ± calcium on fractures in postmenopausal women (≥50 years) and elderly men (≥65 years) (33;36-38), two of which favoured vitamin D doses of 700 IU to 800 IU/day. (33;37)

Table 6: Adequate Daily Intake of Vitamin D (from diet or supplements), Canada

Age group	Adequate Intake Canada, 1997† (70)	Health	Vitamin D supplementation for breastfed infants, Health Canada, 2004 (17)	Upper Tolerable Level, Health Canada, 1997 (70)
0-1 year	200 IU*		All breastfed, full term infants 400 IU (from supplement) Starting at birth and continuing until the infant's diet includes at least 400 IU vitamin D/day from other dietary sources or until 1 year of age.	1,000 IU
1- 50 years	200 IU		Not applicable	2,000 IU
Over 50 years	51-70 years 400 IU		Not applicable	2,000 IU
> 70 years	> 70 years: 600 IU		Not applicable	2,000 IU

IU refers to international units; max maximum

† based on the absence of adequate sunlight)

Estimate of Vitamin D intake in Ontario

The 2004 Canadian Community Health Survey on Nutrition was designed to estimate the intake of various nutrients, including vitamin D, derived from diet alone (excludes supplements) for Canada and the Provinces. (74) It excluded residents from the territories, Indian reserves or Crown lands, remote areas, individuals living in institutions, and full-time members of the Canadian Forces. People with zero or invalid intake from food, breastfed children and pregnant or breastfeeding women were also excluded. The cross-sectional probability survey selected respondents representing the provinces and each age and sex group. A total of 35,000 individuals were included at the national level including 10,921s Ontarians. Face-to-face interviews were used to estimate the amount of each nutrient with a 24-hour recall. Results are provided for different age and sex groups. Due to its multi-stage sampling design, the variance was estimated using the bootstrap method.

Table 7 shows the estimated vitamin D intake by age group and sex for Ontario residents. Among Ontario males ages 9 to > 70, the median daily dietary vitamin D intake ranged from 196 IU to 272 IU per day. Between 43% and 69% of males 9 to 50 years old, depending on age group, kept a daily intake above the recommended adequate intake of 200 IU/day (data not provided for men > 50 years). For females between

9 and > 70 years old, the median daily dietary intake varied from 152 IU to 196 IU/day, also depending on the age. Less than 50% of females between 9 and 50 years had an intake above the recommended adequate intake of 200 IU/day. In boys and girls 1 to 3years old, the median daily dietary vitamin D intake was 248 IU, while it was 224 IU among 4 to 8 year-olds. Of these age groups, 60% and 65%, respectively, were above the daily adequate vitamin D intake of 200 IU.

Table 7: Estimated Vitamin D Intake from Diet Alone in Ontario

	Males				Females			
Age group	5th percentile IU (SE)	Median IU (SE)	95th percentile IU (SE)	% > AI (SE)†	5th percentile IU (SE)	Median IU (SE)	95th percentile IU (SE)	% (SE) > AI†
9-13 yrs N=1,174	116 (20) ‡	**248 (12)**	444 (36) ‡	69.4% (6.2)	84 (20) ‡	**196 (12)**	384 (40)	48.8% (5.3)
14-18 yrs N=1,284	112 (20) ‡	**272 (16)**	564 (56)	73.2% (5.9)	60 (20) ‡	**176 (12)**	468 (84) ‡	40.9% (5.5)
19-30 yrs N=995	132 (44) ‡	**232 (28)**	380 (80)	67.7% (16) ‡	68 (20) ‡	**156 (12)**	292 (36)	25.0% (6.6) ‡
31-50 yrs N=1,464	72 (20) ‡	**184 (16)**	444 (60)	43.6% (6.2)	68 (16) ‡	**168 (12)**	384 (40)	36.2% (4.8)
51-70 yrs N=1,713	72 (16) ‡	**192 (20)**	616 (124)‡	§	64 (16) ‡	**152 (12)**	372 (40)	§
> 70 yrs N=2,079	80 (20) ‡	**196 (16)**	436 (52)	§	68 (8)	**168 (8)**	408 (32)	< 3¦
Values for boys and girls								
1-3 yrs N=644	68 (16) ‡	**248 (12)**	524 (3.2)	65% (3.4)				
4-8 yrs N=956	108 (16)	**224 (8)**	428 (32)	60.1% (5.1)				

Source: Canadian Community Health Survey, Cycle 2.2, Nutrition (2004). (74)
* AI refers to adequate intake ; IQR interquartile range; IU international units; SE standard error
† Adequate intake per day: 1-50 year-old: 200 IU, 51-70 year-old: 400 IU, > 70 years: 600 IU
‡ Values with a coefficient of variation between 16.6% and 33.3% should be interpreted with caution
§ Values with a coefficient of variation > 33.3% and a 95% confidence interval not entirely between 0 and 3%. Data suppressed.
¦ Values with a coefficient of variation > 33.3% and a 95% confidence interval entirely between 0 and 3%. Interpret with caution.

Ontario Context

Two vitamin D laboratory tests are available in Ontario, 25(OH)D and 1,25-dihydroxyvitamin D. The Ministry of Health and Long-Term Care Health Data Branch was contacted in order to provide the number of vitamin D laboratory services (both types) that were billed to the Province over the last 5 years. From those data the average change in the number of services provided each month of each year was calculated in order to project an estimate of the number of services that may be provided in the later months of 2009. These services were then multiplied by the unit cost of each test obtained from the Ministry of Health and Long-Term Care Schedule of Laboratory Fees (last updated Sept 30th, 2009; accessed Oct 13th, 2009) to project total billings to the Province from 2004 to 2009. (75) Different vitamin D test kits are also approved by Health Canada such as radioimmunoassay, liquid chromatography-tandem mass spectrometry, high pressure liquid chromatography, etc. (76)

Volume of Vitamin D Tests

The volume of vitamin D tests performed in Ontario has steadily increased over the past 5 years, with a steep increase of 169,000 tests in 2007 to more than 393,400 such tests in 2008 (up 133%). This figure has

continued to increase, such that the projected number of tests for 2009 is in excess of 731,000 (up 85%). Actual data up to July 2009 is available and the number of tests between August and December 2009 was extrapolated based on the average change in the number of tests between 2004 and 2008. These values include both vitamin D tests available, but the increase is largely derived from the 25(OH)D test (see Table 8 and Figure 2). The majority of the 25(OH)D tests were requested by general practitioners (87.4% in 2009, 84.8% in 2008). Notably, the percentage of 25(OH)D tests requested by general practitioners had previously increased from 59.5% in 2004 to 77.0% in 2007.

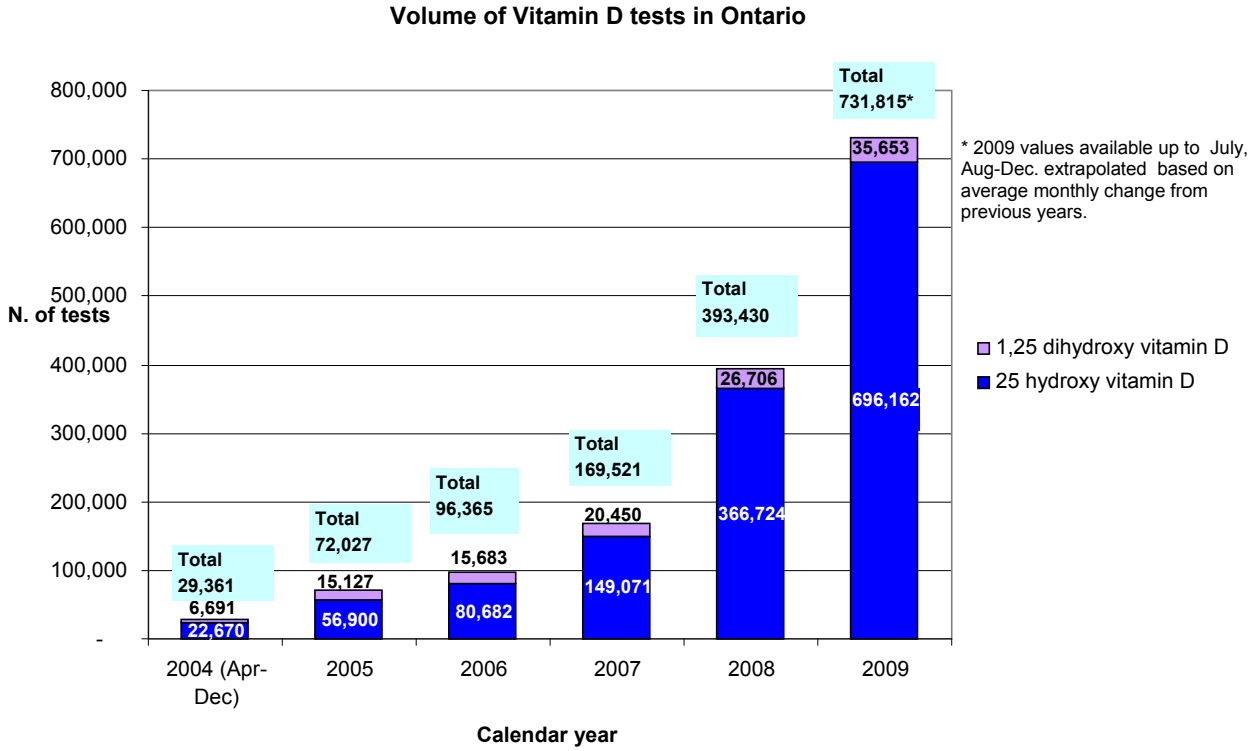
Table 8: Number of Vitamin D Laboratory Tests per Calendar Year†

Laboratory test	N. of vitamin D tests per Calendar Year - Ontario					
	2004 (Apr-Dec)	2005	2006	2007	2008	2009 (real data Jan-Jul, projected‡, Aug-Dec)
25-hydroxyvitamin D (25(OH)D	22,670	56,900	80,682	149,071	366,724	696,162
1,25-dihydroxyvitamin D	6,691	15,127	15,683	20,450	26,706	35,653
Total	29,361	72,027	96,365	169,521	393,430	731,815

Source: Health Data Branch, Ministry of Health and Long-Term Care, Ontario. All fiscal years assessed to M7 where possible, fiscal 2008 assessed to M5, fiscal 2009 includes services up to 30-jun-2009, assessed to M2.

† Includes both community and hospital laboratories. Hospital laboratories provide information about number of tests once a year and may not represent fiscal year. The number of tests done in hospital laboratories constitutes approximately 20% of all tests in 2005 and 7% in the 2008 fiscal years.

‡ Projected data for August-December was based on the 2008 month-by month increase ratio.



Source: Health Data Branch, Ministry of Health and Long-Term Care, Ontario. All fiscal years assessed to M7 where possible, fiscal 2008 assessed to M5, fiscal 2009 includes services up to 30-jun-2009, assessed to M2.

Figure 2: Annual Number of Vitamin D Tests in Ontario 2005-2009.

Cost of Vitamin D Tests

The annual billing costs of vitamin D tests was estimated by multiplying the unit cost of the test by the number of vitamin D laboratory tests performed in a given year. The unit cost of each test was, derived from amounts billed by community laboratories in the Ontario Ministry of Health and Long-Term Care Schedule of Laboratory Fees, was found to be $51.7 for 25(OH)D (L606, 100 LMS units, $0.517/unit) and $77.6 for 1,25-dihydroxyvitamin D (L605, 150 LMS units, $ 0.517/unit). (75) It was assumed that the costs of tests done in community and hospital laboratories would be similar. Approximately 92% of the vitamin D tests were performed in community laboratories over the 5-year period. The annual billings of vitamin D tests has increased from $1.7M in 2004 to $21.0M in 2008. The projected annual billing for 2009 is $38.8M, based on the number of tests reported between January and July 2009 and an extrapolation of the volume of tests for August to December 2009 (see Table 9).

It is unknown if a follow-up physician visit may be required after the vitamin D test. The actual number of follow-up visits that were incurred as a consequence of vitamin D tests cannot be accurately estimated. We therefore assumed different scenarios. Specifically, it was assumed that 5%, 10%, and 25% of the vitamin D tests would require a follow-up visit. We used applied a unit cost of $29.20 for each follow-up visit, which was based on the general practitioner's fee according to the Ontario Schedule of Benefits. (77) Table 10 summarizes the annual costs of vitamin D testing including the follow-up visits.

Table 9: Annual Billings of Vitamin D Laboratory Tests in Ontario

Year	25-hydroxy Vitamin D Tests	1, 25 dihydroxy Vitamin D Tests	Total
2004 (Apr – Dec.)	$1,172,039	$518,887	$1,690,926
2005	$2,941,730	$1,173,099	$4,114,829
2006	$4,171,259	$1,216,217	$5,387,476
2007	$7,706,971	$1,585,898	$9,292,868
2008	$18,959,631	$2,071,050	$21,030,681
2009 (projected)	$35,991,586	$2,764,859	$38,756,445

Source: Health Data Branch, Ministry of Health and Long-Term Care, Ontario. The same unit costs used for community and hospital laboratories.

Table 10: Annual Billings of Vitamin D Laboratory Tests and Follow-up Physician Visits in Ontario

Year	Annual Billings of Vitamin D tests	Annual cost of vitamin D tests + follow-up visits (assuming 1 visit in 5% of the tests)	Annual cost of vitamin D tests + follow-up visits (assuming 1 visit in 10% of the tests)	Annual cost of vitamin D tests + follow-up visits (assuming 1 visit in 25% of the tests)
2004 (Apr-Dec)	$1,690,926	$1,733,793	$1,776,660	$1,905,261
2005	$4,114,829	$4,219,988	$4,325,148	$4,640,626
2006	$5,387,476	$5,528,169	$5,668,862	$6,090,941
2007	$9,292,868	$9,540,369	$9,787,870	$10,530,372
2008	$21,030,681	$21,605,089	$22,179,497	$23,902,720
2009 (projected)	$ 38,756,445	$39,824,894	$40,893,344	$44,098,693

Source: Ministry of Health and Long-Term Care, Ontario.

Evidence-Based Analysis

Research Questions

The objective of this report is to evaluate the clinical utility of vitamin D testing in the average risk population and in those with kidney disease. As a separate analysis, this report also sought to evaluate the prevalence of vitamin D deficiency in Canada. The specific research questions addressed were:

1. What is the clinical utility of vitamin D testing in the average risk population and in subjects with kidney disease?
2. What is the prevalence of vitamin D deficiency in the average risk population in Canada?
3. What is the prevalence of vitamin D deficiency in subjects with kidney disease in Canada?

The clinical utility of vitamin D testing was defined as the ability to improve bone health outcomes with the focus on the average risk population (excluding osteoporosis) and kidney disease.

Methods

Literature Search

A literature search was performed on July 17th 2009 using OVID MEDLINE, MEDLINE In-Process and Other Non-Indexed Citations, EMBASE, the Cumulative Index to Nursing & Allied Health Literature (CINAHL), the Wiley Cochrane Library, and Centre for Reviews and Dissemination/International Agency for Health Technology Assessment for studies published between January 1, 1998 and July 17th 2009. Details of the keywords used in the literature search are provided in Appendix 2. Studies published in the grey literature were included if no other studies in the peer-reviewed literature were identified for specific outcomes or subgroups.

Observational studies that evaluated the prevalence of vitamin D deficiency in Canada in the population of interest were included based on the criteria listed below. When evaluating the prevalence of vitamin D deficiency, in the case of studies that evaluated the effect of vitamin D use on serum levels, the baseline levels were used. The eligibility of studies was judged based on the information provided in the study title and abstract. Abstracts were reviewed by a single reviewer and, for those studies meeting the eligibility criteria, full-text articles were obtained. Reference lists were also examined for any additional relevant studies not identified through the search. Articles with an unknown eligibility were reviewed with a second clinical epidemiologist and then a group of epidemiologists until consensus was established. Some studies have evaluated different outcomes in addition to the prevalence of vitamin D deficiency; in such cases, only the latter outcome was used. If studies evaluated the effects of vitamin D supplementation on serum 25(OH)D levels, the baseline (before treatment) information was used in our report.

The search focused on prevalence studies published in Canada. In cases where no Canadian studies could be identified, comparable studies from the United States were included as the vitamin D food fortification practices and recommended adequate daily intake of vitamin D are similar for the two nations. (7)

Inclusion Criteria

- Studies published in English
- Publications that reported the prevalence of vitamin D deficiency in Canada.
- Studies that included subjects from the general population or with kidney disease.
- Studies in children or adults.
- Studies published between January 1998 and July 17th 2009.

Exclusion Criteria

- Studies that included subjects defined according to a specific disease other than kidney disease.
- Letters, comments, and editorials.
- Studies that measured the serum vitamin D levels but did not report the percentage of subjects with serum levels below a given threshold.

Outcomes of Interest

- Prevalence of serum vitamin D deficiency (defined as serum levels < 25 nmol/L).
- Given the ambiguity in the appropriate target serum vitamin D level, the prevalence of serum vitamin D levels below a range of 40 to 50 nmol/L was also evaluated.
- Serum 25-hydroxyvitamin D was used to measure the subjects' vitamin D status. Results in adults and children were reported separately. Subgroup analyses were performed for factors that affect serum vitamin D levels such as seasonal effects, skin pigmentation, and vitamin D intake if sufficient data was available from the included studies.

Statistical Analysis

The results were presented as reported in the studies. In some cases, the weighted average results from different studies were calculated using the inverse variance method.

