Vitamin D

A review prepared for the New Zealand Food Safety Authority and the Ministry of Health

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Abbreviations

$1,25(OH)_2D_3$	calcitriol
$25(OH)D_3$	calidiol
7-DHC	7-dehydrocholesterol
ALP	alkaline phosphatase
BMC	bone mineral content
BMD	bone mineral density
BSAP	bone specific alkaline phosphatase
CI	confidence interval
CPB	competitive protein binding
CT	computer tomography
CTx	C-telopeptide of collagen cross-links
DEXA	dual energy x-ray absorptiometry
DPA	dual photon absorptiometry
Dpd	deoxy-pyridinolines
EPA (US)	Environmental Protection Agency (United States)
HPLC	high performance liquid chromatography
ICTP	cross-linked C-telopeptide of type 1 collagen
IDDM	insulin dependent diabetes mellitus
IU	international unit
MS	multiple sclerosis
NHANES (US)	National Health and Nutrition Examination Survey (United States)
NIDDM	Non-insulin dependant diabetes
NTx	N-telopeptide of collagen cross-links
OC	osteocalcin
PCIP	carboxyterminal propeptide of type 1 collagen
PINP	aminoterminal propeptide of type 1 collagen
Pyd	pyridinolines
PSA	prostate specific antigen
PTH	parathyroid hormone
QUS	quantitative ultrasound
RCT	randomized controlled trial
RIA	radioassay
RNI (UK)	recommended nutrient intake (United Kingdom)
SPA	single photon absorptiometry
TRAP	tartrate-resistant acid phosphatase
UL	upper level
US	United States
USSR	Union of Soviet Socialist Republic
UVB	ultra violet B radiation
VDR	vitamin D receptor

Introduction

Cholecalciferol, or 'vitamin' D as it is commonly referred to, is a popular research topic. While it has long been known that vitamin D deficiency causes rickets in children, there is now renewed interest in this pro-hormone as potential health roles for vitamin D increase, with a concomitant rise in prevalence estimates for insufficiency. An informed discussion on vitamin D by public health professionals is now especially relevant because of recent evidence that many New Zealanders have low vitamin D status (1, 2).

The following literature review encompasses a summary of vitamin D metabolism and its determinants, the current areas of research into the impacts of vitamin D on health, both in the well-described role in skeletal health, and in the more uncertain roles it may play in other aspects of health, such as certain cancers and autoimmune disease. The means to defining vitamin D status are discussed, followed by a review of the major national surveys on vitamin D status abroad and in New Zealand. Finally, international experience with strategies for improving vitamin D status are discussed and related to the New Zealand situation.

1 Vitamin D Metabolism

The major physiologically relevant forms of vitamin D are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_2 is a less common form of vitamin D, produced by the action of ultraviolet light on the plant steroid ergosterol. Vitamin D_3 is synthesised through the action of ultraviolet B (UVB) light on 7-dehydrocholesterol, which is distributed throughout the epidermis and dermis in the skin of animals (3, 4). Upon exposure of skin to sunlight in the UVB range (290-315nm), 7-dehydrocholesterol is converted to previtamin D_3 and further rearranged in a thermally induced reaction to vitamin D_3 (5). Vitamin D_2 and vitamin D_3 were believed to have equal bioavailability, but vitamin D_3 may have greater potency (6, 7).

Vitamin D is found naturally in few foods. Major natural food sources of vitamin D are limited to the oil and flesh of fatty fish and animal liver. In countries where it is permitted, foods

fortified with vitamin D_2, such as milks, margarines and cereals are major contributors to vitamin D intake (3, 8). Vitamin D_2 is also the form most widely used in pharmaceutical preparations (9).

Vitamin D (D_3 or D_2) from sunlight or dietary sources is biologically inert, and is hydroxylated twice before it becomes metabolically active. It enters the circulation from the skin or the lymph via the thoracic duct and is transported (by vitamin D- binding protein) to the liver and hydroxylated to 25-hydroxyvitamin D, the major circulating form of the vitamin (10) **(Figure 1)**. The second hydroxylation occurs in the kidneys to form the biologically active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol). This second hydroxylation is tightly regulated under the control of serum phosphorus levels and parathyroid hormone which is released in response to the body's demand for calcium and phosphorous. The conversion of 25-hydroxyvitamin D to calcitriol was first believed to occur only in the kidney. However, more recent studies have shown that other tissues in the body, the prostate and colon, can express 25-hydroxyvitamin D -1$\propto$-hydroxylase and thus also produce calcitriol (11-13).

Receptors for calcitriol (also known as Vitamin D receptors, or VDR) are located in more than 30 tissues including the intestine, bone, and kidney (10). The best-described actions of calcitriol are calciotropic, incorporating increased calcium and phosphate absorption in the small intestine, maintenance of calcium homeostasis in the extracellular fluid, and bone resorption (14). Other actions of vitamin D are paracrine, involving local synthesis of calcitriol that appear to be important for cell cycle functions, immune function, and proliferation (15).

2 Markers of vitamin D status and bone metabolism

2.1 Calcitriol (1, 25 Dihydroxyvitamin D_3)

In the intestine, calcitriol enhances absorption of calcium and phosphorus from the diet. In the bone marrow, calcitriol initiates the transformation of monocytic stem cells to mature osteoclasts, which are involved in bone resorption. In this way, calcitriol regulates serum calcium concentrations. Circulating calcitriol concentrations should not be used to assess vitamin D status because the half-life of calcitriol is less than four hours. Furthermore, with mild vitamin D

deficiency, blood concentrations of calcitriol remain normal due to tight regulation of this form of vitamin D. Concentrations of calcitriol may rise and then fall only as vitamin D deficiency becomes more severe (9, 16).

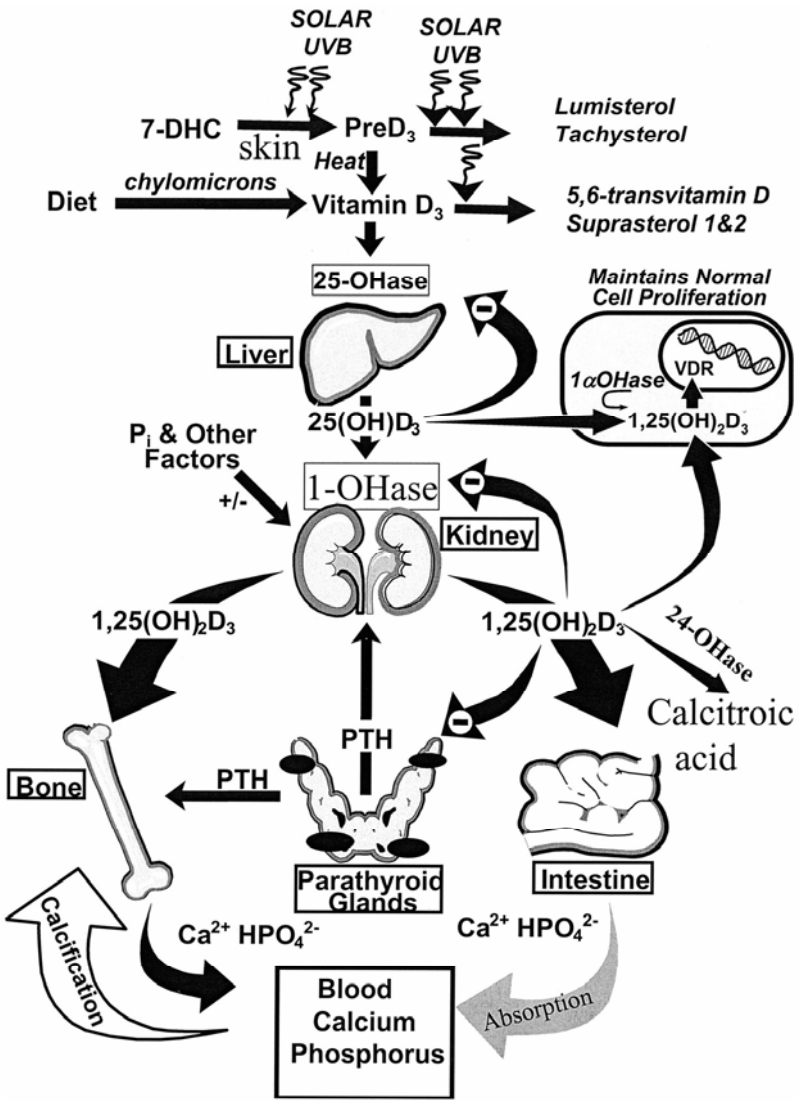

Figure 1. Schematic diagram of cutaneous production of vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth (16).

2.2 Calcidiol (25-Hydroxyvitamin D)

Plasma or serum 25-hydroxyvitamin D is the best indicator of vitamin D status and reflects vitamin D derived from both dietary intake and sunlight exposure (17, 18). Circulating 25-

hydroxyvitamin D is a transport form of vitamin D and it is also a direct measure of stores. The two most popular methods for determining 25-hydroxyvitamin D are the competitive protein–binding (CPB) assay or radioimmunoassay (RIA). However, an international comparison of methods for vitamin D determination reported the CPB assay to measure higher than RIA in the lower range of measurement (19). With the exception of HPLC (a method which is costly and requires a large sample volume), clinical assays do not differentiate between vitamin D_2 and D_3 in serum, and reported 25- hydroxyvitamin D concentrations are generally a total of the two forms (9). However, some immunoassays may underestimate total 25-hydroxyvitamin D because of a lesser reactivity to vitamin D_2 (19). Total 25-hydroxyvitamin D may therefore be underestimated in persons whose vitamin D source is largely from fortified foods and/or supplements, when compared to those whose major vitamin D source is from sun exposure. Comparisons between studies that use different assays are somewhat impeded, adding fuel to the current debate about what is the most appropriate serum concentration of 25-hydroxyvitamin D to use as a cut-off value to define vitamin D deficiency (discussed further in section 3).

2.3 Parathyroid hormone

Parathyroid hormone can be measured from serum or plasma using RIA (9). Serum PTH is useful for assessing vitamin D status because PTH is indirectly associated with 25-hydroxyvitamin D, in its response to circulating calcium levels (9). However, PTH should be measured together with 25-hydroxyvitamin D because factors other than lack of vitamin D may increase PTH concentrations (such as low calcium intake or primary hyperparathyroidism), or prevent their increase (for example physical inactivity) (20). An elevated PTH in conjunction with a low 25-hydroxyvitamin D concentration provides a good indication of the body trying to mobilize calcium from bone in an attempt to maintain plasma calcium levels. If prolonged, this condition, known as secondary hyperparathyroidism, leads to bone demineralization (21). PTH is often used as a means to defining cut-offs for 25-hydroxyvitamin D, though the majority of these studies have been in elderly populations and may not be relevant for younger age groups, particularly children.

2.4 Markers of bone turnover

In order to keep bone healthy and robust, bone is constantly being broken down (or resorbed) by cells called osteoclasts and reformed by cells called osteoblasts. Bone mass remains stable when the activities of these two types of cells are evenly matched. For healthy young adults in a 'normal' state, bone formation and resorption are in balance. However, disease states, aging, or increased/decreased activity levels can offset this balance (22). Regulation of this bone remodelling process is a complex system involving many hormones and chemical messengers, some of which can be used as biochemical markers of bone turnover (23), and provide useful information when used in conjunction with 25-hydroxyvitamin D and PTH. Some currently available biomarkers of bone formation and resorption are given in Table 1.1, although clinically relevant cut-offs for these biomarkers have not been established for all (24).

2.4.1 Alkaline phosphatase

Alkaline phosphatase (ALP) is produced by a variety of tissues in the body. Bone specific alkaline phosphatase (BSAP) is a specific product of osteoblasts; it is therefore used as a marker for bone turnover, though there is 15-20% cross-reactivity between liver alkaline phosphatase and BSAP. As a result, some studies use the cheaper method for total ALP, which, once liver disease is ruled out, provides a fair estimation of bone formation. However, BSAP has a higher specificity and is can detect smaller changes in bone turnover (22). ALP is generally normal in osteoporosis, but elevated with vitamin D deficiency and is recommended mainly for a clinical confirmation of the diagnosis (9).

2.4.2 Osteocalcin

Osteocalcin is a protein produced by osteoblasts, odontoblasts and hypertrophic condrocytes, and is involved in bone mineralisation. However its precise function is not yet determined. Its use is limited due its low specificity and the lack of comparability between different assays (22).

Table 1.1 Currently available biomarkers of bone turnover

Formation	**Also influenced by:**
Serum	
Bone specific alkaline phosphatase (BSAP)	Liver function
Osteocalcin (OC)	Renal function, haemolysis
Carboxyterminal propeptide of type I collagen (PCIP)	Liver function
Aminoterminal propeptide of type I collagen (PINP)	Liver function
Resorption	
Urine	
Hydroxyproline	Liver function, diet, inflammation
Free and total pyridinolines (Pyd)	Liver function, active arthritis, UV radiation
Free and total deoxy-pyridinolines (Dpd)	Liver function, UV radiation
N-telopeptide of collagen cross-links (NTx)	Liver function
C-telopeptide of collagen cross-links (CTx)	Renal function, liver function
Serum	
Cross-linked C-telopeptide of type I collagen (ICTP)	Renal function, liver function
Tartrate-resistant acid phosphatase (TRACP)	Haemolysis, blood clotting
N-telopeptide of collagen cross-links (NTx)	Liver function
C-telopeptide of collagen cross-links (CTx)	Renal function, liver function

2.5 Bone densiometry

Bone mass is usually expressed as bone mineral content (BMC), which refers to the amount of mineral in a length of bone (g/cm), or expressed as bone mineral density (BMD). Biopsies of bone can be analysed to ascertain 'true' bone density or material BMD. Material BMD (bone mass/volume) is a reflection of the degree of bone mineralisation in the organic bone matrix (25). This method however, is often impractical for the research setting.

A variety of non-invasive methods now exist for measuring what is known as compartment bone density. These non-invasive methods included single and dual-photon absorptiometry (SPA and DPA, respectively), dual energy x-ray absorptiometry (DEXA), quantitative computer tomography (CT) and quantitative ultrasound (QUS), which vary in their accuracy and precision (26). Measures of compartment BMD tend to overestimate bone volume in the calculation of bone density because they do not differentiate between bone mineral and marrow spaces (in trabecular bone) and osteonal canals (in cortical bone), which are known to vary with the extent of remodelling activity (25).

BMD measured by DEXA refers to areal BMD (g/cm^2) (aBMD), which is the ratio of BMC: projected area of bone. This measurement is inappropriate for longitudinal studies in children, as it fails to take into account the age-related increase in bone thickness during growth. Additionally, population variance in bone structure means that caution should be exercised when comparing different studies using aBMD (25). However, aBMD is a useful and frequently used tool for comparing within groups, and an argument made for its continuing use is the high correlation it has with bone strength (27, 28), which is applicable in studies of osteoporosis. Bone density predicts approximately half the risk of osteoporotic fracture (29).

To account for some of the size-related differences in aBMD, it has been recommended that bone area, weight and height are incorporated into all regression models of BMC (30). An alternative adjustment is to calculate volumetric or apparent BMD (g/cm^3) (vBMD), which is based on the assumption that the measured site is cylindrical (31).