Quality of Evidence

The quality of prevalence studies was based on the method of subject recruitment and sampling method, possibility of selection bias, and generalizability to the source population. The overall quality of the studies was examined according to the GRADE Working Group criteria (44) in which quality refers to factors such as the adequacy of allocation concealment, blinding and follow-up; and consistency refers to the similarity of estimates of effect across studies. If there was important unexplained inconsistency in the results, confidence in the estimate of effect for that outcome decreases. Differences in the direction of effect, the size of the differences in effect, and the significance of the differences, guide the decision about whether important inconsistency exists. Directness refers to the extent to which the intervention and outcome measures are similar to those of interest.

As stated by the GRADE Working Group, the following definitions of quality were used in grading the quality of the evidence:

High	Further research is very unlikely to change confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate.
Very Low	Any estimate of effect is very uncertain

Results of Evidence-Based Analysis

Table 11 below summarizes the number and types of publications identified through the systematic literature review.

Table 11: Quality of Evidence of Included Studies

Study Design	Level of Evidence†	Number of Eligible Studies
Large RCT, systematic review of RCTs	1	0
Large RCT unpublished but reported to an international scientific meeting	1(g)	0
Small RCT	2	0
Small RCT unpublished but reported to an international scientific meeting	2(g)	0
Non-RCT with contemporaneous controls	3a	0
Non-RCT with historical controls	3b	0
Longitudinal (non-comparative) prevalence study Adults, general population	3c	1
Cross-sectional (non-comparative) prevalence studies Adults and children, general population	3d	11
Cross-sectional (non-comparative) prevalence studies Adults and children, kidney disease	3d	5
Cross-sectional (non-comparative) prevalence studies, unpublished but reported to an international scientific meeting (children)	3(g)	1
Surveillance (database or register) published in the grey literature	4(g)	1
Case series (multisite)	4b	0
Case series (single site)	4c	0
Retrospective review, modelling	4d	1
Case series presented at international conference	4(g)	0
	Total	20

RCT refers to randomized controlled trial;
Source: Goodman. (78)

Prevalence of Vitamin D Deficiency in Canada

Fourteen prevalence studies met the eligibility criteria examining the general Canadian population, including adults and children. (79-92) All but one consisted of cross-sectional measures of serum vitamin D levels (80-92), while the remaining was a longitudinal study in which serum vitamin D levels were measured once during each season (79). A summary of the results of vitamin D tests performed in community laboratories in the Province of Ontario between October 2008 and September 2009 were also included (test data from October 2008 to September 2009 were provided by the Ontario Association of Medical Laboratories through its participating member laboratories).

Two studies specifically looked at Canadian adults with renal disease (93;94) but none examined Canadian children with the same condition, although three studies in children with kidney disease in the United States were identified. (95-97) No systematic reviews or health technology assessments evaluating the prevalence of vitamin D deficiency in Canada were identified.

General Population – Study Characteristics

Of the 14 Canadian studies evaluating the prevalence of vitamin D deficiency (79-92), 12 were peer-reviewed (79-90). The two other studies published in the grey literature were a study of infants presented as an abstract at a conference (91) and a Canadian survey of serum vitamin D levels (92). In addition, a summary of the results of vitamin D tests performed in community laboratories in the Province of Ontario between October 2008 and September 2009 was also included. Test data from October 2008 to September 2009 were provided by the Ontario Association of Medical Laboratories through its participating member laboratories. Despite not being published in peer-reviewed literature, these studies were included in our analysis as the first evaluated the serum vitamin D levels in Canadian children 24-30 months old (91) and no other studies were identified for the age group; the second was representative of the Canadian provinces and territories (92); and the third evaluated of a database that represented approximately 90% of the vitamin D tests performed in Community Laboratories in Ontario.

The studies examined vitamin D levels in persons from across all age categories, including newborns, (82;88), children and adolescents, (82;89-91), and adults/elderly (79-88). Subjects were either randomly or consecutively selected from lists of patients seeking medical care at hospital or private clinics, sub-samples of cohort studies, municipal/provincial registration lists, and through media advertisement. One Quebec study used a province-wide, school-based cluster sampling method but excluded non-French Canadian subjects. (89) In one study, serum levels of vitamin D were assessed in a sample of the Canadian population as part of the Canadian Health Measures Survey (CHMS). (92) In only one study was the sampling method not clearly stated. (84)

In all studies serum 25(OH)D levels were measured but the method by which this was achieved varied. Amongst the laboratory assays used were radioimmunoassay, high pressure liquid chromatography, competitive binding assays, and liquid chromatography-tandem mass spectrometry. This have led to different varying results for serum level. For instance, the inter-assay coefficient of variation ranged from 6% to 17.3% as reported in eight studies (79;81;85-90), while the intra-assay coefficient of variation reported in four studies ranged from 5% to 12.5%. (79;85-87)

The studies used different serum vitamin D thresholds, varying from 25 to 50 nmol/L. The percentage of subjects with serum levels varying between 25 to 30 nmol/L and 37.5 to 50 nmol/L were included in this report. Some also provided the percentage of subjects with serum levels <75 nmol/L, but as previously mentioned, this threshold was not included in this report. Further details regarding the design and characteristics of the included studies are provided in Appendix 3.

General Population – Study Results

The Canadian Health Measures Survey (CHMS) included approximately 5,000 Canadians, age 6 to 79 years, living in the community[7], representing 97% of the population in ten provinces and three territories. (92) Probability sampling stratified across 10 age and gender groups was performed (92) and the preliminary results for 2,673 Canadians are provided in Table 12. Note that the number of individuals who declined participating in the survey was not reported.

Blood samples were collected throughout the year between March 2007 and February 2008. (92) Results stratified by gender are also available but not reported here as they did not vary considerably. Additional demographic information and information regarding vitamin D intake of the participants is not currently available.

[7] Excludes people living in Indian Reserves or Crown lands, people living in institutions, full-time members of the Canadian Forces, and people living in remote regions.

The preliminary results showed that the median serum 25(OH)D level for males and females age 6 to 79 was 66.3 nmol/L (5th percentile: 29.5 , 95th percentile: 111.5). (92) The median levels did not vary considerably across the age groups, with the exception of the youngest (6-11 years) and oldest (60-79 years) groups, with means of 76.0 nmol/L (38.5 , 121) and 74 nmol/L (33.9 , 112.3), respectively. (92) These findings have not been addressed at this point. Additional information on all 5,000 subjects and on vitamin D consumption, outdoor activities, and sunscreen use are expected to be released in 2010. (92)

Table 12: Preliminary Results of the Canadian Health Measures Survey, 2007-2008: Serum Vitamin D Levels

Age Group, Both Sexes	5th Percentile, mol/L (95% CI)	10th Percentile, mol/L (95% CI)	25th Percentile, nmol/L (95% CI)	Median nmol/L (95% CI) [mean; 95% CI]	75th Percentile, nmol/L (95% CI)	95th Percentile, nmol/L (95% CI)
Overall N=2,673	29.5 (24.8 , 34.2)	36.6 (32.5, 40.8)	48.0 (43.9 , 52.1)	66.3 (61.9 , 70.6) [66.9; 63.2,70.7]	82.8 (79.8 , 85.8)	111.5 (106.4 , 116.7)
6-11 yrs N=435	38.5† (17.0 , 60.0)	45.5 (32.2, 58.8)	61.8 (51.1 , 72.5)	76.0 (70.6 , 81.5) [76.0; 67.4,84.6]	88.1 (82.2 , 94.0)	121.0 (109.5 , 132.5)
12-19 yrs N=428	26.5 (21.6 , 31.4)	32.0 (25.3, 38.7)	43.4 (39.7 , 47)	60.4 (56.3 , 64.5) [64.0; 58.9,69.1]	78.9 (71.3 , 86.5)	107.7 (97.8 , 117.6)
20-39 yrs N=611	29.4 (26.3 , 32.4)	35.2 (32.0, 38.5)	45.1 (41.5 , 48.7)	61.0 (56.0 , 65.9) [63.5; 60.5,66.5]	78.8 (75.0 , 82.5)	108.3 (97.2 , 119.3)
40-59 yrs N=683	28.8 (23.0 , 34.5)	36.3 (31.1, 41.5)	47.7 (43.7 , 51.6)	66.1 (59.7 , 72.5) [66.1; 61.1,71.1]	80.7 (73.8 , 87.7)	110.9 (100.6 , 121.2)
60-79 yrs N=516	33.9 (25.8 , 42.0)	42.6 (37.8, 47.3)	55.3 (53.1 , 57.5)	74.0 (70.5 , 77.4) [73.5; 70.8,76.3]	90.9 (88.7 , 93.1)	112.3 (106.7 , 118)

Source: Canadian Health Measures Survey (92)
*25(OH)D 25-hydroxyvitamin D ; CI confidence interval;
† Estimate to be used with caution according to the authors. (92)

The summary of more than 624,000 vitamin D tests performed among male and female patients in community laboratories in Ontario between October 2008 and September 2009 are presented in Table 13 (test data provided by the Ontario Association of Medical Laboratories through its participating member laboratories). Included are the percent of subjects with serum vitamin D levels below 25 nmol/L, 50 nmol/L, and above 250 nmol/L, which is believed to be a risk factor for adverse effects such as hypocalcaemia. (15) Overall, 16,697 (2.7%) of the test individuals had serum levels < 25 nmol/L, 1,272 (4.4%) among individuals younger than 19 years and 14,413 (2.6%) among individuals 19 years or older. In general, there was not a large difference in prevalence among the age groups, although there was a trend towards a slightly lower prevalence among younger (0-10 years old) and older (51 to > 70 years) individuals (Table 13). Between 10% and < 25% of the individuals had serum levels between 39 and 50 nmol/L. In 725 (0.12%) tests, serum 25(OH)D exceeded 250 nmol/L, which is the level above which the risk of adverse events is believed to increase. Demographic information such as ethnicity and underlying conditions, or data on vitamin D intake was not available for the individuals tested.
It was assumed that in most cases, individuals were tested just once as the statistics provided by the

Health Data Branch show that during that period, there were approximately 1.03 vitamin D tests per individual tested in Ontario. It should also be noted that there was a slight seasonal variation in the prevalence of vitamin D levels below 25 nmol/L or above 250 nmol/L (see Figure 3).

Table 13: Serum Vitamin D Levels in Ontario Community Laboratories, Oct. 2008 – Sept. 2009

Age group, both sexes	N	% Serum Vitamin D < 25 nmol/L	% Serum Vitamin D Level > 250 nmol/L
0 to 10 years	7,591	105 (1.4%)	26 (0.34%)
11 to 18 years	21,363	1,167 (5.5%)	22 (0.10%)
19 to 35 years	79,066	3,905 (4.9%)	71 (0.09%)
36 to 50 years	164,400	5,904 (3.6%)	149 (0.09%)
51 to 70 years	244,162	4,068 (1.7%)	292 (0.12%)
> 70 years	107,873	1,536 (1.4%)	165 (0.15%)
Total	**624,455**	**16,697 (2.7%) 95% CI: 2.63, 2.72**	**725 (0.12%) 95% CI: 0.10, 0.12**
Children 0 to 18 years	**28,954**	**1,272 (4.4%) 95% CI: 4.15, 4.63**	**48 (0.17%) 95% CI: 0.11, 0.21**
Adults > 18 years	**595,501**	**15,413 (2.6%) 95% CI: 2.54, 2.63**	**677 (0.11%) 95% CI: 0.10, 0.12**

Data from Oct.2008 to Sept. 2009 was provided by the Ontario Association of Medical Laboratories through its participating member laboratories.

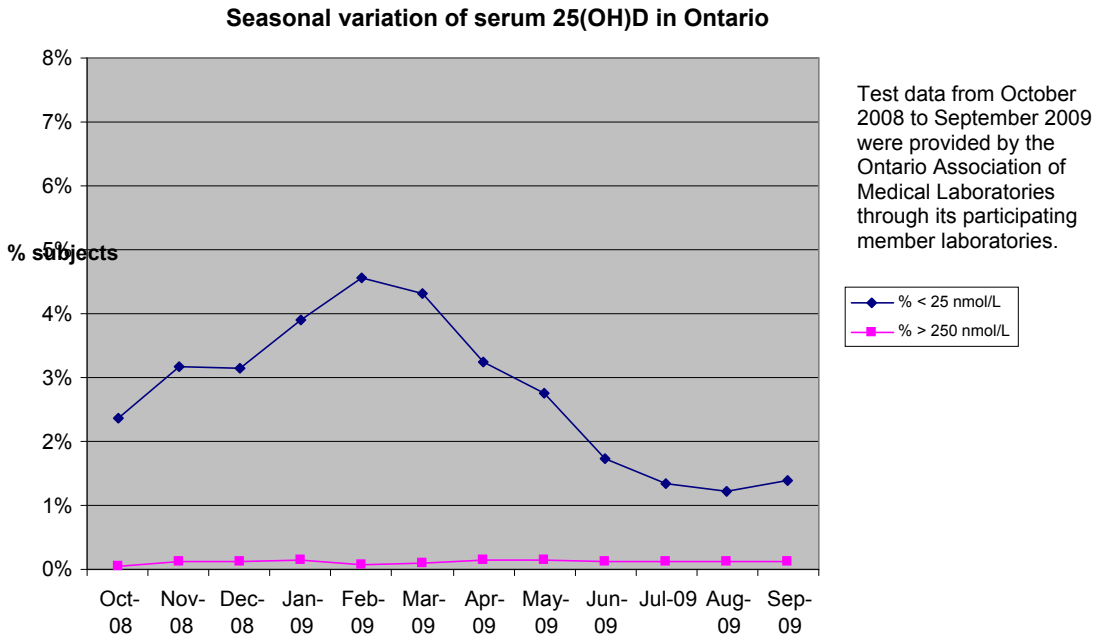


Figure 3: Seasonal Variation in Serum Vitamin D Levels from Ontario Community Laboratories (Oct. 2008 – Sept. 2009)

Adults

Ten peer-reviewed prevalence studies conducted among Canadian adults were identified. (79-88) Four used a 50 nmol/L threshold (79;82;86;87), while four others used a 37.5 to 40 nmol/L threshold. (80;81;85;88) Five studies also provided data on the prevalence of vitamin D levels below 25 nmol/L - 30 nmol/L. (80;82-84;86) Study participants generally consisted of community-dwelling healthy subjects and most studies excluded individuals with conditions or medications that alter either vitamin D and/or bone metabolism such as kidney or liver disease among other conditions. This included conditions such as Crohn's disease, rheumatoid arthritis, bilateral oophrectomy among others or use of corticosteroids, estrogens, anticonvulsants, biphosphanates, loop diuretics etc… Only one study provided information on the number of subjects who declined participation (55.9%), but the demographic characteristics were not compared between participants and non-participants. (79)

Six studies reported mean baseline vitamin D intake from either diet, supplements, or both. The mean daily vitamin D intake reported in five studies varied from 133-556 IU/day, depending on the subgroup; however, the standard deviation for the estimate was wide in most studies (Table 14). One study reported that 34% of the subjects consumed 1-2 glasses of milk per day and 34% took 50 to 400 IU of vitamin D supplements per day. One study excluded subjects with vitamin D intake > 200 IU or serum 25(OH)D levels ≤ 25 nmol/L, as measured during a pre-screening phase. (79)

Although the studies were conducted in different parts of Canada (Calgary, Edmonton, Toronto, Montreal, Winnipeg, St. John's, Inuvik, Manitoba and Newfoundland and Labrador), most of them were done at latitudes ranging between 43°N and 53°N, with only one relatively small study (N=121) at a higher latitude (Inuvik, Northwest Territories, approximately 60°N latitude).

The number of subjects included in the studies ranged from 50 to 1,433. Although different age groups were represented, six of the ten studies included only young adults (18-35 years), and four included only pregnant women (see Appendix 3). (82-84;88). Some studies stratified the results according to factors that affect vitamin D status including season, skin pigmentation, age, and vitamin D intake. (79-81;85;86;88) Two studies also used multivariate models to investigate the effects of different subject characteristics such as age, skin pigmentation, and BMI (79), as well as vitamin D intake and season on serum 25(OH)D levels (86), although it is unclear if these studies were adequately powered for subgroup analyses.

Serum 25(OH)D levels < 25-30 nmol/L were observed in 0 to 25.5% of the subjects from five studies. (80;82-84;86) The weighted average of 3.8% (95% CI: 3.0, 4.6) is consistent with the results of the CHMS survey in which approximately 5% had serum 25(OH)D levels below 29.5 nmol/L (5^{th} percentile: 29.5 nmol/L, 95% CI: 24.8, 34.2). The results of vitamin D tests done in community laboratories across Ontario between October 2008 and September 2009 showed a slightly lower prevalence with 2.6% (95% CI: 2.54, 2.63) exhibiting serum 25(OH)D levels < 25 nmol/L (see Table 13 above).

The prevalence of serum vitamin D levels below 37.5 to 50 nmol/L reported in eight adult studies varied widely from 8.0% to 73.6%. (79-82;85;87;88) with a weighted average of 22.5% (95% CI: 21.2 , 23.9). The preliminary results of the CHMS survey showed that between 10% and 25% of subjects had serum levels below 37 to 48 nmol/L (10^{th} percentile: 36.6 nmol/L, 25^{th} percentile: 48.0 nmol/L; see Table 14). (92) The results of vitamin D tests performed in community laboratories in Ontario between October 2008 and September 2009 showed that 10% to 25% of the individuals had serum levels between 39 and 50 nmol/L (test data provided by the Ontario Association of Medical Laboratories through its participating member laboratories).

In an attempt to explain the wide variation in study results, subgroup analyses, stratified according to the variables reported in the studies (e.g. seasonal variation, skin pigmentation, and vitamin D intake), are summarized in Table 14 below.