3 The Consequences of Low Vitamin D Status

3.1 Non-Skeletal Health

The presence of 1,25-dihydroxyvitamin D_3 in numerous tissues of the body, including the pancreas (32), the ability of some tissues other than the liver to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (33), and the recent discovery of VDRs in tissues such as the parathyroid gland, skin, thymus and ovarian cells (32) supports the notion that vitamin D has functions unrelated to the skeleton. However, this is an emerging area of research and at present many of these functions are not well understood. A lower incidence of certain diseases such as specific types of cancer, diabetes and multiple sclerosis, in regions of low latitude or greater UV exposure, indirectly supports a role of low vitamin D status in the aetiology of certain diseases because UV exposure, which is affected by latitude, is a determinant of vitamin D status (Section 5.1). However, studies using UV exposure or latitude as a proxy for vitamin D status, should be interpreted with caution as vitamin D status has a number of other determinants (see section 6), and disease risk factors, such as diet, can vary by geographic region (34, 35).

A number of studies attempt to relate dietary vitamin D to various non-skeletal diseases. For example, there is uncertainty with regards to the protective effect of dietary vitamin D against cancers, because of both their multi-factorial etiology and because the contribution of diet to vitamin D status is usually small, relative to sunlight, with a comparatively small variation in intake within a population. Furthermore, dietary intake of vitamin D is notoriously hard to measure; food composition databases for the vitamin D content of foods is often presumed or derived from other sources (36), and the actual content of fortified foods can vary considerably from the label declaration (37-39). Unfortunately, there are very few studies to date of sufficient quality that provide substantial evidence for a role of vitamin D in diseases unrelated to the skeleton.

3.1.1 Cancer

Although a positive association exists between sunlight exposure and skin cancers such as melanoma (40-42), there is a negative association between direct or inferred measures of UV exposure and cancers of the colon, breast, prostate, and lymph system, suggesting a role of vitamin D in these cancers. Ecologic, case control and prospective cohort studies associating non-skin cancer incidence or mortality and indices of UV exposure are summarized in Table 1.2 and 1.3. As

early as 1941, Apperly (43) reported an association between geographic location and cancer mortality. Since then, cancer mortality has been shown to be higher with increasing distance from the equator (44, 45), or with decreased UV-B exposure (46). A large number of studies have also examined the association between vitamin D intake and cancer (Tables 1.4-1.7), with mixed results. While cancers of the bladder, ovary and non-Hodgkin's lymphoma have been associated with sun exposure or latitude (40, 41, 46-49), this review focuses on the three cancers that have been most studied: colorectal, breast and prostate cancer.

3.1.1.1 Colorectal cancers

Of all cancers, the strongest evidence for a protective effect of vitamin D appears to be for colorectal cancers. Garland (45) and Grant (46) have reported that risk of colon cancer mortality is significantly lower in areas of greater solar radiation. Acid haze air pollution can limit UV exposure at ground level. An analysis of sulfur dioxide and ultraviolet-light-blocking aerosols in 20 Canadian cities, found that population exposed to greater pollution had higher rates of both colon and breast cancer (50). Incidence rates of colon and rectal cancer among men from 1973-84, were shown to be associated with regions that receive lower levels of solar radiation (51); a similar trend was observed in women for colon but not rectal cancer. Later, researchers from the National Cancer Institute (NCI) found that colon cancer mortality in 24 of the United States states, from 1984-95, was inversely associated with residential and occupational exposure to solar radiation (41). It is important to bear in mind that UV exposure is only a proxy for vitamin D status and that there may be benefits of UV unrelated to vitamin D.

Significant negative associations between vitamin D intake and colorectal cancer have been found in some (52-58), though not all (55, 59-66), descriptive studies (Table 1.5). In a cohort study, 1954 men who had completed 28-day dietary histories were followed up for 19yrs. Men within the lowest quartile (2-30IU/kcal) of dietary intakes of vitamin D had twice the risk of developing colon cancer than those in the highest quartile (75-208IU/kcal) (52). In the Nurses Health Study, 501 cases of colorectal cancer were reported in a cohort of 89,448 women followed for eight years (57). Long-term nutrient intake was quantified using semi-quantitative food frequency questionnaires (FFQs) in 1980, 1984 and 1986; nutrient intakes were classified into quintiles.

Table 1.2 Ecologic studies relating geographic region or UV exposure, and various cancers

Type of Cancer	Author, Yr,	Location, time span, Incidence or Mortality	Index of region/UV	Associations/ Main findings
Numerous cancers	Gorham, 1989 (50)	Canada, 20 cities 1966-76 (M)	Average annual total solar energy (cal/cm^2/d) Average ambient winter sulphur dioxide concentrations (1974)	Total solar radiation: NS Sulphur dioxide and haze coefficients: Colon m: r=0.61 P=0.03; f: r=0.74 P=0.003 Breast f: r=0.69 P=0.007 Lung m: r=0.79 P=0.001; f: NS Oral m: r=0.77 P=0.001 Stomach m: NS; f: r=0.58 P=0.04 8 other cancers, NS
	Doll, 1991 (67)	13 populations worldwide (I or M)	Urban *vs.* rural residence (ratio)	*Extreme urban excess:* Lung, liver, larynx, mouth and pharynx, bladder, oesophagus *Moderate urban excesses*: colon, rectum, cervix, kidney, breast, ovary, brain, pancreas *Little urban excess:* Hodgkin's disease, testis, stomach, melanoma, other skin, corpus uteri, prostate, leukemia, Non-Hodgkin's Lymphoma
	Grant, 2002 (46)	US, 1970-94, (M)	UV-B data for July 1992	Inversely correlation for 8 types of cancer in men and 9 cancer types in women, (all p<0.001) (data for white Americans) Breast r= -0.67 (f) Colon r= -0.62 (m), r= -0.63 (f) Ovary r= -0.63 (f) Prostate r= -0.32 (m) Bladder r= -0.57 (m), r= -0.40 (f) An estimated 23,600 'premature' cancer deaths from insufficient UV-B
Colon	Garland, 1980 (45)	US, 1959-61 (M)	Average annual solar radiation gm-cal/cm^2/d Metropolitan *vs.* non-metro	17 metropolitan states, white m: r = -0.9 32 non-metropolitan states, white m: r = -0.6 (age adjusted mortality rates)
Colon & rectal	Emerson, 1992 (51)	US, 9 centers (I)	Solar radiation	m: 50-80% increase with decreasing radiation f: NS

Breast	Gorham, 1990 (68)	USSR, 15 republics 1969-71 (I)	Average annual total solar energy (cal/cm^2/d)	r = -0.75, P = 0.001
	Garland, 1990 (69)	US, 87 urban regions (M)	Average annual total solar energy (cal/cm^2/d)	r = -0.80, P = 0.0001
	Morabia, 1992 (70)	USSR, 15 republics, 1986 (I)	Average annual solar energy (cal/cm^2/d)	r = -0.81, P < 0.001
	Grant, 2002 (71)	35 countries world-wide; 1989-96 (M)	Latitude	r= 0.66 P < 0.001. When E. Europe omitted: r= 0.78, P < 0.001 When Scandinavian countries omitted: r= 0.73, P < 0.001
	Sturgeon, 1995 (34)	US by geographic region, 1987 (M)	4 regions: S, W, Mid-W & NE	No difference in rates after adjustment for regional differences in established risk factors. NB regions do not correspond to latitude.
	Sturgeon, 2004 (59)	US by geographic region, 1950-99 (M)	4 regions: S, W, Mid-W & NE	NE *cf* South 1950-1959: RR= 1.48; 1990-99: RR= 1.15 Authors concluded that the historically lower breast cancer mortality rates in the South have been diminishing, via relatively less favorable trends in the South.
Prostate	Hanchette, 1992 (72)	US, 3073 counties (M)	*UV count* (altitude & latitude); *Epidemiologic index* (cloud cover & latitude); *Latitude*	*UV count:* r =-0.15, r^2 =0.02 P=0.0001 *Epidemiologic Index:* r =-0.25 r^2 =0.06 P=0.0002 *Latitude*: r =0.19 r^2 =0.04 P=0.0001
Ovarian	Lefkowitz (48)	US main city in 100 counties; 1979-88 (M)	Average annual sunlight, ozone thickness & sulphur dioxide pollution	*Sunlight* r= -0.42 P < 0.001 *Ozone* r= 0.42 P < 0.001 *Sulphur dioxide* r = 0.51 P<0.01 (all age adjusted)
Non-Hodgkin's Lymphoma	Hartge, 1996 (49)	US by state economic area, 1970-89 (M)	Latitude; UV radiation adjusted for latitude, altitude & cloud cover ('RB unit')	m: Each additional RB unit corresponded to a 0.16% lower rate of NHL mortality P <0.0001 f: similar findings, 0.17% P <0.0001

(I) Cancer Incidence (M) Cancer Mortality
f Female m Male

Table 1.3 Descriptive (case-control and cohort) studies associating geographic region or UV exposure, and various cancers

Type of Cancer	Author, Yr	Location; Study Design	Participants	Index of region/UV	Main Findings
Numerous cancers	Freedman, 2002 (41)	Case-control, 1984-95; 24 US States (M)	m&f: 427212 cases, matched to controls,	Low, Med & High exposure estimated by residence at birth and usual occupation (indoor, mixed, outdoor, farm), recorded on death certificate.	OR (95% CI) Residence (*cf* 'Low'): Breast: Med. 0.84 (0.82 – 0.86) High 0.74 (0.72 – 0.76) Prostate: Med. 0.89 (0.86 – 0.91) High 0.90 (0.87 – 0.93) Colon: Med. 0.90 (0.88 – 0.92) High 0.73 (0.71 – 0.74) Ovary: Med. 0.90 (0.87 – 0.93) High 0.84 (0.81 – 0.88) Occupation: ('Outdoor' *cf* 'Indoor'): Breast: 0.82 (0.70 – 0.97) Prostate: 1.00 (0.96 – 1.05) Colon: 0.90 (0.86 – 0.94) Ovary: 0.94 (0.75 – 1.17)
Breast	John, 1999 (73)	Prospective cohort; NHANES I 1971-75, follow-up in 1992; US (I)	f: 5009 ~20yrs, white. (Incl. 190 cases)	Occupational and recreational sun exposure (3 grps); sun-induced skin damage (4 grps); & region of residence (4 regions)	Sun exposure, age-adj RR (95% CI) 0.50 (0.29- 0.86), P for trend = 0.01 Region South *vs.* NE: 0.66 (0.44-1.00), P for trend = 0.06
	Hansen, 2001 (74)	Population-based case-control; Denmark (I)	f: 7035, 30-54yrs & age-matched controls	Employment history	OR (95% CI) = 1.5 (1.2-1.7)
Prostate	Luscombe, 2001 (75)	Case-control; UK (I)	210 sporadic prostate cancer; 155 controls (benign prostatic hypertrophy)	Lifetime UV exposure questionnaire;	Lowest quartile of exposure: OR (99% CI) = 3.03 (1.59-5.78) P = 0.008
Bladder	Michaud, 2001	12yrs prospective	m: 49455 US health	Zip code and urban *vs.*	all adj. for smoking, RR (95% CI):

	(47)	study; US (I)	professionals; 40-75yrs (incl. 328 cases)	rural	NE *vs.* W of US, 1.65 (1.18-2.30) Strictly urban, NE *vs.* W of US, 199 (1.23-3.20) Long residence in NE *vs.* W of US 1.77 (1.15-2.71)
Non-Hodgkin's Lymphoma	Freedman, 1997 (40)	Case-control, 1984-91; 24 US states (M)	m&f: 24 state mortality database. Controls 2:1 selected from non-cancer deaths.	Residence at birth and usual occupation recorded on death certificate.	Sun exposure by residence (High *cf* 'Low'): OR (95% CI) = 0.83 (0.81-0.86) Sun exposure by occupation (outdoor non-farm workers *cf* 'Indoor'): OR = 0.88 (0.81-0.96)

(I) Cancer Incidence
(M) Cancer Mortality
m male f female

Table 1.4 Descriptive studies associating vitamin D intake & colorectal cancer incidence, or adenoma recurrence

Author, Yr	Study Design	Participant characteristics	Measured variables for vit D intake & cancer	Main Findings
Ferraroni, 1994 (63)	Hospital based case-control; Italy	m&f, 828 colon & 498 rectal cancer, 2024 controls, 19-74yrs	FFQ (diet); Colon & rectal	NS
La Vecchia, 1997 (58)	Continuation of (63), see above	m&f, 1225 colon & 728 rectal cancers, 4,154 controls, 23-74yrs	FFQ (diet); Colon & rectal	*Diet*, high *vs.* low quintiles, OR (95% CI) Multivariate adjusted; Colorectal 0.77 (0.6-0.9) Women 0.73 (0.6-0.9), Men 1.00 (0.9-1.1); All: Colon 0.81 (0.7-0.9); Rectal 1.03 (0.9-1.2)
Peters, 1992 (66),	Case control; LA, US	m&f, 746 cases 746 controls, 45-69yrs	FFQ, Suppl; Colon	NS
Pritchard, 1996 (56)	Population-based case-control, 1986-88; Sweden	m&f, 352 colon, 217 rectal cancers, ~67yrs	FFQ (diet) average over past 5yrs; Colon & rectal	*Diet*, high *vs.* low quartiles, OR (95% CI) Colon 0.6 (0.4-1.0) Rectal 0.5 (0.3-0.9)
Marcus, 1998 (76)	Population based case-control; Wisconsin, US	f, 348 colon & 164 rectal, 678 controls, <74yrs	FFQ (diet) 2y prev, Suppl., Colon & rectal	NS
Kampman, 200 (65)	Case-control; Utah, US	m&f, 1,993 colon cancer, 2,410 controls, ~65yrs	FFQ (diet), Suppl. (Y/N); Colon	NS
Levine, 2001 (77)	Screening sigmoidoscopy study. Case control, US	m&f, 55-74yrs 467 cases, 500 controls	FFQ Colorectal adenomas	NS
Garland, 1985 (52)	Prospective cohort 1957-78; Chicago, US	m, 1954, white, 40-55yrs at baseline	28-d diet histories (Vit D & Ca combined); Colorectal	Age-adjusted incidence rates, low 30.7/1000 *vs.* high 16.4/1000 quartiles of intake, $p \leq 0.05$
Heilbrun, 1985 (64)	Nested case-control 13-16yrs follow-up; Hawaii	m, Japanese n=8006, 100 colon 7 59 rectal	24-hr recall	NS
Bostick, 1993 (78)	Prospective cohort, 1986-90; Iowa, US.	f, 35216, 212 cases, 55-69yrs	FFQ (diet), Suppl.; Colon	RR (95% CI) *Total* intake, high *vs.* low quintiles: age adjusted: 0.54 (0.35-0.84) multivariate adjustment: 0.73 (0.45-1.18)