Table 14: Baseline Mean Vitamin D Intake (Adults)

Study (year) Location, N	Source of Vitamin D	Estimated Vitamin D Intake at Baseline, mean ± SD
Genuis et al. (80) (2009) Edmonton N=1433	Supplements Milk Fish oil	<u>VD supplement use:</u> None: 714 (50.0%) 50-400 IU: 487 (34.0%) > 400 IU: 210 (14.7%) <u>Glasses of milk/day</u> None: 713 (49.8%) 1-2: 492 (34.3%) > 2: 206 (14.3%) <u>Fish oil supplement use:</u> No: 1,074 (74.9%) Yes: 337 (23.5%)
Weiler et al. (81)(2007) Manitoba N= 356	Diet (FFQ questionnaire), 24-hour recall	Urban white: 424 ± 404 IU Urban aboriginal: 432 ± 492 IU Rural aboriginal: 556 ± 584 IU <u>% using less than adequate intake for VD*</u> Urban white: 42% Urban aboriginal: 44% Rural aboriginal: 27%
Waiters et al.(84) (1998) Northwest Territories N=121	Total vitamin D intake (diet and supplements)	Caucasian: 528 ± 236 IU Native Indian: 312 ± 240 IU Inuit: 328 ± 200 IU
Weiler et al. (88) (2005) Winnipeg N=50	Use of VD Supplements Mean VD intake	Use of VD supplement: 78% VD deficient: 149 ± 145 IU Adequate VD level: 242 ± 218 IU
Vieth et al. (85) (2001) Toronto, N=796	Total vitamin D intake (diet and supplements)	Mean total VD intake: 184 IU
Gozdzik et al. (86) (2008) Toronto N=107	Total vitamin D intake (diet and supplements)	Mean (5^{th}, 95^{th} percentile) Overall: 171.7 IU (19.7 , 464.3) European: 231.0 ± 174 (34.3, 583) East Asian: 133.4 ± 102 (8, 311.5) South Asian: 164.3 ± 144.3 (27.7, 391.7)

FFQ refers to food frequency questionnaire; SD standard deviation; IU international units; VD vitamin D

* Adequate intake for vitamin D: 200 IU for women ages 25-50 years and 400 IU for ages above 50 years.

<u>Seasonal variation</u>

In three studies that tested serum levels over different seasons, the prevalence of serum vitamin D levels less than 40 to 50 nmol/L varied between 21% and 39% for subjects tested during winter and spring, and between 8% and 14% for subjects tested during the summer (Table 15). The weighted averages were 23.6% (95% CI: 21.4, 25.9) during the winter/spring and 9.6% (7.7, 11.6) during the summer. The difference between the seasons was not statistically significant in one study (80) and not reported in the other two. (79;85) With the exception of one longitudinal study in which subjects were tested each season (79), the studies generally used different subjects for each season.

Table 15: Serum Vitamin D Levels in Adults, Seasonal Variation, Canada

		% Serum Vitamin D < 40-50 nmol/L	
Study (year) City, N	**Thresholds**	**Winter-Spring†**	**Summer**
Longitudinal study			
Rucker et al. (79) (2002) Calgary (51° N) N=188	< 50 nmol/L Test: RIA (DiaSorin)	Winter: 73 (39%) Spring: 70 (37%)	26 (14%)
		115 (61%) at least once during the year	
Cross-sectional studies			
Genuis et al. (80) (2009) Edmonton (53° N) N= 1,433	< 40 nmol/L Test: liquid chromatography	Winter: 78 (21%) Spring: 94 (22%)	30 (10%) p=.1447 (comparing all 4 seasons)
Vieth et al. (85) (2001) Toronto (43° N) N=796	< 40 nmol/L Test: RIA (DiaSorin)	100 (23%)	29 (8%)
	Weighted average (95% CI)‡	**23.6% (21.4 , 25.9)**	**9.6% (7.7 , 11.6)**

RIA refers to radioimmunoassay.

† Prevalence of serum levels < 40-50 nmol/L was 43% (79) and 12% (80) as reported in two studies.

‡ Weighted average calculated using the inverse variance method. 95% CI calculated using the largest variance among studies.

Skin Pigmentation

Four Canadian studies observed a trend toward lower serum vitamin D (< 37.5 to 50 nmol/L) in subjects of darker skin pigmentation (Table 16). (80;81;85;86) The observed weighted averages were 46.8% (95% CI: 42.7, 50.8) among individuals with darker skin pigmentation (defined as urban or rural Aboriginal, non-white, Asians, and 'dark skin tone') and 15.9% (95% CI: 14.4, 17.4) for individuals with lighter skin pigmentation (defined as white Europeans or 'light to medium skin tone'). One of these studies also showed a "dose-response" relationship with skin tone (80), though it is unclear how skin tone was defined in this study.

It is important to note that these studies were not designed to determine causation. Their results were not adjusted for other factors that may affect serum vitamin D levels. Multivariate analyses would be necessary to determine the contribution of each risk factor to lower serum 25(OH)D levels.

These results have been corroborated in six international studies published since 2008 from Northern European countries, which evaluated the prevalence of vitamin D deficiency among immigrants from Africa, Middle-East, and South Asia (Tables 17 and 18). (12;98-101) The subjects included in these studies were primarily adults 18 to 50 years old, with two studies including subjects > 50 years old. (12;101) The prevalence of serum vitamin D levels < 50 nmol/L was high among immigrants with a weighted average of 94.7% (95% CI: 88.5 , 100). In contrast, one study reported a 12.5% prevalence of 25(OH)D < 50 nmol/L among Norwegians. (99) A third study showed a higher prevalence of serum vitamin D levels < 50 nmol/L among Sri Lankans that immigrated to Norway (90.5%) compared to native Sri Lankans who stayed in the country (48%), though the authors point out that this is based on non-concurrent controls. (99) In a separate study, the prevalence of 25(OH)D < 50 nmol/L was found to be 60% among Belgians; but the authors noted that the subjects were recruited from areas of low social status and may not be representative of all of Belgium. (101) The results of these latter two studies were not adjusted for other factors affecting serum vitamin D levels. Lastly, the prevalence of serum vitamin D < 25 nmol/L was also higher in immigrants (weighted average 64.9%) compared to Northern Europeans (weighted average 14.6%). (12;98-101)

Vitamin D intake and serum levels

Four studies reported the percentage of subjects with serum vitamin D < 37.5 to 50 nmol/L according to vitamin D intake. (80;81;85;86) There was an overall trend towards a lower prevalence of serum levels below this range with higher levels of vitamin D intake (Table 19). One study observed a dose-response relationship between higher vitamin D intake from supplements, diet (milk), and sun exposure, not adjusted for other variables. In Genuis et al. it was observed that just 6% of those subjects taking 50 to 400 IU exhibited serum levels < 40 nmol/L and that this figure dropped to 3% among those taking > 400 IU per day. In comparison, 29% of subjects not on vitamin D supplementation exhibited serum levels of < 40 nmol/L. (80) Similarly, the prevalence of serum vitamin D levels < 40 nmol/L was 15% among subjects who drank one or two glasses of milk per day, 6% among those who consumed more than two glasses per day, but 21% in those not who do not drink milk. The study included subjects from different age groups, from < 19 years to ≥60 years (29% were ≥ 60 years). (80)

In contrast, a separate study observed little variation in serum vitamin D levels among young women (18-35 years) with different levels of milk intake. For those consuming up to two glasses per day, the prevalence of low serum vitamin D (< 40 nmol/L) was 21%, compared to 26% among those who drank in excess of two glasses per day and 20% among those who drank no milk. (85) It was observed by Gozdzik et al. (2008) that the prevalence of serum vitamin D < 50 nmol/L among subjects using > 200 IU of vitamin D supplementation was considerable (40%), although it was lower than the overall sample (73.6%). (86) The statistical significance and number of subjects using > 200 IU/day was not reported. (86)

Additional information on study results and subgroup analyses is provided in Appendix 4.

Table 16: Serum Vitamin D Levels According to Skin Pigmentation in Adults (Canada)

Study (year) City, N	Mean Vitamin D Intake (SD) Baseline Characteristics	Serum 25(OH)D Threshold	% Serum Vitamin D < 37.5 – 50 nmol/L
Genuis et al. (80) (2009) Edmonton N= 1,433	<u>VD supplement use</u> None: 714 (50.0%) 50-400 IU: 487 (34.0%) > 400 IU: 210 (14.7%) <u>Glasses of milk/day</u> None: 713 (49.8%) 1-2: 492 (34.3%) 2: 206 (14.3%) < 19 yrs: 87 (6.1%) 19-60yrs:926 (64.6%) ≥ 60 yrs: 421 (29.4%)	< 40 nmol/L	<u>By skin tone†</u> Light: 173/1,179 (15%) Medium: 43/185 (23%) Dark: 8/18 (44%) First Nations: 16/33 (48%) p<.0001 among different categories
Weiler et al. (81) (2007) Manitoba N= 356	<u>VD intake IU/day</u> Urban white:424 ± 404 IU Urban aboriginal: 432 ± 492 IU Rural aboriginal: 556 ± 584 IU	< 37.5 nmol/L	Urban white: 27/146 (18.6%) Urban aboriginal: 56/184 (30.4%) Rural aboriginal: 8/26 (32%) p=.001 (for differences in serum 25(OH)D levels)
Vieth et al. (85) (2001) Toronto N=796	Mean total VD intake: 184 IU		<u>Winter</u> White: 81/380 (21.3%) Non-white‡: 15/47 (31.9%) Black: 2/8 (25%) <u>Summer</u> White: 23/322 (7.1%) Non-white†: 6/35 (17.1%)
Gozdzik et al. (2008) (86) Toronto N=107	Mean total VD intake ± SD (5^{th}, 95^{th} percentile)*: 171.7 IU (19.7-464.3) European: 231 ± 174 (34.3, 583) East Asian: 133.4 ±102 (8, 311.5) South Asian: 164.3 ±144.3 (27.7, 391.7) Mean age (5^{th}, 95^{th} percentile): 21 yrs (18 , 25)	< 50 nmol/L	European: 11/32 (34.4%) East Asian: 23/27 (85.2%) South Asian: 29/31 (93.5%) P=.001 (Fisher exact test)
	Weighted average (95% CI)‖		Lighter skin pigmentation¶: 15.9% (14.4, 17.4) Darker skin pigmentation¶: 46.8% (42.7, 50.8)

25(OH)D refers to 25-hydroxyvitamin D; CI confidence interval; IU international units; NR not reported; SD standard deviation; VD vitamin D; yr year

* Total vitamin D intake includes the amount from both diet and supplements.

† Method of determination of skin tone not reported.

‡ Non-white: Asian, First Nations, and Indo-Asian

¶ Lighter skin pigmentation subgroup includes subjects classified as white, urban white, European, and those with light to moderate skin tone. Darker skin pigmentation subgroup includes urban and rural Aboriginal, non-white, Asians, First Nations and dark skin tone as defined by the authors.

‖ Weighted average calculated using the inverse variance method. 95% confidence interval calculated using the largest variance among the studies included.

Table 17: Serum Vitamin D Levels in Adults According to Skin Pigmentation (Other Countries).

Study (year) Location, N	Patient Characteristics	% Serum Vitamin D < 50 nmol/L			Comments
		Native Europeans	Immigrants	Non-Europeans in Country of Origin	
Madar et al. (98) (2009) Norway 1st generation immigrants from Turkey (n=25), Pakistan (n=45) and Somalia (n=10)	Women Mean age: 28 ± 5.2 yrs Mean time in Norway: 10.9 ± 8 yrs Skin mostly covered: 39 (49%) Daily VD supplementation 21 (26%) Fatty fish >= 2x/wk: 29 (36%)	Not reported	< 50 nmol/L 73 (91%)	Not reported	▪ Higher mean serum levels with supplementation and longer education. ▪ No association with fatty fish or fortified food intake, sun exposure, clothing habits, and season. May be due to lack of statistical power, or imprecise estimate of vitamin D intake from food. ▪ Women with higher education also used more vitamin D supplementation.
Meyer et al. (99) (2008) Norway Immigrants from Sri Lanka (n=242), Sri Lankans in Sri Lanka (n=196), and Norwegians (n=580)	Men and women Mean age (men): Sri Lankans: 47 ± 8 (Sri Lanka), 39.5 ± 6.2 (Norway) Norwegians: 45-60 yrs Education: 12 ± 4 yrs (Norway) 10 ± 3 yrs (Sri Lanka) VD supplementation: 37 (20%) Norway (fish oil, typically has 200 IU – 400 IU) 1 (0.5%) Sri Lanka	< 50 nmol/L 73 (12.5%)	< 50 nmol/L 219 (90.5%)	< 50 nmol/L 94 (48%)	▪ Statistically significant seasonal variation (strong in Sri Lanka, significant but less pronounced in Norway), use of supplements, fatty fish consumption (men), BMI (men) ▪ Not statistically significant association with education.
Belaid et al. (100) (2008) France Women using veil (N=96)	Women 18-49 yrs Mean age: 35 ± 8 yrs Veil covering the face: 9.4% VD supplements: NR	Not reported	≤ 53 nmol/L 95 (99%)	Not reported	▪ 72.6% of the women with ≤ 53 nmol/L had >= 1 clinical signs (asthenia, bone or muscular pain, muscle weakness) ▪ Values measured during the winter.
Moreno-Reyes (101) (2008) Belgium 1st generation immigrants from Morocco, Turkey, Congo, and Belgians (N=100 each)	Men and women Mean age: 49 – 52 yrs VD supplements: NR	< 50 nmol/L 60 (60%)	< 50 nmol/L 76 (77%) Congolese 90 (90%) Moroccan 80 (79%) Turkish p< .001 (Moroccans and Turkish differ from Belgium and Congolese)	Not reported	▪ Multivariate analyses: origin, male sex, BMI, and season predictors of serum levels. ▪ More pronounced increase in serum levels during the summer was observed in Belgians than in immigrants. ▪ Subjects recruited from low social status areas, may not represent Belgium population (including Belgian subgroup).
	Weighted average†	**16% (6.4 , 25.5)**	**94.7% (88.5 , 100)**	**48% (39.6 , 53) 1 estimate**	

IU refers to international unit; NR not reported; VD vitamin D; wk week; yr year.

† Weighted average calculated using the inverse variance method.

Table 18: Vitamin D Deficiency Prevalence in Adults According to Skin Pigmentation.

Study (year) Location, N	Patient Characteristics	% Serum Vitamin D < 25 nmol/L		
		Native Europeans	Immigrants	Non-Europeans in Country of Origin
Madar et al. (98) (2009) Norway 1st generation immigrants from Turkey (n=25), Pakistan (n=45) and Somalia (n=10)	Women Mean age: 28 ± 5.2 yrs Mean time in Norway: 10.9 ± 8 yrs Skin mostly covered: 39 (49%) Daily VD supplementation: 21 (26%) Fatty fish >= 2x/wk: 29 (36%)	Not reported	< 25 nmol/L 46 (57%)	Not reported
Meyer et al. (99) (2008) Norway Immigrants from Sri Lanka (n=242), Sri Lankans in Sri Lanka (n=196), and Norwegians (n=580)	Men and women Mean age (men): Sri Lankans: 39-40 yrs Norwegians: 45-60 yrs VD supplementation: 37 (20%) Norway (fish oil‡) 1 (0.5%) Sri Lanka	Not reported	< 25 nmol/L 79 (33%)	< 25 nmol/L 7 (3.6%)
Belaid et al. (100) (2008) France Women using veil N=96	Women 18-49 yrs Mean age: 35 ±8yrs Veil covering the face: 9.4% VD supplements: NR	Not reported	≤ 30 nmol/L 79 (82.3%)	Not reported
Moreno-Reyes (101) (2008) Belgium 1st generation immigrants from Morocco, Turkey, Congo, and Belgians (N=100 each)	Men and women Mean age: 49 – 52 yrs VD supplements: NR	< 25 nmol/L 13 (13%)	< 25 nmol/L 14 (14%) Congolese 54 (54%) Moroccan 54 (53%) Turkish p< .001 (Moroccans and Turkish differ from Belgium and Congolese)	Not reported
Denmark (12) (2002) Immigrants from Pakistan, N=247	Men and women 18-65 yrs	Not reported	< 25 nmol/L 187 (75.7%)	Not reported
Denmark (12) (1996/97) Immigrants from Middle-East (n=69) and Danish women	Women > 18 yrs	< 25 nmol/L 18.5% (not veiled: 9%, veiled:60%)	< 25 nmol/L 95.6% (not veiled: 89%, veiled: 96%)	
Weighted average†		**14.6% (6.4 , 25.5)**	**64.9% (54.1 , 75.6)** **71.3% (60.5 , 82.1)** **(excludes Congolese)**	**3.6% (1.0, 6.2)** **1 estimate**

NR refers to not reported; VD vitamin D; wk week; yr year.
† Weighted average calculated using the inverse variance method.
‡ Fish oil typically contains 200 IU – 400 IU

Table 19: Prevalence of Serum Vitamin D Levels < 37.5-50 nmol/L According to Vitamin D Intake (Adults)

Study (year) City, N	Baseline Characteristics	% Serum Vitamin D < 37.5- 50 nmol/L
Genuis et al. (80) (2009) Edmonton, N= 1,433	< 19 yrs: 87 (6.1%) 19-30yrs:172 (12.0%) 30-60 yrs: 754 (52.6%) ≥ 60 yrs: 421 (29.4%) Skin tone: Light: 1,179 (83.3%) Medium: 185 (13.1%) Dark: 18 (1.3%) First Nations: 33 (2.3%)	**Univariate analyses (< 40 nmol/L)** VD supplementation† (p<.0001) None: 204 (29%) 50-400 IU: 30 (6%) > 400 IU: 6 (3%) No. glasses of milk/day (p<.0001) None: 151 (21%) 1-2: 76 (15%) > 2: 13 (6%) Recent sun exposure (p<.0001) Minimal: 201 (23%) Moderate: 33 (9%) Lots of sun: 6 (4%)
Weiler et al. (88)(2005) Winnipeg, N=50 Test: RIA (DiaSorin)	Mothers of term newborn babies. Mean age: 25-29 yrs White: 30 (60%) First Nations: 8.4% Use of VD supplement: 78% Mean VD intake (mothers): VD deficient: 149 ±145 IU Adequate VD level: 242 ± 218 IU	< 37.5 nmol/L Using supplements: 14/39 (36%), inferred from the information provided. Mean dose of vitamin D supplementation not provided. None: 9/11 (81.2%) Overall: 46%
Vieth et al. (85) 2001 Toronto N=796 43° N latitude Test: RIA (DiaSorin)	Healthy women, 18-35 yrs Community-based Excluded: conditions associated with bone loss Female: 796 (100%) White: 702 (88%) Non-white:82(10.3%) Black: 12 (1.7%) Mean total VD intake: 184 IU	< 40 nmol/L By N. glasses of milk/d (during winter) None: 31/146 (21%) 0-2: 37/140 (26%) > 2: 30/149 (20%) Overall: 23%
Gozdzik et al. (86) (2008) Toronto N=107 Test: RIA (DiaSorin)	Young adults (18-30 yrs) Community-based Mean age (5^{th}, 95^{th} percentile): 21 yrs (18 , 25) Mean total VD intake ± SD (5^{th}, 95^{th} percentile)*: 171.7 IU (19.7-464.3) European: 231 ± 174 East Asian: 133.4 ± 102 South Asian: 164.3 ± 144.3	< 50 nmol/L > 200 IU: 40% (number of subjects: NR) Overall: 73.6% Measured during winter. Multivariate model, factors associated with serum levels: vitamin D intake (p<.001, explained 28.9% of variance) skin pigmentation (p=.033, explained 4.5% of variance)

CI refers to confidence interval; d day; IU international unit; NR not reported; RIA radioimmunoassay; SD standard deviation; VD vitamin D; yr year
† Use of supplements defined as any dose of vitamin D supplementation or any milk intake if use of vitamin D supplements was not available. The estimate from the study by Gozdzik et al. could not be used since the number of subjects using supplementation was not provided.