				Supplements, high (>400IU/d) *vs.* low (0IU/d): age adjusted: 0.56 (0.34-0.92) multivariate adjustment: 0.67 (0.40-1.13)
Kearney, 1996 (79)	Health Professionals Follow-up Study. Prospective cohort, 1986-92; US	m, 47935 professionals, 203 cases, 40-75yrs	FFQ (diet), Suppl.; Colon	*Total* intake, high *vs.* low quintiles, RR (95% CI): adjusted for age & TE intake 0.54 (0.34-0.85) multivariate adjustment 0.66 (0.42-1.05)
Martinez, 1996 (57)	Nurses Health Study, Prospective cohort, 1980-88; US	f, 89448 nurses; 396 colon & 105 rectal	3xFFQ (diet), Suppl.; Colon & rectal	High *vs.* low quintiles of intake, RR (95% CI) *Diet* 0.72 (0.34-1.54), *Total* 0.42 (0.19-0.91)
McCullough, 2003 (54)	Cancer Prevention Study II Nutrition Cohort, 1992-97; US	m, 60866, 66,883 f, 50-74yrs (421 & 262 cases)	FFQ (diet), Suppl; Colon & rectal	*Total* intake, high>525 *vs.* low<110IU/d quintiles; Multivariate adjusted, RR (95% CI), P for trend Men colorectal 0.71 (0.51-0.98) P=0.002 colon 0.58 (0.39-0.86) P<0.001 rectum 1.01 (0.54-1.88) NS Women all NS
Lin, 2005 (61)	Prospective cohort, US Women's Health Study, (10yrs follow-up)	f, 36976, ≥45yrs 223 cases	FFQ; Colorectal	NS
Lieberman, 2003 (53)	Prospective cross-sectional study; 1994-97	3121 patients 50-74yrs (97% m); 329 cases, 1441 controls	FFQ (total); Polyps *vs.* none (colonoscopy)	*Total* intake, high > 644.9IU/d *vs.* low < 179.5IU/d quintiles , OR (95% CI) = 0.61(0.39-0.97)
Martinez, 2002 (62)	Wheat Bran Fibre Trial; US. Reexamined as case-control study.	m&f, 1034, 40-80yrs, with previous adenoma(s) removal	FFQ (total) ; Adenoma recurrence	*Total* intake, high >455IU/d *vs.* low <86IU/d quartiles, NS
Hartman, 2005 (55)	Multi-center Polyp Prevention Trial; 1991-94, US. Reexamined as cohort	m&f, 2079, ≥35yrs, with ≥1 confirmed colorectal adenoma	FFQ (total) BL+ annual, 4DDR subset, use; Adenoma recurrence	*Diet*: NS *Total,* high >11.7µg/d *vs.* low <3.35µg/d quintiles OR = 0.84, P for trend = 0.03

m male, f female

Table 1.5 Studies associating serum 25-hydroxyvitamin D with colorectal cancer

Author, Yr	Type of Study	Participant characteristics	Cancer variable	Association with 25(OH)D
Garland, 1989 (80)	Nested case-control, 1974-83; Washington County, Maryland, US.	m&f, n=25620, 34 cases, controls matched 2:1. Bloods collection in Autumn months	Colon	Mean 25(OH)D: cases=76 nmol/L, controls = 83 nmol/L, P < 0.05 Relative odds ≥50 *vs.* <50 nmol/L, 0.5, P = 0.05
Braun, 1995 (60)	Extension of above study, 1974-91	m&f, n=20305, 57 cases between 1984-1991, controls matched 2:1	Colon	Mean 25(OH)D: cases =59 nmol/L, controls = 58 nmol/L NS
Peters, 2004 (81)	Cancer Screening trial, examined case control; US multimember	m&f 394 cases and 397 controls. 55-74yrs	Colorectal	High *vs.* low quintile, OR (95% CI) f: 0.27 (0.11-0.69) P for trend = 0.0002, m: 1.10 (0.60-2.05) P for trend = 0.85
Tangrea, 1997 (82)	ATBC Study, clinical trial cohort, 8yrs, examined as nested case control; Finland	m, smokers, 91 colon, 55 rectal, 292 controls, age ~ 60yrs	Colon & rectal	High vs. low quartile RR: Rectal cancer 0.37, P for trend = 0.06 Distal colon & rectal 0.5 (0.2-0.9) P for trend = 0.03 Proximal colon NS
Feskanich, 2004 (83)	Nurses Health Study, nested case-control; US	f, n=32826, 43-70yrs, 1989-1990. 193 colon & 44 rectal cases	Colon & Rectal	High vs. low OR (95% CI), P for trend Colorectal (quintiles): 0.53 (0.27-1.04) P = 0.02 Colon (quartiles): 0.70 (0.35-1.38) P = 0.17 Rectal (tertiles): 0.31 (0.08-1.31) P = 0.03
Levine, 2001 (77)	Screening sigmoidoscopy study. Case control, US	m&f, 55-74yrs, 473 cases, 507 controls	Colorectal adenoma	High vs. low quartile, OR (95% CI) (multivariate adjustment) 0.74 (0.51 – 1.09) Ca < 744 mg/d: 0.40 (0.22 – 0.71), P for trend = 0.005 Ca > 744 mg/d: 1.17 (0.69 – 1.99), P for trend = 0.94
Grau, 2003 (84)	Calcium polyp Prevention Study, 4yrs RCT of Ca supplementation; US	m&f, 405 placebo, 398 Ca suppl, mean age 61yrs, 87% white	Colorectal adenoma	Adenoma recurrence and a 1 std. dev. Δ in [25(OH)D]: Ca supplemented group, RR = 0.88 (0.77 – 0.99) Placebo group, NS

m male, f female

Table 1.6 Descriptive studies associating vitamin D intake and breast cancer, or mammographic breast density*

Author, Yr	Study Design	Participant characteristics	Vitamin D measure & cancer variable	Main Findings
Simard, 1991 (85)	Case-control; Montreal, Canada	40 cases, 322 controls, 40-59yrs	1DDR; breast cancer	mean (sd) intake Vit. DIU/kg/d: cases: 1.65 (2.48) controls: 1.34 (1.17) NS
John, 1999 (73)	Prospective cohort; NHANES I 1971-75, follow-up in 1992; US (I)	n=5009 ~20yrs at BL, white, (177 cases with diet data)	24-hr recall, supplement use; breast cancer	NS
Levi, 2001 (86)	Case-control, hospital based, 1993-99; Switzerland	289 cases, 442 controls, 23-74yrs	FFQ (diet); breast cancer	NS
Shin, 2002 (87)	Nurses Health Study, Prospective cohort, 1980-88; US	88691 (incl. 3482 cases, of which pre-menopausal = 827, post-menopausal = 2345, and uncertain = 310), mean age 47yrs in 1980	5xFFQ (diet), Suppl.; breast cancer	*Total* vit D ref ≤150IU/d, RR (95% CI) Pre-menopausal: 350-500IU/d, 0.77 (0.60 – 0.99) >500IU/d, 0.72 (0.55 – 0.94) P for trend = 0.01 Post-menopausal: NS
Vachon, 2000 (88)	Minnesota Breast Cancer Family Cohort, cross-sectional; US	1508, with a family history of breast cancer, 40-90yrs	FFQ; % breast density (mammogram)	NS
Berube, 2004 (89)	Case-control, Screening Study, 1989-90; Massachusetts, US	few densities n=287, extensive densities n=256, 40-60yrs,	FFQ; % of the breast showing densities (mammogram)	Vit. D (<50, 50-99, 100-199 & ≥200IU/d), adj. OR=1.00 (ref), 0.51, 0.37, & 0.24, respectively (P for trend = 0.0005). Ca intake ≥750 mg/d & vit. D ≥100IU/d (ref <750mg/d and <100IU/d): OR=0.28 (0.15-0.54)

*All studies summarised in this table are for *female* breast cancer, and are for incidence, not mortality.

Table 1.7 Studies associating serum 25-hydroxyvitamin D with prostate cancers

Author, Yr	Type of Study	Participant characteristics	Main Findings
Corder, 1993 (90)	Nested case-control n=250,000. Sampled between 1964 and 1971, follow-up 1987; US	181 cases, 181 matched controls, median age 57yrs	NS
Braun, 1995 (91)	Nested case-control, n=20,305 residents, Washington County, US. Sampled 1974, diagnosis between 1980-92	61 cases, matched controls 2:1, <45-70+y	NS
Gann, 1996 (92)	Nested case-control study n=14,916, Physicians' Health Study; US. Sampled 1982-83, follow-up 1992	232 cases, 414 matched controls, 40-84yrs	NS
Nomura, 1998 (93)	Nested case-control, Hawaii, n=3737 Sampled 1974, 23yrs follow-up	Japanese-American, 136 cases, 136 matched controls	NS
Ahonen, 2000 (94)	Nested case-control, Helsinki Heart Study; Finland n=18966, Sampled 1981/82, follow-up 1995	149 cases, 566 matched controls, age range 40-57yrs	low *vs.* ref high quartile IV, OR (95% CI): 1.7 (1.0 – 3.0) P for trend 0.01 Ref ≤ 40nmol/L: All 1.7 (1.2 – 2.5) <52yrs 3.1 (1.6 – 6.1) >51yrs 1.2 (0.7 – 2.1)
Jacobs, 2004 (95)	Nested case-control, Nutritional Prevention of Cancer trial, n=1312, Sampled 1983 and 1989, follow-up 2002	83 cases, 166 matched controls, mean age 68yrs	NS
Platz, 2004 (96)	Nested case-control; Health Professionals Follow-up Study, 1993/95-98; US	460 cases, 460 matched controls	NS
Tuohima, 2004 (97)	Nested case-control, Norway, Finland and Sweden	622 cases, 1,451 matched controls	nmol/L OR (95% CI) ≤ 19 1.5 (0.8 – 2.7) 20 – 39 1.3 (0.98 – 1.6) 40 – 59 1 60 – 79 1.2 (1.9 – 1.5) ≥ 80 1.7 (1.1 – 2.4)

Vitamin D intake in 1980 from diet or supplements was not significantly associated with colorectal cancer. However, after exclusion of women who had changed their milk intake over subsequent years (*n* = 26,980), the effect was significant for total vitamin D intake (supplements + diet), (RR = 0.42, 95% CI 0.19-0.91). In the Cancer Prevention Study II Nutrition Cohort (54) a large cohort of 184,192 men and women were followed for 4-5yrs. A protective effect of total vitamin D intake was detected, but in men only, and in colon, but not rectal cancer (RR = 0.58, 0.39-0.86).

A weak effect of dietary vitamin D on risk of colon cancer was observed in a cohort study of more than 35,000 Iowa women aged 55-69yrs, followed for four years (78). Dietary questionnaires (as used in the Nurses Health Study) were completed at baseline. 212 cases of colon cancer were documented. After adjustment for age, total (diet + supplements) vitamin D intake at baseline was associated with a reduced risk of colon cancer, (RR = 0.54, 0.35-0.84), but this relationship was non-significant after multivariate adjustment. Similar results were reported in a prospective study of 47,935 US male professionals aged 40-75yrs (79). After adjusting for age and total energy intake, the relative risk (RR) for the highest versus lowest quintile of total vitamin D intake was 0.54 (0.34-0.85), but in the multivariate model this effect disappeared (RR = 0.66, 0.42-1.05).

The mixed results yielded from the aforementioned studies may reflect the difficulties in dietary analysis but are more likely to be due to the small contribution that diet usually makes to vitamin D status. Indeed, in a report from a sigmoidoscopy screening case-control study, Levine (77), found no association between vitamin D intake and the odds of colorectal adenoma, but also found only a small correlation between vitamin D intakes and plasma 25-hydroxyvitamin D concentrations (r = 0.08, P = 0.01).

Blood concentrations of 25-hydroxyvitamin D are the best indicator of vitamin D status. A protective effect of high 25-hydroxyvitamin D concentrations against colon cancer was reported in a prospective nested case control study where blood was collected from 25,620 volunteers in 1974 (80). Thirty-four cases of colon cancer were diagnosed between 1975-83. These cases were matched to 67 controls by age, race, sex, and month of blood collection. When subjects were categorised by serum 25-hydroxyvitamin D concentrations of ≥50 nmol/L, the RR of colon cancer was 0.30 (P = 0.05). However, there was not a clear trend in reduced risk of colon cancer by quintiles of 25-hydroxyvitamin D concentrations. The third and fourth quintiles of vitamin D concentration conferred RR of 0.25 and 0.21, respectively (P ≤ 0.05), when compared to the first quintile (10-49 nmol/L). However, the 5th quintile (105-227 nmol/L) did not follow this trend (RR = 0.73, NS). In the same cohort but with different cases and controls, and a longer interval (10-17yrs)

between blood collection and cancer diagnosis, Braun *et al* (60) found no significant difference in vitamin D status between cases and controls. These studies' results are inconclusive because the number of cases (57 by 1991) was very small.

A larger case control study that included 394 cases, did find a significant association between high 25-hydroxyvitamin D concentrations and reduced odds of colorectal cancer in women (OR=0.27, 95% CI 0.11- 0.69) but not in men (81). Case control studies, while having greater statistical power with more numbers of cases, can be prone to bias because a disease state may change the variable of interest. However as this was a screening study, its authors argue that sun exposure or dietary patterns were not likely to have changed with the onset of the cancer (81).

A clinical trial of vitamin E and B-carotene (not vitamin D) supplementation on male Finnish smokers, was reinterpreted as a nested case-control study to look for an association between vitamin D status and both colon and rectal cancers separately (82). In eight years there were 91 cases of colon cancer and 55 of rectal cancer. Two controls were matched to each case by age, date of baseline blood draw, and intervention type. Mean serum 25-hydroxyvitamin D concentrations at baseline were only slightly lower in cases than controls (30 *vs.* 34 nmol/L, paired t-test, $P < 0.01$). After univariate regression there was a decrease in risk of rectal cancer by increasing quartile of serum 25-hydroxyvitamin D concentration (< 24 nmol/L *vs.* > 50 nmol/L of 25-hydroxyvitamin D concentration, (RR = 0.37; trend P = 0.06), but this association was not significant for colon cancer. Dietary intake of vitamin D was similar between cases and controls at baseline (~5ug/d), and only 4% of the study group took supplements.

In 2004, the Nurses Health Study cohort was re-examined to look for a relationship between circulating 25-hydroxyvitamin D and colorectal cancer in women (83). In order to compare results with the Finnish study, the researchers reported OR for colon and rectal cancers both separately and together. Blood samples were collected from 32,826 participants aged 43 – 70yrs, from June 1989. One year later, 193 cases of colon or rectal cancer had been reported. Two controls were matched by age and month of blood draw to each case. In the highest *vs.* lowest quintile of plasma 25-hydroxyvitamin D concentrations the fully adjusted OR (95 % CI) for colorectal cancer was 0.53 (0.27 – 1.04), P for trend = 0.02. Like the Finnish study, when analysed separately, higher 25-hydroxyvitamin D concentrations were significantly associated with risk of rectal cancer, but not colon cancer (Table 1.7). Interestingly, the controls in this study had a mean 25-hydroxyvitamin D concentration of 68 nmol/L, considerably higher than those in the Finnish study. It is not known why vitamin D concentrations appear to be more protective against rectal than colon cancer.

Emerson (51), found the opposite result when associating solar radiation and colon or rectal cancer. It has been suggested that differences in large bowel physiology and morphology, such as the frequency of VDR by location, account for a varying susceptibility to the effect of low vitamin D (82).