Newborn, Children and Adolescents

Five studies evaluated serum 25(OH)D levels among newborns, children and adolescents (82;88-91), one of which has not been published in the peer-reviewed literature. (91) The studies included subjects from Edmonton (90), St. John's (82), Toronto (91), Winnipeg (88), and the province of Quebec (89). Although a province-wide school-based cluster sample of 9, 13, and 16-year old children was used in the study from Quebec, only children of French-Canadian origin were included. (89) The other studies recruited participants from lists of subjects that sought medical care at hospitals or clinics. (82;88;90;91) Two studies provided information on the number of subjects who declined participation, which were reported to be 25% and 62%. (89;90) Only one of these studies compared the demographic characteristics between participants and non-participants, concluding that both groups had similar characteristics. (89)

Two studies employed a threshold of 50 nmol/L (82;91), while two others used 37.5 and 40 nmol/L as the threshold. (89;90) Four studies provided the percentage of patients with serum levels below 25 nmol/L. (82;88-90) Between 0 and 36% of the children included in four studies had serum vitamin D levels below 25 to 27.5 nmol/L across different age groups (82;88-90) with a weighted average of 6.4%. The results of over 28,000 vitamin D tests performed in children 0 to 18 years old in Ontario community laboratories between October 2008 and September 2009 showed that 4.4% (95% CI: 4.1, 4.6) had serum levels < 25 nmol/L (Table 22; additional information on demographics and vitamin D intake were not available). In two studies, vitamin D levels of less than 50 nmol/L were observed among infants 24 to 30 months old (32%) and newborns (35.3%), both measured during the winter/spring seasons. (82;91)

Among children 2 to 16 years old, two studies reported that 24.5% and 34% of the subjects had serum levels below 37.5 nmol/L or 40 nmol/L, during the winter/spring seasons. (89;90) In both studies, older children were more likely to be below this threshold than younger children (Table 20). The authors of one of the studies concluded that the vitamin D dose should be weight-adjusted in children and adolescents. (90)

Although many of the studies recorded their measurements in the winter and spring seasons, the specific effects of season, as well as skin pigmentation and vitamin D intake, were not explored in the Canadian pediatric studies.

The overall weighted average of the prevalence of serum vitamin D levels < 37.5 to 50 nmol/L in the Canadian pediatric studies was 25.8% (95% CI: 14.6 , 37.0). The preliminary results of the CHMS survey showed that between 10% and 25% of subjects 6 to 11 years of age (N= 435) had serum levels below 50 nmol/L (10^{th} percentile: 38.5, 25^{th} percentile: 61.8; see Table 17). (92) Between 25% and 50% of adolescents 12 to 19 years old had serum vitamin D levels below 50 nmol/L (5^{th} percentile: 26.5, 25^{th} percentile: 60.4). (92) A similar trend towards a higher prevalence of serum vitamin D levels < 25 nmol/L was observed in the data from community laboratories in Ontario (Table 22).

Table 20: Serum Vitamin D Levels in Children According to Age Groups

Study (year) City, N	Population	% Serum Vitamin D , 37.5 - 40 nmol/L Younger children	 Older children
Mark et al. (89) (2008) Québec province N=1,753	Ages: 9, 13 and 16 yr-olds School-based sampling All French Canadian	≤ 37.5 nmol/L 9 yr olds: 58 (10%)	≤ 37.5 nmol/L 13 yrs: 161 (29.7%)† 16 yrs: 219 (33.6%)† p< .0001 between age groups
Roth et al. (90) (2005) Edmonton N=68	Ages 2 – 16 yrs Children who had ED visit Origin: West/East Europe: 60% First Nations: 19%	< 40 nmol/L 2-8 yrs: 6 (17%)	< 40 nmol/L 9-16 yrs: 17 (52%) p<.01 between age groups
	Weighted average (95% CI)‡	**10.3% (0 , 22.6)**	**34.4% (17.7 , 51.2)**

Results for both genders shown. *CI refers to confidence interval; ED emergency department; yr year
† Results for both genders shown. Significant differences were observed between males and females (details in Appendix 5).
‡ Weighted average calculated using the inverse variance method.

Table 21: Preliminary Results of the Canadian Health Measures Survey: Serum Vitamin D Levels, 2007-2008

Age group, both sexes	Percentile, nmol/L (95% CI) 5th	 10th	 25th	 Median and Mean	 75th	 95th
6-11 yrs N=435	38.5† (17.0, 60.0)	45.5 (32.2, 58.8)	61.8 (51.1, 72.5)	**Median: 76.0** **(70.6, 81.5)** Mean: 76.0 (67.4,84.6)	88.1 (82.2, 94.0)	121.0 (109.5, 132.5)
12-19 yrs N=428	26.5 (21.6, 31.4)	32.0 (25.3, 38.7)	43.4 (39.7, 47.0)	**Median: 60.4** **(56.3, 64.5)** Mean: 64.0 (58.9,69.1)	78.9 (71.3, 86.5)	107.7 (97.8, 117.6)

Source: Canadian Health Measures Survey (92)
*25(OH)D 25-hydroxyvitamin D ; CI confidence interval;
† Estimate to be used with caution according to the authors. (92)

Table 22: Serum Vitamin D levels from Ontario Community Laboratories, Oct. 2008 – Sept. 2009

Age group, both sexes		% Serum Vitamin D < 25 nmol/L
0 to 10 years	N=7,591	105 (1.4%)
11 to 18 years	N=21,363	1,167 (5.5%)
0 to 18 years	N=28,954	1,272 (4.4%)

Test data from October 2008 to September 2009 were provided by the Ontario Association of Medical Laboratories through its participating member laboratories.

A surveillance study from the Canadian Paediatric Surveillance Program reported 104 confirmed cases[8] [2.9 cases per 100,000 children (95% CI: 2.2 , 3.7)] of vitamin D-deficient rickets in children ages 1 to 18 in Canada between 2002 and 2004, 57 (55%) of which were in Ontario. (27) The highest incidence of cases was seen in the Northern parts of the country (Yukon, Northwest Territories, and Nunavut). (27) In 92 (89%) cases, the skin was intermediate to dark, 98 (94%) had been breastfed, and 25 (24%) were offspring of immigrants to Canada. (27) More cases were reported during the winter/spring months with a mean of 14 ± 1.9 cases/month between February and May and 6.9 ± 2.1 cases/month between June and January of each year. (27) The median serum 25(OH)D level was 15 nmol/L (range: 1-84) among the 78 (75%) cases for which information was available . (27)

There were three cases of rickets diagnosed within the first few weeks of life among newborns considered to have received enough infant formula. (27) The authors believe that, given the timing of the diagnosis in these cases, rickets developed due to an insufficient transfer of vitamin D from the mother to the fetus and that the deficiency in the children was too severe to be resolved by vitamin D-fortified formula. (27) The mean age of the mothers was 28 years (range: 15-39). Twenty-one (20%) wore head covers, 13 (12.5%) received vitamin D supplementation during pregnancy, 5 (5%) received it after delivery, and 79 (76%) did not drink milk before or after delivery. (27) According to the authors, only pediatricians were surveyed and, since rickets is more likely to be diagnosed by family doctors in remote areas, the results may be an underestimate of the actual number of cases. (27) Additional details in of these cases are supplied in Appendix 5.

Kidney Disease Patients

Adults

Two studies evaluated the prevalence of vitamin D deficiency in Canadian adults with renal disease. (93;94) In the first, serum vitamin D level was measured in 128 patients with chronic kidney disease[9] stages 3 to 5 and a glomerular filtration rate of < 30 ml/min at a renal insufficiency clinic in Edmonton. (93) Patients with vitamin D intake above 400 IU /day were excluded. (93) Serum levels below 37.5 nmol/L were observed in 38% of these patients, as measured between April and July months. (93) This is higher than the results among healthy Canadians previously presented, which showed that between 8% and 14% of subjects had serum 25(OH)D levels of less than 40 nmol/L during the summer.

The second study was comprised of 419 subjects attending a renal transplant clinic who had received a renal transplantation > 1 month prior to enrolment. The mean time since transplantation was 7.2 yrs (SD 6.4). (94) In total, 27.3% had serum 25(OH)D levels < 40 nmol/L. (94) It was assumed that levels were measured between December and March since this was the period of recruitment. Most patients (93%) did not receive vitamin D supplementation as, according to the authors, this was not recommended at the time. (94) This results were similar to what was previously presented for healthy Canadians, that is, 21% to 39% exhibited serum 25(OH)D levels of less than 40 to 50 nmol/L during the winter/spring. The authors concluded that the observed prevalence was similar to what is expected in the general population. (94)

[8] Rickets were confirmed by radiographic signs at the wrist or knee by a radiologist. Serum levels of calcium, phosphate, alkaline phosphatase, parathyroid hormone, and 1,25-dihydroxyvitamin D were included if available. Serum 25(OH)D levels had to be below 27.5 nmol/L or > 27.5 nmol/L in absence of isolated dietary calcium-deficient rickets. VD-deficient rickets associated with underlying diseases were excluded.

[9] CKD stage 3 is defined by the United States National Kidney Foundation as moderately decreased GFR (30-59 ml/min/1.73m^2), stage 4 severely decreased GFR (15-29 ml/min/1.73m^2), and stage 5 is defined as kidney failure (GFR < 15 ml/min/1.73m^2 or dialysis). (29)

Children

No studies evaluating serum vitamin D levels in Canadian pediatric patients with kidney disease were identified, although three US studies examining children with chronic kidney disease (CKD) stages 1 to 5 were. (95-97) The design of the studies was cross-sectional in two (95;96) and retrospective in one. (97) The cross-sectional studies were conducted in Michigan and used a threshold of 50 nmol/L (95;96), while the retrospective study carried out in Florida used a threshold of 37.5 nmol/L. Participants included children attending either a dialysis or a chronic renal insufficiency clinic (unclear in one study) with mean ages ranging from 10.7 to 12.5 years. (95;97) The season in which serum 25(OH)D levels were measured, however, was unclear in all three studies.

In the first study, 34 of 88 (39.0%) children had serum 25(OH)D levels < 37.5 nmol/L (2005 to 2006 sample). (96) In patients evaluated in previous years (1987 to 1996), the prevalence varied between 20 and 75% depending on the year. (96) In the second study, 12 of 54 (21.1%) children with CKD stages 2 to 4 had serum 25(OH)D levels below 37.5 nmol/L, however, only vitamin D_3 was measured in this study. (95) The third study reported that 72 of 258 (28.0%) children had 25(OH)D levels ,< 50 nmol/L. (97)

The authors of all three studies noted that individuals with darker skin pigmentation tended to exhibit lower serum 25(OH)D levels than those with lighter skin pigmentation, although actual figures were not provided. (95-97) The prevalence rates were not considerably different from what was previously presented in healthy children (24-35%). Further details of these studies are provided in Appendix 6.

Clinical Utility of Vitamin D Testing

The clinical utility of vitamin D testing was defined as the ability to improve bone health outcomes with the focus being on the average risk population (excluding osteoporosis) and patients with kidney disease. A previously described comprehensive systematic review published in August 2007 by AHRQ evaluated the association between serum vitamin D levels and different bone health outcomes and falls. (33) It included a total of 72 studies evaluating different bone health outcomes across different age groups. The authors observed that there was a trend towards improvement in some bone health outcomes with higher serum vitamin D levels (excludes fractures for which the evidence was found to be inconsistent). Nevertheless, an overall vitamin D threshold level for improved bone health outcomes could not be determined across age groups. (33;67) No new studies on this association were identified through a systematic review on vitamin D published in July 2009. (11).

As stated elsewhere, no high quality or even moderate quality evidence was found to support an association between vitamin D and non-bone health outcomes such as cancer, cardiovascular outcomes, and all-cause mortality. Even if there is any residual uncertainty, there is no evidence that testing vitamin D levels encourages adherence to Health Canada's guidelines for vitamin D intake. The normal threshold for vitamin D levels to prevent non-bone health related conditions cannot be resolved until a causal effect or correlation has been demonstrated between vitamin D levels and these conditions and a normal threshold established. This is regarded as an ongoing research issue with too much uncertainty on which to base any conclusions that would support routine vitamin D testing.

In patients with chronic kidney disease, there is also a lack of high and moderate quality evidence to show improved outcomes (reduction of fractures and survival) following treatment with calcitriol or vitamin D analogs. (18;29) In the absence of such data, guidelines for CKD patients consider best practice to maintain serum calcium and phosphate at normal levels and supplementation with active vitamin D (1,25-dihydroxyvitamin D, calcitriol) should be considered if the serum parathyroid hormone level is elevated. (29) As previously stated guidelines for CKD patients believe that there is insufficient evidence to support routine 25(OH)D testing for this patient group. (18;29) Decisions regarding the commencement or discontinuation of treatment with calcitriol or vitamin D analogs should be based on serum PTH, calcium, and phosphate levels. (18;29)

Other limitations associated with vitamin D testing include ambiguity on the adequate threshold level mentioned above, as well as inter- and intra-assay variability. These limitations and the lack of a consensus on target serum vitamin D levels undermine the clinical utility of such tests. Overall, the evidence for the clinical utility of vitamin D testing is thus considered to be of very low quality.

Daily vitamin D intake, either through diet or supplementation, should thus follow Health Canada recommendations for healthy individuals of different age groups. Individuals with conditions such as renal and liver disease, malabsorption syndromes, or other conditions/medications affecting vitamin D absorption or metabolism should follow the guidance of their attending physician with regards to both testing and supplementation.

Grading of Evidence

Tables 23 to 26 show the evaluation of the quality of the evidence for the studies identified in this report based on the GRADE Working Group criteria. (44)

Table 23: GRADE Quality of Evidence: Prevalence of Vitamin D Deficiency in Canada – Adults (Peer-Reviewed Literature)

Outcome	Design	Quality	Consistency	Directness Appropriate Range of Subjects	Other Modifying Factors	Overall Quality
Vitamin D Deficiency Adults General	10 observational 1 longitudinal 9 cross-sectional	No serious limitations.*	Not largely inconsistent†, especially when results were separated by season.	No major limitations‡	N/A	
	Low →					Low
Vitamin D Deficiency Adults Seasonal effects	3 observational 1 longitudinal 2 cross-sectional	No serious limitations.*	Consistent	No serious limitations‡	Seasonal gradient observed in 5/10 studies§. **+ 1**	
	Low →			Low	Moderate	Moderate
Vitamin D Deficiency Adults Skin pigmentation	4 observational 1 longitudinal 3 cross-sectional	No serious limitations.*	Consistent	No serious limitations‡	Higher prevalence in darker skin pigmentation (skin pigmentation gradient in 1 study **+ 1**	
	Low →			Low	Moderate	Moderate

* Recruitment seemed to have been done appropriately since subjects were either randomly or consecutively from the source population. Although the percentage of subjects that declined participation was not reported, which may result in selection bias, it appears that this does not represent serious limitations in this case, therefore, the evidence was not further downgraded.

† The results of 1 study were different from the other 9 studies, however, the higher prevalence of deficiency in one study (73.6%) compared to the others (21-43.6% < 40-50 nmol/L) seems to be skewed by skin pigmentation (serum level < 50 nmol/L in winter: overall: 73.6%, Caucasian: 34.4%, Asian: 85-93%). When results are stratified by skin pigmentation, the results don't seem to be as different to the other studies.