Calcium intake and vitamin D status may be interrelated in preventing colon cancer. Holt (98) conducted a clinical trial measuring the effects of treatment with either calcium, both cholecalciferol (400IU/d) and calcium, or 1,25-dihydroxyvitamin D on rectal epithelial cell proliferation in subjects at risk for colonic neoplasia (*n*=39). The researchers found no reduction in cell proliferation between groups. However, they did find that in calcium supplemented groups there were significant negative correlations between serum 25-hydroxyvitamin D concentrations and indices of epithelial cell proliferation at 6 months.

Colon polyps are associated with an increased the risk of colon cancer. The Calcium Polyp Prevention Trial found that calcium supplementation prevented reoccurrence of colon polyps only in those with serum 25-hydroxyvitamin D concentrations >73 nmol/L with a RR = 0.71 (0.57–0.89) (84). Furthermore, serum 25-hydroxyvitamin D concentrations were only associated with a risk reduction in the calcium supplemented group (RR = 0.88, 0.77-0.99). Levine *et al* (77) also investigated the associations between plasma 25-hydroxyvitamin D concentrations and risk of colorectal adenomas in individuals with differing calcium intakes, and found the protective effect of 25-hydroxyvitamin D was limited to those with calcium intakes below the median of 744 mg/d. Conversely, the Nurses Health Study found no difference in the relationship between plasma 25-hydroxyvitamin D concentrations and cancer risk for those with calcium intakes above or below 900 mg/d (83).

With the exception of Holt (98), who supplemented with only 400IU/d cholecalciferol, there are no published RCTs that investigate the effects of vitamin D supplementation and prevention or progression of colorectal cancer. Such studies are necessary to establish the importance of vitamin D in the etiology and treatment of this disease.

3.1.1.2 Breast cancer

Breast cancer, like colorectal cancer, has been associated with UV or latitude. In ecologic studies, average annual solar energy (cal/cm^2/d) was inversely correlated with breast cancer incidence in the USSR (68, 70) and breast cancer mortality in the US (69). Sturgeon *et al* (34, 59) investigated breast cancer mortality rates by geographic region over time, and found that the while

breast cancer mortality rates were lower in the South compared to the Northeast of the US in 1950-69, there was little regional variation by 1990-99. However, the regions of the US (NE, S, W, Mid-W) were not divided according to latitude in that study. Grant (46) found a correlation between latitude and breast cancer mortality ($r=0.66$, $P < 0.001$) in a study of 35 countries worldwide. This association became stronger when E. European or Scandinavian countries were omitted, which led the authors to suggest that high fish consumption in these countries overwhelmed the contribution of solar radiation to vitamin D status.

Occupational and recreational sun exposure has been associated with breast cancer. A Danish population-based case-control study found women working jobs that are commonly worked night shift had 50% greater odds of breast cancer (74). In the US, women (n=5009) who had completed dermatological examination and a 24-hr recall were followed for 20yrs as part of the NHANES I study (73). By 1992, 190 women had developed breast cancer. Using a combined estimate of recreational and occupation exposure, the age-adjusted RR (95% CI) (high *vs.* low exposure) was 0.50 (0.29-0.86) P for trend $P = 0.01$, but this association was not significant using multivariate analysis, RR = 0.67 (0.42-1.06). In this study, region of residence was not significantly associated with breast cancer incidence. In contrast, a case-control study across 24 US states found a significant inverse association between breast cancer mortality and area of residential and occupational sun exposure, using multivariate analysis (41). However, studies that use mortality rather than incidence of cancer are prone to bias, because there may be regional differences in the accuracy of death information and in the screening and treatment of the disease.

As for colon cancer, studies looking for an association between dietary vitamin D and breast cancer have yielded mixed results (Table 1.3). The largest study to associate vitamin D intake (diet and supplements) with breast cancer incidence was the Nurses Health Study (87). From a prospective cohort of 88691 women, pre-menopausal women with previously reported vitamin D intakes of >500IU/d had a significantly lower risk of developing breast cancer (RR=0.72, 95% CI 0.55 – 0.94) than those with intakes <150IU/d. This protective effect was not apparent in postmenopausal women. In contrast, a smaller prospective cohort of US women (n=5009) followed for 8-11yrs demonstrated no protective effect of vitamin D intake measured by 24-hr recall. Increased density of the breast observed through mammography indicates a higher risk of developing breast cancer. A cross-sectional study of women who had a screening mammogram, found that high dietary intakes of vitamin D were associated with less extensive breast density (89).

However, a study of 1508 women with a family history of breast cancer found no effect of vitamin D intake and breast density (88).

Animal models support an association between vitamin D status and breast cancer, suggesting that low vitamin D status results in suboptimal generation of calcitriol in the mammary gland, and predisposes mammary cells to tumorigenesis (99).

Interest in vitamin D in cancer prevention and treatment has for some time focused on calcitriol. Using data from a nested case-control study, Hiatt *et al* (100), reported no difference in serum calcitriol concentrations of 95 women 15yrs prior to diagnosis, than age-matched controls. The small participant numbers and long time between blood draw and follow-up limits the interpretation of this study. In a university-based case-control study, whole blood concentrations of calcitriol were significantly lower in white women diagnosed with breast cancer than controls matched for age, race, clinic and month of blood draw (101). To date, this is the only published breast cancer study that also measured 25-hydroxyvitamin D concentrations. No relationship was found between cases and controls in whole blood 25-hydroxyvitamin D. However, the metabolite was measured in whole blood not serum or plasma.

Calcitriol arrests the progression of breast cancer by regulating cell growth, apoptosis, and preventing metastasis (102). Further, it has been suggested that vitamin D status may influence the progression or prognosis of breast cancer. Women with breast tumours that had no measurable VDR had shorter disease free intervals than women who had breast tumours with VDR (103). A case-control study in Norway found that women diagnosed with breast cancer during the summer and autumn had a better prognosis than those diagnosed at other times of the year (104). In a study of 129 women with breast cancer, calcitriol concentrations fell in those whose disease progressed, but remained constant in those women whose disease state was stable or responded to treatment (105).

Blood concentrations of calcitriol are tightly regulated and do not reflect 25-hydroxyvitamin D concentrations except when very low (106). Therefore, in an analysis of vitamin D status and cancer, studies associating calcitriol and breast cancer are of limited use.

3.1.1.3 Prostate cancer

Vitamin D deficiency is hypothesized to be a risk factor for prostate cancer (107). In the US, prostate cancer mortality has been inversely correlated with UV radiation [r=-0.15, P=0.001 (72); r= -0.32, P < 0.001 (46)]. Established risk factors for prostate cancer include race (being African American), and (increasing) age; both are factors which are associated with lower vitamin D status. 'Lifetime' UV exposure based on a questionnaire has also been linked to prostate cancer in a UK case-control study OR=3.03 (95% CI 1.59 – 5.78) (108, 109).

Biochemical evidence supports a role of vitamin D in prostate cancer. Expression of 1α-hydroxylase prostate tissue (11) demonstrates local conversion of 25-hydroxyvitamin D to calcitriol, and VDR are present in the epithelial and stromal cells of the prostate (110). Calcitriol has been demonstrated to have antiproliferative and apoptic effects on prostate cancer cells *in vitro* and in *in vivo* animal models (111). It is therefore plausible that adequate 25-hydroxyvitamin D concentrations decrease the risk of prostate cancer.

Of the nested case controls looking at prediagnostic serum 25-hydroxyvitamin D concentrations (90-97), just two found a significant protective effect of vitamin D status against prostate cancer (94, 97), both in Scandinavian countries. These studies must be interpreted with caution because they assume that all controls are free of prostate cancer. A more thorough examination of these men might yield a considerable number with clinically significant lesions (112). While clinical trials continue to investigate the effects of calcitriol on prostate-specific antigen (PSA) or tumour mass in prostate cancer patients (113-116), there are as yet no clinical trials supplementing with cholecalciferol, to then ascertain effects in prevention or progression of prostate cancer.