‡ Subjects were selected randomly from different sources, however, the source population they were selected from may not adequately represent the population in the area since they were chosen from lists of subjects who sought medical care, sub-sample of cohort studies etc. Different subgroups were included in the different studies; however, taken together, they may be a closer representation of the Canadian population. Therefore it was decided not to downgrade the evidence further.

§ A seasonal gradient was observed in 5/10 studies, however, a summary estimate for prevalence during different seasons cannot be provided.

Table 24: GRADE Quality of Evidence: Prevalence of Vitamin D Deficiency in Canada – Adults and Children (Grey Literature)

Outcome	Design	Quality	Consistency	Directness Appropriate Range of Subjects	Other Modifying Factors	Overall Quality
Vitamin D Deficiency – Adults and children Grey literature	1 observational cross-sectional (Canadian Survey) Preliminary results	1 large study (N=2,673) of good quality, sampling method designed to represent the 97% of Canadian population	N/a (1 study) Results not inconsistent with peer-reviewed studies.	Appropriate as an overall age-gender specific estimate†	N/A	
	Low →					Low

† Individuals ages 6-79 years included; results stratified by age and gender. Results not available for subgroups defined by latitude, skin pigmentation or seasonal variation. Children < 6 years not included.

Table 25: GRADE Quality of Evidence: Prevalence of Vitamin D Deficiency in Canada – Children

Outcome	Design	Quality	Consistency	Directness Appropriate range of subjects	Other modifying factors	Overall quality
Vitamin D Deficiency – Children Peer reviewed	4 observational cross-sectional studies Newborn to adolescents	Relatively small sample sizes (48-68) in 3 studies and 1,753 in 1.	Fairly consistent in 3/4 studies†.	Serious limitation: Risk that different subgroups not adequately represented‡	N/A	
	Low	Low	Low	Very low →		Very low
Vitamin D Deficiency – Children Grey literature	1 observational cross-sectional study Children 24-30 months old	Relatively small sample size (n=82)	Fairly consistent with peer-reviewed studies	Serious limitation: Risk that different subgroups not adequately represented‡	N/A	
	Low	Low	Low	Very low →		Very low

* Recruitment seemed to have been done appropriately since subjects were either randomly or consecutively selected from the source population.

† One study showed very different results, 36% of 50 newborn with serum levels < 27.5 nmol/L (88) (other studies in children: 0-6.3% with levels < 25-27.5 nmol/L. (82;89;90) The discrepancy cannot be explained from the information given.

‡ One large Quebec study used a province-wide sampling method but included only French Canadian children and may not represent the Canadian population. The other 3 studies were small (48-68) and may also not adequately represent the Canadian population. Moreover, non-participation rate was reported in 3 studies (25%, (89) 62%, (90) and 78%(88)), which may result in selection bias. Therefore, the level of evidence was downgraded to very low.

Table 26: GRADE Quality of Evidence: Prevalence of Vitamin D Deficiency in Canada – Adults and Children with Kidney Disease

Outcome	Design	Quality	Consistency	Directness Appropriate range of subjects	Other modifying factors	Overall quality
Vitamin D deficiency – Adults Kidney disease	2 observational cross-sectional	No serious limitations*	Not largely inconsistent	Sparse data (only 1 study in patients with chronic kidney disease and 1 in patients with renal transplantation)	Not applicable	
	Low	Low	Low	Very Low →		Very Low
Vitamin D deficiency – Children Kidney disease	3 observational studies, 2 cross-sectional, 1 retrospective	No serious limitation*	Not largely inconsistent	Serious limitation No studies in Canadian patients.	Not applicable	
	Low	Low	Low	Very Low →		Very Low

* Recruitment seemed to have been done appropriately since subjects were either randomly or consecutively from the source population. Although the percentage of subjects that declined participation was not reported, which may result in selection bias, we believe that this does not represent serious limitations in this case, therefore, the evidence was not further downgraded.

Discussion

The currently available evidence indicates that vitamin D, alone or in combination with calcium, may decrease the risk of fractures and falls in postmenopausal women and elderly men. With regards to other outcomes such as cancer (colorectal, breast, prostate, pancreatic), cardiovascular outcomes, and all-cause mortality, there is no high or even moderate quality evidence to support the benefits of vitamin D. Even if there is any residual uncertainty, there is no evidence to show that testing vitamin D levels encourages adherence to Health Canada's guidelines for vitamin D intake.

Health Canada currently recommends a daily vitamin D intake of 200 IU for Canadians. (68-70) Men and women over the age of 50 should take an additional daily supplementation of 400 IU. (69) Further recommendations are available for breastfed infants. (102) These recommendations are based on the evidence of health effects and the safety of vitamin D in healthy individuals. (71) Health Canada is currently reviewing the evidence of vitamin D's safety and effectiveness in order to decide if the recommendations should be revised. (68)

The results of a 2004 survey suggest that, in Ontario, the median dietary vitamin D intake is below that which is recommended by Health Canada. (74) Less than 50% of females age 9 to 50 included in the survey in Ontario had a daily intake equal to or above the 200 IU recommended, in fact it was as low as 25% in the 19 to 30 age group. (74) Approximately 44% to 69% of Ontarian males in the same age groups met Health Canada's requirements. (74) Similarly, four studies included in the MAS evaluation observed a mean vitamin D intake below Health Canada's recommended guidelines. (80;85;86;88)

The Canadian studies included in our evaluation suggest that approximately 5% of adults and children are vitamin D deficient and between 10% and 25% have serum levels below the 40 to 50 nmol/L bracket. This is consistent with both the results of the Canadian Health Measures Survey and the results of vitamin D tests performed in community laboratories across Ontario. A trend toward a higher prevalence of serum vitamin D of less than 37.5 to 50 nmol/L was observed during the winter-spring months (weighted average: 23.6%; 95% CI: 21.4, 25.9), compared to the summer months (weighted average 9.6%; 95% CI: 7.7, 11.6). This is consistent with an earlier study that indicated that the production of vitamin D through dermal synthesis is practically non-existent during the winter and spring months at higher latitudes (103), such as is the case in Canada. Two studies also showed a higher prevalence of serum levels less than the 37.5 to 40 nmol/L bracket among older children compared to their younger counterparts (weighted averages of 34.4% and 10.3%, respectively).

Some studies indicated that individuals with darker skin pigmentation may exhibit lower serum vitamin D levels. (80;81;85;86) Notwithstanding its biological plausibility, the cause-effect relationship of this association is still unclear and such results mainly stem from univariate analyses that do not account for other factors that could contribute to the differences, such as vitamin D intake and sun exposure. Melanin, which is present in larger amounts in darker skin appears to act as a filter to UV radiation leading to decreased dermal production of vitamin D (19), nevertheless, the amount of skin pigmentation that affects dermal vitamin D production is not well documented and an objective measure of skin pigmentation was not used among these studies.

It is difficult to compare the prevalence of vitamin D deficiency between countries due to differences in food fortification, type of vitamin D assay, lifestyle, and cultural factors etc… Nevertheless, trends observed in Canadian studies were also seen in other Northern hemisphere nations such as the effects of season and skin pigmentation. (9)

There is also a paucity of strong evidence to determine the target serum level of vitamin D. No precise targets across age groups could be established from a comprehensive systematic review performed by the

AHRQ (33), which evaluated the association between serum vitamin D levels and different bone health outcomes and falls. The normal threshold for vitamin D levels to prevent non-bone health related conditions cannot be resolved until a causal effect or correlation has been demonstrated between vitamin D levels and these health conditions. This is an ongoing research issue around which there is presently too much uncertainty to form any conclusions that would support routine vitamin D testing. The lack of consensus on target serum vitamin D levels, as well as inter- and intra-assay variations, directly affect and undermine the clinical utility of vitamin D testing.

Conclusions

1. Studies indicate that vitamin D, alone or in combination with calcium, may decrease the risk of fractures and falls among older adults.
2. There is no high or moderate quality evidence to support the effectiveness of vitamin D in other outcomes such as cancer, cardiovascular outcomes, and all-cause mortality.
3. Studies suggest that the prevalence of vitamin D deficiency in Canadian adults and children is relatively low (approximately 5%), and between 10% and 25% have serum levels below 40 to 50 nmol/L (based on very low to low grade evidence).
4. Given the limitations associated with serum vitamin D measurement, ambiguities in the definition of a 'target serum level', and the availability of clear guidelines on vitamin D supplementation from Health Canada, vitamin D testing is not warranted for the average risk population.
5. Health Canada has issued recommendations regarding the adequate daily intake of vitamin D, but current studies suggest that the mean dietary intake is below these recommendations. Accordingly, Health Canada's guidelines and recommendations should be promoted.
6. Based on a moderate level of evidence, individuals with darker skin pigmentation appear to have a higher risk of low serum vitamin D levels than those with lighter skin pigmentation and therefore may need to be specially targeted with respect to optimum vitamin D intake. The cause-effect of this association is currently unclear.
7. Individuals with medical conditions such as renal and liver disease, osteoporosis, and malabsorption syndromes, as well as those taking medications that may affect vitamin D absorption/metabolism, should follow their physician's guidance concerning both vitamin D testing and supplementation

Appendices

Appendix 1: Effects of Vitamin D ± Calcium on Non-Bone Health Outcomes (based on the AHRQ 2009 Systematic Review)

A comprehensive systematic review published in July 2009 by the Agency for Healthcare Research and Quality (AHRQ) evaluated the effects of vitamin D, calcium alone, and a combination of the two in different bone and non-bone health outcomes including cancer, all-cause mortality, cardiovascular disease, growth, body weight, blood pressure, and autoimmune and infectious diseases. (11) The overall quality of the systematic review was considered high, rating 10 out of 11 based on the AMSTAR (a measurement tool to assess systematic reviews) criteria. (41) The systematic literature search extended between 1969 and April 2009. (11) Observational or interventional studies published in English in a generally healthy population were included. Studies that evaluated the effects of vitamin D either through serum markers [25(OH)D or 1,25-dihydroxyvitamin D] or known vitamin D doses were included. Studies that based vitamin D doses on dietary intake were excluded due to the possibility of imprecision in the estimation of food vitamin D content. Vitamin D combinations other than with calcium (e.g., multivitamins) were also excluded unless the independent effects of vitamin D could be determined. (11) In addition to meeting these eligibility criteria, included studies must also have contained: a clear research question, a description of the literature search, a list of their inclusion criteria, and defined outcomes. Pooled analyses were included if they were based on a systematic review that satisfied the eligibility criteria listed above. (11)

In total, over 165 interventional and observational studies were included. (11) In most of these, the evaluation of non-bone health outcomes was drawn from studies originally designed to evaluate bone health outcomes. No eligible studies examining the effects of vitamin D ± calcium in type 1 diabetes or multiple sclerosis patients seem to have been identified. The analysis included the results of studies on growth, body weight, cardiovascular outcomes, cancer, immunologic outcomes (infectious disease mortality and eczema), pregnancy-related outcomes, all-cause mortality, hypertension and blood pressure. (11)

The authors of the review concluded that the evidence available did not permit firm conclusions to be drawn on the effects of vitamin D on non-bone health outcomes such as cancer, overall and specific sites (colorectal, breast, prostate, and pancreatic), cardiovascular outcomes, all-cause mortality among others. (11) Considerable heterogeneities were encountered among studies, as well as inconsistent results and limitations in study design, which precluded any conclusions regarding the association between serum 25(OH)D levels and vitamin D supplementation (alone or combined with calcium), on the different non-bone health outcomes evaluated. (11)

The results of the studies identified in the AHRQ 2009 systematic review are described below. The quality of evidence of these studies was appraised by MAS according to the GRADE criteria. (44) For each given outcome, where available, RCTs were the focus of the appraisal as they are considered to be of greater evidentiary value than observational studies. (44) In the absence of RCTs, observational studies identified were described.

The effects of vitamin D in colorectal cancer and colorectal adenocarcinoma

Two RCTs (46;47) evaluating the effects of vitamin D ± calcium in colorectal cancer and colorectal adenocarcinoma in men and women ages 50 and older were identified in the AHRQ review (Table A1). (11) In both RCTs (46;47), colorectal cancer was evaluated as a secondary outcome, the studies being originally designed to evaluate the effects of vitamin D on fracture risk.

No association was found in the two RCTs between vitamin D ± calcium and the incidence of colorectal cancer or colorectal adenocarcinoma with a mean follow-up of 5 to 7 years. (46;47) Despite not finding an association between vitamin D use and the risk of colorectal cancer, one of the RCTs found that subjects with higher baseline serum 25(OH)D had a lower risk of colorectal cancer (p for trend:0.02). (46) This discrepancy was not discussed by the authors. In a letter to the editor about another cancer outcome (breast cancer) in the same RCT, Speers and Brown pointed out that there was an overlap between in the self-reported vitamin D intake among the different serum level quintiles used. (55) According to them, factors other than intake may affect serum vitamin D levels such as sunlight exposure, BMI, physical activity, and genetic factors – none of which were adjusted for in the analysis (55), which may affect the validity of the study results.

Vitamin D ± Calcium and Breast Cancer Risk

Two RCTs (47;104) evaluating the effects of vitamin D ± calcium in breast cancer with mean follow-ups of 5 and 7 years were identified through in the AHRQ review (Table A2). (11) Again, the RCTs were originally designed to evaluate bone health outcomes. (47;104) Neither of the RCTs showed an association between vitamin D and breast cancer risk. In one of them (104), a higher baseline vitamin D level was not associated with a decrease in breast cancer risk after adjusting for BMI and physical activity, in addition to other variables (nested case-control analysis). In a letter to the editor Olsen et al. point out that the vitamin D dose (400 IU/day) may not have been sufficient to generate improved outcomes (105), although the other RCT used a 2-fold higher dose (800 IU/day) with equally inconclusive results. (47) On the other hand, Olsen et al. also cautioned that an observational study has already shown an increased risk of prostate cancer in subjects with higher serum vitamin D levels. (105) They further commented on the importance of adjusting for confounders such as body mass index and physical activity, (105) which again may affect the validity of the results if not accounted for.

Vitamin D ± Calcium and Prostate Cancer Risk

No RCTs evaluating the effects of vitamin D ± calcium on prostate cancer risk could be identified through the systematic review by the AHRQ. (11) The systematic review did, however, identify 12 nested case-control studies that evaluated the association between baseline serum vitamin D levels and prostate cancer risk. (11) Prostate cancer risk was a secondary outcome in most of these studies. Sample sizes ranged from 61 to 749 subjects with mean ages between 44 and 68 years. Follow-up periods ranged from 2 to 16 years. (11)

The results obtained in the observational were inconsistent. While one study found an increased risk of prostate cancer in subjects with higher serum vitamin D levels [OR: 1.7; 95% CI: 1.1, 2.4; 25(OH)D 80 nmol/L vs. 40-49 nmol/L] another study found a protective effect but only in men younger than 52 years [OR 3.5; 95% CI: 1.7, 7.0; ≤ 40 nmol/L vs. > 40 nmol/L]. In the latter study, no association between vitamin D levels and prostate cancer risk was found in men over the age of 51. (11) The remaining 10 observational studies did not find a significant association between serum vitamin D and prostate cancer risk. (11)

Table A1: Vitamin D Effects on Colorectal Cancer Incidence or Mortality

Study	Study Characteristics	Study population	Exposure assessment	Outcome ascertainment	Statistical analysis	Study Results (By 25(OH)D nmol/L)	Summary of conclusions
Wactawski-Wende (46) (2006) United States N= 36,282	▪ RCT ▪ Nested case-control [25(OH)D] ▪ Mean f-up: 7 yrs ▪ F-up: 1-12 yrs	▪ Age: 50-79 yrs	▪ VD 400 IU + Ca 1 g vs. ▪ Placebo ▪ Baseline 25(OH)D	▪ Colorectal cancer ▪ Self-reported and confirmed in medical records.	▪ Cox proportional hazards (VD vs. placebo) ▪ Matched: age, study centre, race. ▪ Logistic regression [25(OH)D quintiles]	▪ VD vs. Placebo ▪ Invasive colorectal cancer incidence: HR: 1.08 (0.86 , 1.34) ▪ Colon cancer: HR 1.00 (0.78, 1.28) ▪ Rectal cancer: HR 1.46 (0.92, 2.32) ▪ Colorectal adenocarcinoma: HR 1.00 (0.78, 1.26) ▪ Invasive colorectal cancer: OR by 25(OH)D quartile) (nmol/L) < 31 nmol/L: 2.53 (1.49, 4.32) 31-42.3: 1.96 (1.18, 3.24) 42.4-58.3: 1.95 (1.18, 3.24) > 58.3: reference p for trend: .02	▪ No association between VD + Ca and invasive colorectal cancer vs. placebo. ▪ However, association between baseline 25(OH)D and the outcome – this does not take into account use of VD. Very limited adjustment for confounders. ▪ Discrepancy between lack of effect of VD use and effect of baseline 25(OH)D not clarified by authors.
Trivedi (47) (2003) UK N= 2,686	▪ RCT ▪ Mean f-up: 5 yrs ▪ N= 2,686	▪ Mean age: 75 yrs (65-85) ▪ Men and women	▪ Vitamin D3 100,000 IU ev. 4 mos vs. ▪ Placebo ▪ Baseline characteristics self reported by questionnaire. ▪ Adjusted for age	▪ Colorectal cancer mortality ▪ Secondary endpoint ▪ From death certificate or self-reported in questionnaire ▪ Multiple outcomes evaluated	▪ Cox proportional hazards, age-adjusted.	▪ Colorectal cancer incidence: HR (95% CI) 1.02 (0.60, 1.74) ▪ VD (28 cases) vs. Pl (27 cases) ▪ Colorectal cancer mortality: HR (95% CI) 0.62 (0.24, 1.60) ▪ VD (7 cases) vs. Pl (11 cases) ▪ ITT analysis	▪ No significant effect of VD on total mortality or cancer incidence.

25(OH)D refers to 25-hydroxyvitamin D; Ca calcium; F-up follow-up; ITT intention-to-treat; RCT randomized controlled trial; VD vitamin D; yr year

Table A2: Vitamin D Effects on Breast Cancer Risk

Study	Study Characteristics	Study population	Intervention	Outcome ascertainment	Statistical analysis	Study Results	Summary of conclusions
Triverdi et al. (2003) RCT evaluates fractures and mortality N=2,686	RCT to evaluate the effect on fractures Secondary endpoint F-up: 5 yrs Double-blind	Mean age: 75±4.6 yrs Women: 24% Similar demographics and co-morbidities between groups	VD3 100,000 IU every 4 months (equivalent to ~ 800 IU/day) ± Ca vs. Placebo ± Ca Mean Ca: 742 mg/day (no difference between groups) Cointerventions: VD < 200 IU possible in control group	▪ Breast cancer ▪ Secondary endpoint ▪ From death certificate or self-reported in questionnaire ▪ Multiple outcomes evaluated	▪ Cox proportional hazards, age-adjusted. ▪ Intent-to-treat analysis	Breast cancer RR 0.99 (0.25 , 3.99) # Events 4 (1.2%) / 4 (1.2%)	Secondary endpoint No significant effect of VD on total mortality or cancer incidence. Losses to f-up: 23.5% mostly deaths Compliance: 66% at last dose (80% excluding deaths)
Chlebowski et al. (2007) Women's Health Initiative (WHI) to evaluate dietary modification and HRT on different outcomes	RCT Secondary endpoint f-up: 7 years Double-blind	Women: 100% Mean age: 62 yrs CV risk factors: 30% Diabetes: 4.9% N. chronic conditions: 1.2±0.9 Well-balanced groups Free of breast cancer at entry.	VD 400 IU + Ca 1 g vs. Placebo (PI) Cointerventions: VD 600 IU/day increased to 1,000 IU/day during study + 1,000 mg Ca/day	▪ Medical records (blinded investigators)	▪ Cox proportional hazards, stratified by age, disease prevalence, treatment assignment. ▪ Intent-to-treat analysis	▪ Breast cancer HR: 0.96 (0.86 , 1.07) ▪ Several subgroups evaluated ▪ Age 70-79 yrs HR: 1.08 (0.82 , 1.43) ▪ Baseline VD ≥ 600IU 1.34 (1.01 , 1.78)	▪ Compliance (≥80%): 60-63% ▪ Authors mention association between higher calcium and lower risk. ▪ No association between lower breast cancer risk and higher serum vitamin D levels after adjusting for BMI and physical activity in addition to other variables (nested case-control).

25(OH)D refers to 25-hydroxyvitamin D; BMI body mass index; Ca calcium; CV cardiovascular; F-up follow-up; ITT intention-to-treat; RCT randomized controlled trial; VD vitamin D; yr year

Vitamin D ± Calcium and Pancreatic Cancer Risk

No RCTs evaluating the effects of vitamin D ± calcium on pancreatic cancer risk were identified through the AHRQ systematic review, although two nested case-control studies were. (11) The two studies yielded conflicting results. While the first observed a higher risk of pancreatic cancer in subjects with a higher baseline serum vitamin D, the second study did not find an association between baseline vitamin D levels and pancreatic cancer. (11)

One of the nested case-control studies included 600 adult male smokers 54 to 62 years old, 200 cases and 400 controls. (11) Subjects with higher serum vitamin D (> 65.5 nmol/L, 5th quintile) had an almost 3-fold higher risk of incident exocrine pancreatic cancer (OR 2.92, 95% CI: 1.56, 5.48) than those in the lowest vitamin D quintile (< 32 nmol/L) over a median 11.6 years of follow-up. (11) The results were adjusted for age, month of blood draw, years smoked, No. cigarettes/day, stopped smoking for > 1 year, occupational physical activity, education, and serum retinol. (11) Islet cell carcinomas were excluded from the analysis. (11)

The second nested case-control study included 552 men and women 55 to 74 years old, 184 cases and 368 controls. (11) Over a median 5.4 years of follow-up no statistically significant association between exocrine pancreatic cancer in subjects in the highest vs. lowest vitamin D quintiles was found (OR: 1.45, 95% CI: 0.66, 3.15). (11) The analysis was adjusted for age, race, sex, date of blood draw, BMI, and smoking. (11) Subjects with higher (vs. lower) serum vitamin D levels and low UVB residential exposure had a higher risk of exocrine pancreatic cancer (OR 4.03, 95% CI: 1.38, 11.79, highest vs. lowest quintile). (11)

Vitamin D ± Calcium and Cardiovascular disease

Two RCTs (three publications) (47;106;107) evaluating the effects of vitamin D ± calcium on cardiovascular disease were identified in the AHRQ systematic review. (11) Again, both were originally designed to evaluate bone health outcomes. No statistically significant association between vitamin D ± calcium use and cardiovascular outcomes were found in the two RCTs. (47;106;107)

The first RCT evaluated the effect of vitamin D3 (100,000 IU every 4 months) compared to placebo on fractures and overall mortality in 2,686 men and women, 65 to 85 years old, selected from the Doctors Study Register in the UK. (47) An intention-to-treat analysis using age-adjusted Cox regression was used. and the two groups were comparable with regards to demographic characteristics. The mean calcium intake at four years of 742 mg/day did not differ between the study groups. The compliance rate was approximately 76% and did not differ between the study groups. After a follow-up of 5 years, there was no statistically significant difference between the study groups with regards to several cardiovascular outcomes and cardiovascular death (see Table A3). (47)

The second RCT was based on the Women's Health Initiative (WHI) trial designed to evaluate the effect of vitamin D ± calcium on fractures (compared to placebo) in postmenopausal women. (106;107) The cardiovascular outcomes evaluated were secondary endpoints specified *a priori*. A total of 36,282 women were included, 18,176 received active treatment and 18,106 placebo. No statistically significant association between vitamin D ± calcium and cardiovascular outcomes were observed. The authors concluded that there was no evidence of increased or decreased risk in cardiovascular outcomes with vitamin D + calcium. (106;107)

Table A3: Vitamin D Effects on Cardiovascular Outcomes and Death

Study	Study Characteristics	Study population	Exposure assessment	Outcome ascertainment	Statistical analysis	Study Results By 25(OH)D nmol/L, HR (95% CI)
Trivedi (47) (2003) UK N= 2,686	RCT Mean f-up: 5 yrs N= 2,686	▪ Mean age: 75 yrs (65-85) ▪ Men and women	▪ Vitamin D3 100,000 IU ev. 4 mos vs. ▪ Placebo (Pl) ▪ Baseline characteristics self reported by questionnaire ▪ Adjusted for age	▪ Cardiovascular outcomes ▪ Secondary endpoint ▪ From death certificate or self-reported in questionnaire ▪ Multiple outcomes evaluated	▪ Cox proportional hazards, age-adjusted. ▪ ITT analysis	▪ Cardiovascular disease (CVD): 0.90 (0.77, 1.06) ▪ Ischemic heart disease (IHD): 0.94 (0.77, 1.15) ▪ Cerebrovascular disease: 1.02 (0.77, 1.36) ▪ CVD death: 0.84 (0.65, 1.10) ▪ IHD death: 0.84 (0.56, 1.27) ▪ Cerebrovascular disease death: 1.04 (0.61, 1.77)
Hsia et al. (107) LaCroix et al. (106)	RCT (VD use) Nested case-control [25(OH)D] Mean f-up: 7 yrs F-up: 1-12 yrs	▪ Age: 50-79 yrs	▪ VD 400 IU + Ca 1 g vs. ▪ Placebo (Pl)	▪ Cardiovascular outcomes and mortality ▪ Self-reported and confirmed in medical records.	▪ Cox proportional hazards, age-adjusted.	▪ MI: 1.05 (0.91, 1.20) ▪ CABG or PCI: 1.08 (0.98, 1.22) ▪ Hospitalized for heart failure: 0.95 (0.83, 1.10) ▪ Angina: 1.08 (0.94, 1.24) ▪ Stroke: 0.95 (0.82, 1.10) ▪ TIA: 1.16 (0.95, 1.42) ▪ Composite (stroke, TIA): 1.02 (0.91, 1.15) ▪ Cardiac composite (MI, CHD, death, CABG, or PCI): 1.08 (0.99, 1.19) ▪ Cardiac composite (MI or CHD death): 1.04 (0.92, 1.18) ▪ Cardiovascular death: 0.92 (0.77, 1.07) ▪ CHD death: 1.01 (0.79, 1.29) ▪ Cerebrovascular death: 0.89 (0.62, 1.29)

25(OH)D refers to 25-hydroxyvitamin D; BMI body mass index; Ca calcium; CABG coronary artery bypass graft; CHD coronary heart disease; CV cardiovascular; F-up follow-up; ITT intention-to-treat; MI myocardial infarction; PCI percutaneous coronary intervention; RCT randomized controlled trial; TIA transient ischemic attack; VD vitamin D; yr year

Vitamin D ± Calcium and All-Cause Mortality

The AHRQ systematic review included a meta-analysis of 11 RCTs (N=44,688) that evaluated the effects of vitamin D (300-880 IU/day) + calcium (500-1,200 mg/day) vs. placebo on all-cause mortality. (11) A second meta-analysis of four RCTs (N=13,833) evaluating the effects of vitamin D (400-880 IU/day) vs. placebo on all-cause mortality was also performed. (11) Most of the RCTs were originally designed to evaluate the effects of the intervention in fractures.

In both analyses, the authors concluded that vitamin D (± calcium) had no effect on all-cause mortality. (11) The risk ratio for all-cause mortality with vitamin D + calcium vs. placebo was 0.93 (95% CI: 0.86, 1.01), and the risk ratio for all-cause mortality with vitamin D vs. placebo was 0.97 (95% CI: 0.92, 1.02). (11) The authors observed little evidence of heterogeneity among the studies in both meta-analyses.

GRADING of the Evidence

The quality of the studies concerning the non-bone health outcomes listed above was examined by MAS according to the GRADE Working Group criteria. (44) In summary, low quality evidence showed that there was no association between vitamin D ± calcium and risk of cancer. Moderate quality evidence has also shown that there is no association between vitamin D + calcium and colorectal/breast cancer, cardiovascular outcomes, and all-cause mortality. Finally, there was very low quality evidence that showed no association between vitamin D ± calcium and prostate or pancreatic cancer (see Table A4).

Table A4: GRADE Quality of Evidence: Effects of Vitamin D ± Calcium in Non-Bone Health Outcomes

Outcome	Design	Quality	Consistency	Directness Appropriate Range of Subjects	Other Factors	Overall Quality	Conclusion
Overall cancer	3 RCTs	Serious limitations in 1 study. (45) 2° outcome in all RCTs.	Important inconsistency in 1 study with serious limitations. (45)	No major limitations although studies in older adults	N/A		No association
	High	Moderate	Low →			Low	
Colorectal cancer	2 RCTs	Cancer was a 2° outcome in all RCTs	Consistent	As above	N/A		No association
	High	Moderate →				Moderate	
Breast cancer	2 RCTs	As above	Consistent	As above	N/A		No association
	Low	Moderate →				Moderate	
Prostate cancer	12 nested control studies	Serious limitations*	Important inconsistency	As above	N/A		Inconsistent results
	Low	Very low	Very low (-1) →			Very low	
Pancreatic cancer	2 nested control studies	Serious limitations*	Important inconsistency	As above	N/A		Inconsistent results
	Low	Very low	Very low (-1) →			Very low	
Cardiovascular outcomes	2 RCTs	2° outcome in all RCTs	Consistent	As above	N/A		No association
	High	Moderate →				Moderate	
All-cause mortality	2 meta-analysis	2° outcome in all RCTs	Consistent	As above	N/A		No association
	High	Moderate →				Moderate	

* Serious limitations included use of a proxy for vitamin D exposure, i.e., serum vitamin D level measured at baseline, lack of accounting for changes in exposure (vitamin D) during follow-up of up to 16 years, limited adjustment for important confounders.

Appendix 2: Literature Search Strategies

Databases searched: OVID MEDLINE, MEDLINE In-Process and Other Non-Indexed Citations, OVID EMBASE, Wiley Cochrane, Centre for Reviews and Dissemination/International Agency for Health Technology Assessment

Database: Ovid MEDLINE(R) < 1996 to July Week 2 2009>
Search Strategy:
---
1 exp Vitamin D Deficiency/ (4311)
2 (vitamin D adj2 (inadequa* or low* or deficien* or insufficien*)).ti,ab. (2367)
3 1 or 2 (5077)
4 exp Prevalence/ (99399)
5 prevalen*.ti,ab. (198412)
6 4 or 5 (229424)
7 6 and 3 (793)
8 limit 7 to (humans and yr="1998 -Current") (756)

Database: EMBASE < 1980 to 2009 Week 28>
Search Strategy:
---
1 exp vitamin D deficiency/ (4121)
2 (vitamin D adj2 (inadequa* or low* or deficien* or insufficien*)).ti,ab. (3671)
3 1 or 2 (5514)
4 exp prevalence/ (162992)
5 prevalen*.mp. (285607)
6 4 or 5 (288862)
7 3 and 6 (868)
8 limit 7 to (human and yr="1998 -Current") (781)
9 from 8 keep 1-781 (781)

CINAHL Search

#	Query	Limiters/Expanders	Results
S10	S9	Limiters - Published Date from: Jan1998 – Dec 2009	168
S9	S5 and S8		173
S8	S6 or S7		42,627
S7	prevalen*		42,627
S6	(MH "Prevalence")		15,464
S5	S1 or S4		1,362
S4	S2 and S3		1,034
S3	(MH "Vitamin D+")		2,953
S2	inadequa* or low* or deficien* or insufficien*		154,201
S1	(MH "Vitamin D Deficiency")		863

Appendix 3: Characteristics of the prevalence studies in Canada

Table A5: Characteristics of Prevalence Studies in Canada: Adults

Study (year) City/region (latitude) N	Study design and Statistical analysis	Population	25(OH)D test / Period of Measurement	Inclusion Criteria
Rucker et al. (79) (2002) Calgary (51° N) N=188	Longitudinal study Statistical analysis Regression model used to test differences in seasonal variation and other predictors such as BMI, season, and travel to lower latitudes Power calculation: NR	Men and women Randomly selected sub-sample of healthy subjects included osteoporosis study cohort in Calgary centres.¶ Subjects invited by telephone contact. Participation rate: 204/463 (44.1%) – characteristics not compared to subjects excluded Withdrawal: 16/204 (7.8%) Reasons for withdrawal: 25(OH)D < 25 nmol/L (n=3), use of VD supplements > 200 IU/day during study (n=3)	Test: RIA (DiaSorin) 2-step Intra-assay CV: 11.7-12.5% Inter-assay CV: 9.4-11% VD deficiency: < 50 nmol/L Measurement period: 1999	Inclusion criteria Participants of osteoporosis study Exclusion criteria Use of VD supplements > 200 IU/d or serum 25(OH)D ≤ 25 nmol/L on prestudy screening. Participants were asked not to exceed 200 IU dose/day during study.
Genuis et al. (80) (2009) Edmonton (53° N) N= 1,433	Cross-sectional study Statistical analysis Cochran Mantel-Haenszel test used for stratified analyses†: age, sex, skin pigmentation, pregnancy status, BMI, season, clinical practice, VD exposure Power calculation: NR	Men and women Patients recruited from the practice of 3 physicians‡ between 2001 and 2007 Participation rate: NR Withdrawal: N/A	Test: HPLC (1 laboratory), mass spectrometry Intra-assay CV: NR Inter-assay CV: NR VD deficiency: < 40 nmol/L Measurement period: June 2001 – March 2007	Inclusion criteria Patients presenting to 3 clinical practices: All new patients (1 clinic) Consecutive patients doing annual check-up (1 clinic) Patients with conditions that may be affected by VD status (1 clinic)
Weiler et al. (81) (2007) Manitoba (~49°N) N= 356	Cross-sectional study Statistical analysis Factorial ANOVA design to test for skin pigmentation and age effect. Power calculation: NR	Aboriginal and non-aboriginal women (urban and rural cohorts) Subject identification: Urban cohort: Status Verification System files and provincial registry files (Winnipeg) Rural cohort: random sample from band lists (Northern and Southern Manitoba) Participation rate: NR Withdrawal: 100/456 (21.9%) due to invalid questionnaire or not attending the visit.	VD2 and VD3: RIA (DiaSorin) (1 lab) Intra-assay CV: NR Inter-assay CV: 6-13% VD deficiency: < 37.5 nmol/L Measurement period: June 2002 – March 2004	Inclusion criteria Non-aboriginal (white) or aboriginal women living in the areas sampled Exclusion criteria Currently or recently pregnant or breastfeeding

Study (year) City/region (latitude) N	Study design and Statistical analysis	Population	25(OH)D test / Period of Measurement	Inclusion Criteria
Newhook et al. (82) (2009) Avalon Peninsula, Newfoundland and Labrador (47° N) N=50	Cross-sectional study (pilot study) Data collected at the end of summer and end of winter (using different subjects) <u>Statistical analysis</u> Rate of VD deficiency reported for summer and winter. Power calculation: NR	Pregnant women undergoing routine prenatal blood test through the provincial public health laboratory. Not clear if a random sample of the women was used. Participation rate: NR Withdrawal: N/A	Liquid chromatography mass spectrometry (Waters) Intra-assay CV: NR Inter-assay CV: NR VD deficiency: < 50 nmol/L or < 25 nmol/L Measurement period: September 2005– March 2006	<u>Inclusion criteria</u> Pregnant women undergoing routine prenatal blood test
Sloka et al. (83) (2009) Newfoundland and Labrador (46-53°N) N=593 (304 winter, 289 summer)	Cross-sectional Data collected in late summer and late winter (using different subjects) <u>Statistical analysis</u> Rate of VD deficiency reported for summer and winter. Power calculation: based on expected mean (50 nmol/L) and SD (20 nmol/L) and 5 nmol/L difference between winter and summer.	Pregnant women Random province-wide sample of pregnant women. Up to 5 samples of each of the 79 census consolidated subdivisions selected through random number generator (both summer and winter) Participation rate: N/A Withdrawal: N/A	Test: RIA (DiaSorin) Intra-assay CV: NR Inter-assay CV: NR VD deficiency: < 25 nmol/L Measurement period: January-March and July-September 2007	<u>Inclusion criteria</u> Pregnant adolescent to young adult
Waiters et al. (84) (1999) (Inuvik) Northwestern Territories N=121	Cross-sectional study Samples collected both during pregnancy and at delivery <u>Statistical analysis</u> Prevalence rate taken from discussion. Pearson correlation coefficients and multiple regression used to test for predictors Power calculation: NR	Pregnant women Sampling method NR Participation rate: NR Withdrawal: NR	Test: Competitive binding assay VD deficiency: < 30 nmol/L Measurement period: NR	<u>Inclusion criteria</u> Pregnant women undergoing prenatal care visit <u>Exclusion criteria</u> History of severe medical conditions
Weiler et al. (88) (2005) Winnipeg 49.54°N N=50	25(OH)D level measured at baseline of longitudinal study <u>Statistical analysis</u> Rate of VD deficiency reported, subgroup analysis presented. Statistical test: chi-square Power calculation: NR	Pregnant women All consecutive women admitted to study hospital for delivery during weekdays. Participation rate (mothers): 72/342 (21%)¶ Withdrawal (mothers): 22/72 (30.6%)	Test: RIA (DiaSorin, Stillwater, MN) Intra-assay CV: NR Inter-assay CV: < 10% VD deficiency: < 37.5 nmol/L Measurement period: August 2001 – April 2003	<u>Inclusion criteria</u> Healthy women§

Study (year) City/region (latitude) N	Study design and Statistical analysis	Population	25(OH)D test / Period of Measurement	Inclusion Criteria
Vieth et al. (85) (2001) Toronto 43° N latitude N=796 (435 in winter, 361 in summer)	Cross-sectional study <u>Statistical analysis</u> Prevalence calculated. Subgroups: skin pigmentation and season. Power calculation: NR	Healthy young adult women (18-35 yrs) Healthy female subjects who responded to advertisements for osteoporosis study. Participation rate: NR Withdrawal: N/A	Test: RIA (DiaSorin, Stillwater, MN), VD2 + VD3 Between assay CV: < 16% Within assay CV: < 10% Low VD: < 40 nmol/L Measurement period: November 1995 – March 1997	<u>Inclusion criteria</u> Healthy women 18-35 yrs <u>Exclusion criteria</u> Conditions and drugs associated with 2. bone loss/use of corticosteroids > 3 months¦ Previous diagnosis of osteopenia
Gozdzik et al. (86) (2008) Toronto N=107	Cross-sectional study Skin pigmentation measured in inner upper arm through band reflectometer. <u>Statistical analysis</u> Prevalence calculated. Differences between subgroups calculated through ANOVA (serum levels log transformed) Multiple linear regression: age, sex, BMI, skin pigmentation and VD intake. Power calculation: 87% power for both ANOVA and multiple regression analysis.	Young adults (mostly University students and staff). Subjects recruited from university campus. Participation rate: all eligible subjects agreed to participate (those who responded to ads) Withdrawal: N/A	Test: Competitive chemiluminescent immunoassay, LIAISON (DiaSorin), VD_2 + VD_3 (total VD) Intra-assay CV: 5% Inter-assay CV: 7% Serum VD thresholds used: < 25 nmol/L, < 50 nmol/L, < 75 nmol/L Measurement period: Winter 2007	<u>Inclusion criteria</u> 18-30 yrs <u>Exclusion criteria</u> Kidney or liver disease or conditions and drugs that affect VD metabolism or absorption‖. Recent UVB exposure, i.e., use tanning bed, travel to lower latitude during 3 months prior to recruitment.
Vecino-Vecino et al. (87) (2006) Montreal (45° N) N= 256	Cross-sectional study <u>Statistical analysis</u> Prevalence calculated, stratified by season. Power calculation: NR	Elderly men and women (≥ 65 yrs) Random selection of elderly referred to ambulatory clinic of 1 hospital. Participation rate: NR Withdrawal: N/A	Test: RIA (Stillwater, Minnesota = DiaSorin), VD3 Intra-assay CV: 8.5% Inter-assay CV: 17.3% Hypovitaminosis D: 25-50nmol/L, VD ≤ 25 nmol/L Measurement period: 1994-99	Elderly (≥ 65 yrs) No known metabolic bone syndrome or other conditions or drugs that affect VD or PTH levels.**

* 25(OH)D refers to 25-hydroxyvitamin D; BMI body mass index; CV coefficient of variation; d day; IU international units; HPLC high pressure liquid chromatography; N/A not applicable; NR not reported; PTH parathyroid hormone; RIA radioimmunoassay; SD standard deviation; VD vitamin D

† Fischer exact test was used for severe vitamin D deficiency ‡ Physician specialties: obstetritian/gynecologist, primary care generalist, family doctor

§ No hypertension, gestational diabetes, and long-term medical therapy. No use of illicit drugs.

¶ Cohort study (Canadian Multicentre Osteoporosis Study (CaMOS) believed to represent population in the area.

¦ Conditions associated with secondary bone loss (exclusion criterion): Crohn's disease, symptom addict hyperthyroidism, rheumatoid arthritis, bilateral oophrectomy, use of systemic corticosteroids for > 3 months at any time in the past. ‖ Exclusion criteria: conditions that may affect vitamin D metabolism or absorption: osteomalacia, osteopenia, Crohn's disease etc. Drugs that may affect vitamin D metabolism: corticosteroids, anticonvulsants etc. .

**Medications that may affect vitamin D or parathyroid hormone levels: estrogens, biphosphanates, loop diuretics, etc.

Table A6: Characteristics of Prevalence Studies in Canada: Adults with Kidney Disease

Study (year) City, N	Study Design and Statistical Analysis	Population	25(OH)D test	Inclusion Criteria
Rucker et al. (93) (2009) Edmonton 53°N latitude N=128	Baseline serum 25(OH)D levels measured at the start of VD supplementation study Statistical analysis Prevalence calculated, no subgroup analysis Power calculation: N/A	Recruited from renal insufficiency clinic Participation rate: NR Withdrawal: N/A	Test: liquid chromatography tandem mass spectrometry Intra-assay CV: 8% Inter-assay CV: 6% VD deficiency: < 37.5 nmol/L	Inclusion criteria Chronic kidney disease stages 3-5 GFR < 30 mL/min Exclusion criteria VD use > 400 IU/d
Boudville et al. (94) (94) (2006) London (ON) 43°2'N latitude N=419	Cross sectional study Statistical analysis Prevalence calculated, no subgroup analysis Power calculation: N/A	All patients from renal transplant clinic in 1 hospital Dec 2003 – Mar 2004 Participation rate: NR Withdrawal: N/A	Test: RIA (DiaSorin) Intra-assay CV: NR Inter-assay CV: NR VD deficiency: < 40 nmol/L	Inclusion criteria > 18 yrs Renal transplant > 1 month

* 25(OH)D refers to 25-hydroxyvitamin D; CV coefficient of variation; d day; GFR glomerular filtration rate; IU international units; HPLC high pressure liquid chromatography; N/A not applicable; NR not reported; SD standard deviation; VD vitamin D

Table A7: Characteristics of Prevalence Studies in Canada: Children

Study (year) City, N	Study Design and Statistical Analysis	Population	25(OH)D test	Inclusion Criteria
Mark et al. (89) (2008) Province of Quebec N=1,753	Cross-sectional survey Statistical analysis Prevalence calculated taking sampling weights and cluster design into account. Subgroup analyses (age and sex): likelihood ratio test using generalized linear regression.	Boys and girls, 9, 13, 16 yrs Province-wide school-based cluster sampling for ages 9, 13, and 16 yrs Participation rate: 63%-75% provided blood sample (no difference in sex, BMI z score, or parental income between included and excluded subjects Withdrawal: N/A	Test: RIA (Immunodiagnostic Systems LTD.) Intra-assay CV: NR Inter-assay CV: 5.9% VD deficiency: < 37.5 nmol/L Measurement period: January – May 1999	Inclusion criteria French Canadian† school children included in the Quebec Child and Adolescent Health and Social Survey. Ages 9, 13, and 16 yrs.
Newhook et al. (82) (2009) Avalon Peninsula (Newfoundland and Labrador) N=48 (children) N=50 (newborn	Cross-sectional study (pilot study) Data collected at the end of summer and end of winter (using different subjects) Statistical analysis Rate of VD deficiency reported for summer and winter. Power calculation: NR	Newborn babies and children 0-14 yrs Sample of children seen or newborn babies delivered at the study hospital and who had a blood test done. Participation rate: NR Withdrawal: N/A	Liquid chromatography mass spectrometry (Waters) Intra-assay CV: NR Inter-assay CV: NR VD deficiency: < 50 nmol/L or < 25 nmol/L Measurement period: September 2005 and March 2006	Inclusion criteria Children 0-14 yrs who presented at the hospital and had a blood test done. OR Newborn delivered at study hospital.
Roth et al. (90) (2005) Edmonton (52°N) N=68	Cross-sectional study Statistical analysis Rate of VD deficiency reported according to age and sex. Power calculation: NR	Children 2-16 yrs Consecutive patients who had ED visit at study hospital. Participation rate: 68/178 (38%) had both blood test and VD use assessment. Withdrawal: N/A	RIA (DiaSorin Incstar, Stillwater MN) Intra-assay CV: NR Inter-assay CV: 12% VD < 40 nmol/L VD < 25 nmol/L Measurement period: April 2003	Inclusion criteria Ages 2- 16 yrs ED visit at study hospital Exclusion criteria Unstable condition and use of feeding tube

Study (year) City, N	Study Design and Statistical Analysis	Population	25(OH)D test	Inclusion Criteria
Maguire et al. (91) (2009) Toronto (43°N) N=92	Cross-sectional study Statistical analysis Rate of VD deficiency reported. Multivariate linear regression including milk intake, BMI, skin pigmentation and VD supplementation, time spent outdoors, TV viewing. Power calculation: NR	Infants 24-30 months old. Children attending a routine (healthy) physician visit at a community-based practice. Participation rate: NR	Not specified	Inclusion criteria 24-30 months old Children attending a routine (healthy) physician's practice visit
Weiler et al. (88) (2005) Winnipeg (49.54°N) N=50	25(OH)D level measured at baseline of longitudinal study Statistical analysis Rate of VD deficiency reported, subgroup analysis presented. Statistical test: chi-square Power calculation: NR	Newborn All consecutive children delivered at study hospital during weekdays. Participation rate (mothers): 72/342 (21%)¶ Withdrawal (mothers): 22/72 (30.6%)	Test: RIA (DiaSorin, Stillwater, MN) Intra-assay CV: NR Inter-assay CV: < 10% VD deficiency: < 27.5 nmol/L Measurement period: August 2001 - April 2003	Inclusion criteria Appropriate weight for gestational age and weight > 3rd percentile. No congenital malformations. Healthy mothers‡

* 25(OH)D refers to 25-hydroxyvitamin D; BMI body mass index;CV coefficient of variation; d day; GFR glomerular filtration rate; IU international units; HPLC high pressure liquid chromatography; N/A not applicable; NR not reported; SD standard deviation; VD vitamin D

† French Canadian children represented 78-80% of all survey subjects.

‡ No hypertension, gestational diabetes, and long-term medical therapy. No use of illicit drugs.

¶ Most common reasons to refuse participation were: time constraints and concerns about X-rays to measure bone measure content included in the longitudinal study.

Table A8: Characteristics of Prevalence Studies: Children with Kidney Disease

Study (year) City/region(latitude) N	Study design and Statistical analysis	Population / Sampling	25(OH)D test / Period of measurement	Inclusion criteria
Ali et al. (96) (2009) Chicago N= 88 (2005-2006) N=79-336/yr (1987-1996	Cross-sectional Random sample of patients who had serum 25(OH)D level measured	Chronic kidney disease stages 1-5	Test: RIA (DiaSorin, Minnesota) Period: 2005-2006	CKD Serum 25(OH)D measured during study period.
Seeherunvong et al. (97)(2009) United States, Florida (25°N) N=258	Retrospective chart review.	CKD stages 1-5 Children attending outpatient dialysis clinic	NR	CKD stages 1-5 attending outpatient dialysis clinic Exclusion criteria Chronic liver disease, gastrointestinal malabsorption, anticonvulsants' use
Menon et al. (95) (2008) United States, Michigan N=57	Baseline 25(OH)D level obtained from longitudinal study.	Children and adolescents seen at the chronic renal insufficiency clinic,	25(OH)D3 measured (chemiluminescent assay)	Children and adolescent followed at chronic renal insufficiency clinic. eGFR < 75 ml/min/1.73 m2

* 25(OH)D refers to 25-hydroxyvitamin D; CKD chronic kidney disease; eGFR estimated glomerular filtration rate; NR not reported; RIA radioimmunoassay

Appendix 4: Results of Prevalence Studies in Adults (General Population, Canada)

Table A9: Serum Vitamin D, Prevalence Studies in Canada: Adults

Study (year) City, N	Baseline Characteristics	% Serum Vitamin D < 25 - 50 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Rucker et al. (79) 2002 Calgary 51° N latitude N=188 Test: RIA (DiaSorin)	Male: 60 (31.9%) Mean age: 63 yrs (Large age range) Lighter skin pigmentation: 185 (98.4%) BMI: 27 kg/m^2 Mean VD intake: NR (excluded use > 200 IU/d from supplements)	< 50 nmol/L Spring: 70 (37%) Winter: 73 (39%)	< 50 nmol/L Summer: 26 (14%) Fall: 81 (43%) NS	Statistically significant predictors: Increased age and BMI, and travel to lower latitude (< 2°N)
		< 50 nmol/L 61% (at least once during the year)		
Genuis et al. (80) (2009) Edmonton N= 1,433 Test: liquid chromatography	Patients from the practice of 3 physicians (obstetritian/gynecologist, primary care generalist, family doctor) Age < 19 yrs: 87 (6.1%) 19-30yrs:172 (12.0%) 30-60 yrs: 754 (52.6%) ≥ 60 yrs: 421 (29.4%) VD supplement use: None: 714 (50.0%) 50-400 IU:487 (34.0%) > 400 IU: 210 (14.7%) Glasses of milk/day None: 713 (49.8%) 1-2: 492 (34.3%) > 2: 206 (14.3%) Fish oil supplement use No: 1,074 (74.9%) Yes: 337 (23.5%) Skin tone Light: 1,179 (83.3%) Medium: 185 (13.1%) Dark: 18 (1.3%) First Nations: 33 (2.3%)	< 40 nmol/L Spring: 94 (22%) Winter: 78 (21%)	< 40 nmol/L Summer: 30 (10%) Fall: 38 (12%) NS across 4 seasons	
		< 25 nmol/L: 3.4%		
		Subgroup analyses (< 40 nmol/L) – seems to be from univariate analysis		
		By age (p=.1996) < 19 yrs: 28% 19-60 yrs: 18-20% ≥ 60 yrs: 11% By skin tone (p<.0001) Dark: 44% Light: 15% Midcolor: 23% First Nations: 48% BMI†, N=704 (p=.2959) Normal - underweight: 16-24% Overweight – obese: 14-22%	VD supplements¦ (p<.0001) None: 204 (29%) 50-400 IU: 30 (6%) > 400 IU: 6 (3%) No. glasses of milk/day (p<.0001) None: 151 (21%) 1-2: 76 (15%) > 2: 13 (6%) Recent sun exposure (p<.0001) Minimal: 201 (23%) Moderate: 33 (9%) Lots of sun: 6 (4%)	

Study (year) City, N	Baseline Characteristics	% Serum Vitamin D < 25 - 50 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Weiler et al. (81) (2007) Winnipeg for white women Northern/southern Manitoba for aboriginal women ~ 49°N N= 356 Test: RIA (DiaSorin)	Women, community-based sampling Mean age: 43-47 yrs (range: 25-76) Urban white: 146 (41.0%) Urban aboriginal:184 (51.7%) Rural aboriginal:26 (7.3%) BMI: 28.6-31.8 kg/m^2 VD intake IU/day – from FFQ questionnaire (24-hour recall period) Urban white:424±404 IU Urban aboriginal: 432±492 IU Rural aboriginal: 556±584 IU % using less than adequate intake for VD* Urban white: 42% Urban aboriginal: 44% Rural aboriginal: 27%	< 37.5 nmol/L Urban white: 27 (18.6%) Urban aboriginal: 56 (30.4%) Rural aboriginal: 8 (32%) p=.001 (for differences in serum 25(OH)D levels)		More urban aboriginal women had VD levels measured during the winter compared to white urban women (72% vs. 55%) Predictors: age

25(OH)D refers to 25-hydroxyvitamin D; BMI body mass index; FFQ food frequency questionnaire; IU international unit; NS not statistically significant; NR not reported; RIA radioimmunoassay; VD vitamin D; yr year.

* Adequate intake for vitamin D: 200 IU for women ages 25-50 years and 400 IU for ages above 50 years.

† BMI definitions: underweight (≤ 18.5), normal (18.5 – 24.9), overweight (25-29.9), obese (≥ 30)

¦ Similar statistically significant trend with increased intake of milk, fish, fish oil, sun exposure, and tanning bed use

Table A10: Serum Vitamin D, Prevalence Studies in Canada: Pregnant Women

Study (year) City, N	Population	% Serum Vitamin D < 25-37.5 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Newhook et al. (82) (2009) Newfoundland and Labrador N=50 Test: liquid chromatography	Pregnant women Age: NR VD supplementation: NR	< 25 nmol/L 2.5% (from graph)	< 25 nmol/L 0 (from graph)	
		< 25 nmol/L 1 (2%) < 50 nmol/L 21 (42%)		
Sloka et al. (83) (2009) Newfoundland and Labrador 46-53°N N= 304 (winter) N= 289 (summer)	Pregnant women sampled from entire province Adolescent – young adults Mean age: 27 yrs Measurements random throughout pregnancy (mostly 2nd trimester)	< 25 nmol/L 20 (6.6%)	< 25 nmol/L 5 (1.7%) p<.001 for mean difference between seasons	There was a trend to lower serum VD levels in more northern parts of the province
		< 25 nmol/L 25 (4.2%)		
Waiters et al. (84) (1998) Northwestern Territories N=121 Test: Competitive binding assay	Women (during pregnancy and at delivery) Community-based Inuit: 51 (42%) Native Indian: 37(30.6%) Caucasian: 33 (27%) Mean total VD intake ±SD: Caucasian:528±236 IU Native Indian: 312 ± 240 IU Inuit: 328 ± 200 IU	Measured throughout the year < 30 nmol/L 7 (6%) Mean ± (nmol/L) Caucasian: 59.8±29.4 Indian: 52.1 ±25.9 Inuit: 48.8 ± 14.2		Trend to lower serum levels in individuals with darker skin pigmentation.
Weiler et al. (88) (2005) Winnipeg (49°N) N=50 Test: RIA (DiaSorin)	Mothers of term newborn babies. Measurements done within 48 hours of delivery. Mean age: 25-29 yrs White: 30 (60%) First Nations: 8.4% Mothers taking VD supplement: 78% Mean VD intake (mothers): VD deficient: 149±145 IU Adequate VD level: 242±218 IU	< 37.5 nmol/L 23 (46%) Using supplements: 14/39 (36%), inferred from information below. VD-deficient mothers Use of supplements: 14/23 (61%) VD deficient infant: 16 (70%) Mothers with adequate serum levels: Use of supplements: 25/27 (93%) VD deficient infant: 2 (7%)		Trend to higher VD intake in mothers with adequate VD levels

IU refers to international unit; NR not reported; RIA radioimmunoassay;SD standard deviation; VD vitamin D; yr year

*Includes both dietary and supplements

Table A11: Serum Vitamin D, Prevalence Studies in Canada: Young Adults

Study (year) City, N	Population	% Serum Vitamin D < 25-37.5 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Vieth et al. (85) (2001) Toronto N=796 (435 winter, 361 summer) 43° N latitude Test: RIA (DiaSorin)	Healthy women, 18-35 yrs Community-based Excluded: conditions associated with bone loss Female: 796 (100%) White: 702 (88%) Non-white:82(10.3%) Black: 12 (1.7%) Mean total VD intake*: 184 IU	<u>< 40 nmol/L</u> 100 (23%) White: 81 (21.3%) Non-white†: 15 (31.9%) Black: 2 (25%) By N. glasses of milk/d None: 31/146 (21%) 0-2: 37/140 (26%) > 2: 30/149 (20%)	<u>< 40 nmol/L</u> 29 (8%) White: 23/322 (7.1%) Non-white†: 6/35 (17.1%) Black: 0/4	Significant association with serum level (only in summer): use of multivitamins and physical activity. VD intake was modestly associated with serum levels only in the summer
Gozdzik et al. (86) (2008) Toronto N=107 Test: RIA (DiaSorin)	Young adults (18-30 yrs) Community-based Excludes conditions and drugs that affect VD metabolism, recent UVB exposure Male: 50 (46%) Mean age (5th, 95th percentile): 21 yrs (18 , 25) BMI: 19.9 (15-26.6) European: 32 (30%) Asian: 59 (55%) African: 7 (6.5%) Mean total VD intake ± SD (5th, 95th percentile)*: 171.7 IU (19.7-464.3) European: 231 ± 174 (34.3, 583) East Asian: 133.4±102 (8, 311.5) South Asian: 164.3±144.3 (27.7, 391.7)	<u>< 25 nmol/L</u> 27 (25.5%) (European: 6.2%, others: 29.6-35.5) <u>< 50 nmol/L</u> 79 (73.6%) (European: 34.4%, Asian: 85-93%, African NR) p.001 (Fisher exact test) By vitamin D intake > 200 IU: 40% (n=NR) Multivariate model, factors associated with serum levels: - vitamin D intake (p<.001, explained 28.9% of variance) - skin pigmentation (p=.033, explained 4.5% of variance)		Factors associated with VD status: Age, BMI, VD intake, skin pigmentation. Only the last 2 statistically significant in linear regression analysis

BMI refers to body mass index; IU international unit; NR not reported; RIA radioimmunoassay;SD standard deviation; UVB ultraviolet B; VD vitamin D; yr year

*Includes both dietary and supplements

† Non-white ethnicity comprised of: Asians, Indo-Asians, and Native American. (85)

Table A12: Serum Vitamin D, Prevalence Studies in Canada: Elderly

Study (year) City, N	Population	% Serum Vitamin D < 50 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Vecino-Vecino et al. (87)(2006) Montreal N= 256 Test: RIA (Stillwater, Minnesota)	Sample of healthy subjects referred to ambulatory clinic Elderly (≥ 65 yrs) No known metabolic bone syndromes or drugs that affect VD metabolism Mean age: 72.8±5.6 yrs BMI: NR VD intake: NR	Only VD_3 measured < 50 nmol/L 111 (43.2%) Statistically significant difference in serum levels between the end of winter and end of fall (values not provided, p<.004)		

BMI refers to body; mass index; NR not reported; VD vitamin D; yr year.

Appendix 5: Results of Prevalence Studies in Children (General Population, Canada)

Table A13: Serum Vitamin D, Prevalence Studies in Canada: Children and Adolescents

Study (year) City, N	Population	% Serum Vitamin D < 25-50 nmol/L		Comments
		Winter-Spring	Summer - Fall	
< 25 – 27.5 nmol/L				
Mark et al. (89) (2008) Québec N=1,753 Test: RIA (DiaSorin)	Ages: 9, 13, and 16 yrs School-based sampling Boys and girls 100% French Canadian Overweight – obese: 22%	≤ 27.5 nmol/L Overall: 6.3% 9 yr-olds: 1.5% (boys and girls) 13 yrs: 10 (3.3%) (boys) 20 (7.9%) (girls) 16 yrs: 38 (12.6%) (boys) 35 (10.1%) (girls)		
Newhook et al. (82) (2009) Newfoundland and Labrador N=48 Test: liquid chromatography	Children 0-14 yrs Cross-sectional Children who had a blood test done at the hospital Patient demographics: NR	0		
Roth et al. (90) (2005) Edmonton N=68 Test: DiaSorin Incstar kit (Minnesota)	Ages 2 – 16 yrs Cross-sectional study Children who had ED visit (excludes unstable condition an use of feeding tube) Male: 68% Mean age: 9.1 ±4.5 yrs Origin: West/East Europe: 60% First Nations: 19% Median VD intake (diet): 359 (173-531)† IU/day No chronic condition/drug that affects VD metabolism	< 25 nmol/L All: 4 (5.9%) Boys: 3 (7.7%) Girls: 1 (3.4%) 2-8 yrs: 1 (2.9%) 9-16 yrs: 3 (9.1%)		Weak association VD intake-serum levels. Association between dose/kg and serum levels moderate and statistically significant, all subjects with > 18 IU/kg/day had serum levels > 40 nmol/L

Study (year) City, N	Population	% Serum Vitamin D < 25-50 nmol/L		Comments
		Winter-Spring	Summer - Fall	
< 37.5 – 40 nmol/L				
Mark et al. (89) (2008) Québec N=1,753 Test: RIA (DiaSorin)	Ages: 9-16 yrs School-based sampling Boys and girls 100% French Canadian Overweight – obese: 22%	≤ 37.5 nmol/L 9 yr olds: 10% (overall) Boys: 22 (7.8%) Girls: 36 (13%) p=NS 13 yrs:30% (overall) Boys: 75 (25.5%) Girls: 86 (34.5%) p=.02 16 yrs: 34% (overall) Boys: 115 (37.8%) Girls: 104 (29.9%) p= .016 P< .0001 among age groups		
Roth et al. (108) (2005) Edmonton N=68 Test: DiaSorin Incstar kit (Minnesota)	Ages 2 – 16 yrs Cross-sectional study Children who had ED visit (excludes unstable condition an use of feeding tube) Male: 68% Mean age: 9.1 ±4.5 yrs Origin: West/East Europe: 60% First Nations: 19% Median VD intake (IQR) (diet†): 359 (173-531) IU/day 2-8yrs: 332.4 (183 , 417) IU 9-16 yrs: 421.2 (140 , 598) IU % < 200 IU/day: 2-8 yrs: 9 (26%) 9-16 yrs: 11 (33%) For boys and girls: No chronic condition/drug that affects VD metabolism	< 40 nmol/L All: 23 (34%) Boys: 16 (41%) Girls: 7 (24%) 2-8 yrs: 6 (17%) 9-16 yrs: 17 (52%) p<.01 between age groups		VD intake was the only statistically significant factor associated with serum levels in multivariate analysis

ED emergency department ; IQR interquartile range; IU international units ; NR not reported; NS not statistically significant; RIA radioimmunoassay; VD vitamin D ; yr year

† Median dietary vitamin D intake obtained through the 7-day recall Food Frequency Questionnaire (FFQ). Median intake obtained through the 24-hour recall (weekday) was 253 IU/day (116 – 520)

Table A14: Serum Vitamin D, Prevalence Studies in Canada: Infants

Study (year) City, N	Population	% Serum Vitamin D < 50 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Maguire et al. (91) (2009) Toronto N=92	Infants 24-30 months old. Children attending a routine (healthy) physician visit at a community-based practice.	November – June < 50 nmol/L 29 (32%)		Factors possibly associated with lower levels: lower milk consumption, higher BMI, eating snacks while watching TV Not associated with skin pigmentation, breastfeeding without VD supplementation, time spent outdoors, TV viewing time.

BMI refers to body mass index; VD vitamin D

Table A15: Serum Vitamin D, Prevalence Studies in Canada: Newborn

Study (year) City, N	Population	% Serum Vitamin D < 25-27.5 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Weiler et al. (88) (2005) Winnipeg 49.54°N N=50 Test: RIA (DiaSorin)	Cross-sectional study Term newborn Sample from cord blood of children born in the hospital Boys: 29 (58%) White: 30 (60%) Mothers taking VD supplement: 78% Mean VD intake (mothers): VD deficient: 149±145 IU Adequate VD level: 242±218 IU	< 27.5 nmol/L Overall: 18 (36%) Lighter skin pigmentation: 6/30 (20%) Darker skin pigmentation:12/20 (60%) VD-deficient mothers Use of supplements: 14/23 (61%) Lighter skin pigmentation: 53.3% VD deficient infant: 16 (70%) Mothers with adequate serum levels: Use of supplements: 25/27 (93%) Lighter skin pigmentation: 81.5% VD deficient infant: 2 (7%)		
		< 27.5 nmol/L 46% (October-March)	< 27.5 nmol/L 19.4% (April-September)	
Newhook et al. (82) (2009) Newfoundland and Labrador (Avalon Peninsula) N=51 Test: liquid chromatography	Cross-sectional study Newborn Sample from cord blood of children born in the hospital VD supplementation (mothers): NR	< 25 nmol/L 2 (3.9%) < 50 nmol/L 18 (35.3%)		

IU refers to international unit; NR not reported; RIA radioimmunoassay; VD vitamin D

Table A16: Incidence of Vitamin D Deficient Rickets in Canadian Children

Author, Year Study design	Population characteristics	# Confirmed cases	Comments
Ward et al. (27) (2007) Canada N= 104 cases Surveillance study (Canadian Paediatric Surveillance Program) VD-deficient rickets	July 2002 – June 2004 Cases reported to 2,325 pediatricians and pediatric subspecialists (84.5% response rate) Confirmed cases† Inclusion criteria: Children 1-18 years old 25(OH)D < 27.5 nmol/L or > 27.5 nmol/L in absence of isolated dietary calcium-deficient rickets Receiving VD therapy before serum test and confirmed intake of age-specific reference intake of calcium (diet or supplements) Excluded if VD-deficient rickets associated with underlying diseases	VD-deficient rickets 104 cases 2.9/100,000 (95% CI: 2.2 , 3.7) Intermediate - darker skin: 92 (89%) Black: 34 (33%) Middle-Eastern: 13 (13%) First Nations: 12 (12%) Inuit: 11 (11%) Caucasian, Latin American, Asian: 1 (1%) each Not reported in 15 cases Breast-fed: 98 (94%) Immigrant to Canada: 25 (24%) Characteristics of the mothers Mean age: 28 yrs (15-39) Wearing a head cover: 21 (20%) VD supplementation: During pregnancy: 13 (13%) After delivery: 5 (5%) Drinking milk before or after delivery: 25 (24%)	Only pediatricians surveyed. According to the authors, in more remote areas rickets may be diagnosed by family doctors, which were excluded from the study – may result in underestimate of the number of cases No cases of rickets in children receiving ≥ 400 IU VD supplementation / day

IU refers to international unit; VD vitamin D

† Rickets were confirmed by radiographic signs at the wrist or knee by a radiologist. Serum levels of calcium, phosphate, alkaline phosphatase, parathyroid hormone, and 1,25-dihydroxyvitamin D were included if available.

Appendix 6: Results of Prevalence Studies in Adults and Children with Kidney Disease (Canada/US)

Table A17: Serum Vitamin D, Prevalence Studies in Canada: Adults with Kidney Disease

Study (year) City, N	Population	% < 37.5 – 40 nmol/L		Comments
		Winter-Spring	Summer - Fall	
Rucker et al. (93) (2009) Edmonton (53°30'N) N=128 Test: liquid chromatography	CKD stages 3-5 (patients of the Alberta Renal Insufficiency Clinic) GFR < 30 ml/min Mean age: 67-71 yrs Male: 57% BMI: 29 VD intake < 400 IU by study design	Not reported	Spring-summer < 37.5 nmol/L 49 (38%) Mean ± (nmol/L) 16-21.6 ±5-9	Part of RCT comparing use of 1,000 IU/ day vs. controls. Baseline results reported here.
Boudville et al. (94) (2006) London (ON) (43°2'N) N=419 Test: RIA (DiaSorin)	Renal transplantation Men: 264 (63%) Mean age: 51±15 yrs Time since transplant: 7.2 ±6.4 yrs White: > 95% (from Tripathi et al.(109)) VD supplementation not recommended at the time (93% not using)	< 40 nmol/L 114 (27.3%)† Mean 57.3 ± 26.2 nmol/L	Not reported	

BMI body mass index ; GFR glomerular filtration rate ; min minute ; NR not reported; RCT randomized controlled trial ; RIA radioimmunoassay ; VD vitamin D ; yr year

† It was assumed that baseline levels were measured between December and March, as this was the time of recruitment.

Table A18: Serum Vitamin D, Prevalence Studies in Children with Kidney Disease (US)

Study (year) Location, Test used	Study / population characteristics	% < 37.5 – 50 nmol/L				Comments
		Winter	Spring	Summer	Fall	
Ali et al. (96) (2009) Chicago N= 88 (2005-2006) N=79-336/yr (1987-1996) Test: RIA (DiaSorin)	Chronic kidney disease stages 1-5 Age: NR Caucasian: 38% Hispanics: 37% African American: 24%	<u>< 37.5 nmol/L</u> 1987-1996 20-75% depending on yr (most values 30-60%) 2005-2006 34 (39%) Black and Hispanic patients had higher prevalence				Not broken down by disease stage
Seeherunvong et al. (97) (2009) United States, Florida (25°N) N=258	CKD stages 1-5 Children attending outpatient dialysis clinic Excluded: chronic liver disease, gastrointestinal malabsorption, anticonvulsants' use Mean age: 12.3 ±5.2 yrs Male: 48% Caucasian: 49.6% BMI: 60th percentile	January – June <u>< 50 nmol/L</u> 72 (28%)				
Menon et al. (95) (2008) United States, Michigan N=57	Children and adolescents seen at the chronic renal insufficiency clinic, eGFR < 75 ml/min/1.73 m2 CKD stages2-4 Mean age: 10.7 ±5.4 yrs Male: 70.2%s Mean GFR: 50.9 ±16.4 ml/min/1.73 m2 Caucasian: 44% African American: 49%	< 37.5 nmol/L (VD_3) 12 (21.1%)				

CKD refers to chronic kidney disease; eGFR estimated glomerular filtration rate ; NR not reported; RIA radioimmunoassay ; VD vitamin D; yr year

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