In summary, current evidence is insufficient to confirm a role of vitamin D in the aetiology of cancer. Biological plausibility for an association is based on both the ability of numerous tissues to convert 25-hydroxyvitamin D to calcitriol, and the *in vitro* studies of calcitriol inhibiting cancer cell proliferation. There have been mixed findings from observational studies and, with the exception of colorectal cancer, there is a shortage of studies that have measured 25-hydroxyvitamin D. Further, clinical trials of vitamin D supplementation are sorely lacking. Until more convincing scientific evidence is available, public health policy with regards to vitamin D is best directed by its comparatively clear role in skeletal health.

3.1.2 Immune Function

Vitamin D receptors have been found to be present in mononuclear cells (117, 118), and recent research implicates a selective role of vitamin D in the regulation of autoimmune response in the gastro-intestinal tract and central nervous system (119). Researchers have linked vitamin D with conditions such as inflammatory bowel disease, asthma, rheumatoid arthritis, Type I diabetes and multiple sclerosis (16, 119, 120).

3.1.2.1 Multiple sclerosis

The aetiology of multiple sclerosis (MS), a demyelinating disease of the central nervous system, is unknown, but is thought by most to be an autoimmune condition (121). A geographic association with the rates of MS exists, with a larger incidence occurring at extremes of latitude, leading some to hypothesise that sufficient sunlight exposure may exert a protective effect (122). In Tasmania, higher sun exposure during childhood and early adolescence was also associated with a reduced risk of MS (123). In Scotland and Canada, patients with MS (n=29376) were more likely to have been born in May (after the winter months) than in November (1257 observed vs. 1373 expected χ^2 =10.67, P = 0.0011) (124). Residence by latitude and subsequent risk of MS was examined in data from the Nurses Health Study I (NHS; women born between 1920-46) and NHS II (1947-64) (125). The incidence of MS among NHS participants (181 cases) increased significantly with latitude (RR= 3.5 (95% CI, 1.1-11.3) for the north, 2.7 (0.8, 8.9) for the middle, relative to the southern tier of latitude (P for trend = 0.03). However, among NHS II women (131 cases), no association between latitude and MS was found. Vitamin D intake and subsequent risk of MS was also examined using combined data from the NHS I and II (126). The highest quintile of total vitamin D intake (diet + supplements) corresponded to a RR of 0.67 (0.40 – 1.12), P for trend = 0.03. Vitamin D intake by supplements alone, a more precise measure of intake, was more strongly associated with risk of developing MS (RR= 0.59 (0.38 – 0.91) P for trend = 0.006. There is insufficient evidence, as yet, to firmly establish vitamin D insufficiency as a causal link to MS, though one researcher is convinced enough to recently recommend vitamin D supplementation in pregnancy and childhood as a preventive measure against developing this condition (127).

3.1.2.2 Type I Diabetes *(IDDM)*

Type 1 diabetes, or insulin dependent diabetes mellitus (IDDM), occurs as a result of autoimmune destruction of the β-cells in the islets of the pancreas, which produce insulin (128). Pharmacologic doses of calcitriol have been shown to arrest disease progression in mice prone to

Type I diabetes (129, 130). Furthermore, when raised vitamin deficient, these mice developed diabetes earlier than non-deficient controls (131).

Associations between latitude and incidence of IDDM are inconsistent (132). No studies have investigate the relationship between infant or maternal vitamin D status by serum 25-hydroxyvitamin D, however, associations have been made between vitamin D or cod liver oil supplementation in pregnancy or infancy, and risk of developing IDDM (Table 1.8). A population-based case-control study in 7 European countries found children who were given vitamin D supplements in the first year of life had lower chance of developing IDDM by age 15yrs (133). Unfortunately the frequency of dose of supplementation was not recorded. Stene *et al* (134) conducted a case control study in Norway, of 85 children with type I diabetes and 1071 controls, and reported that children born to women who took cod liver oil, during pregnancy had a lower risk of developing type I diabetes (OR = 0.36, 95% CI 0.14-0.90). Multivitamin use had no significant effect. Researchers followed a Finnish cohort of 10,366 children born in 1966 and found vitamin D supplementation in the first year of life was associated with a reduced risk of type I diabetes during early adulthood (RR = 0.12; 95% CI 0.03, 0.51) (135). However, only 32 children in this cohort were not supplemented and only two of those developed diabetes. Stene *et al* (136) reported results from another population–based case-control study in Norway, where frequency of maternal (during pregnancy) or infant (in the first year) supplementation with vitamin D or cod liver oil was reported. The researchers found cod liver oil, but not other vitamin D supplementation of the infant in the first year of life had a protective association with IDDM, OR=0.74 (95% CI, 0.56 – 0.99). It may be that that cod liver oil has components, such as long chain n-3 fatty acids, that may also lower the risk of IDDM. Another possible reason suggested for the difference in findings between Stene (136) and Hypponen (135) is that the children in the highest dose category may have been receiving considerably higher doses in the study of Hypponen (~50ug/d) than that of Stene (6-10ug/d) (128).

Table 1.8 Studies associating vitamin D intake and Type 1 Diabetes*

Author, Yr; Study	Country	Type of Study	Participant characteristics	Measured variables	Main Findings
The EURODIAB Substudy 2 Study Group, 1999 (133)*	7 European countries	Popn-based case-control	820 cases 2335 popn-based controls	Vit D supplementation in 1st yr of life (y/n)	By age 15yrs, OR 0.67
Stene, 2000 (134)	Norway	Popn-based case-control	85 cases (~11yrs), 1071 controls (~8.5yrs)	Cod liver oil (y/n) or multivitamin use (y/n) during pregnancy	adj OR (95% CI) cod liver oil 0.36 (0.14-0.90) multivitamin NS
Hypponen, 2001 (135)	N Finland	Prospective birth-cohort 1966-1997	n=10821 (incl 81 cases)	Vit D suppl frequency, dose calculated by brand	Use of suppl (ref none) adj RR (95% CI) Irregular 0.16 (0.04 – 0.74) Regular 0.12 (0.03 – 0.51) Dose (ref Low) Recommended 0.22 (0.05 – 0.89) High (0.14 (0.02 – 1.01) Suspected rickets (ref No) Yes 3.0 (1.0 – 9.0)
Stene, 2003 (136) The Norwegian Childhood Diabetes Study Group	Norway	Popn-based case-control	545 cases (<15y), 1668 controls	Vit D suppl. or cod liver oil, during pregnancy (maternal) and in first yr of life (infant)	*Infant* adj OR (95% CI) Cod liver oil >5/wk 0.74 (0.56 – 0.99) other Vit D suppl NS *Maternal* NS

*Studies in humans only

3.1.2.3 Type II Diabetes (NIDDM)

Vitamin D may be involved in glucose regulation of insulin secretion, as 1,25-hydroxyvitamin D_3 has been detected in the islet cells of the pancreas (32). Deficiency in vitamin D has also been proposed as a risk factor for higher risk of insulin resistance and the metabolic syndrome, which are related to Type 2 diabetes, or non-insulin-dependant diabetes mellitus (NIDDM) (137). Data from the NHANES III has been analysed to show an inverse association between serum 25-hydroxyvitamin D and fasting glucose concentrations of ≥ 7.0 mmol/L in non-Hispanic whites and Mexican-Americans, but not in Non-Hispanic blacks (138). A Dutch study found similar results in 142 elderly Dutchmen (139) but not in a British study (140). Isaia (141) has reported results from a study of 799 ambulatory post-menopausal women in Italy, where serum 25-hydroxyvitamin D concentrations were measured (in late winter), and medical history was used to identify diabetic patients. 25-hydroxyvitamin D concentrations were significantly lower in diabetic patients than non-diabetic patients [mean (sd), 27 (24) vs. 22 (28), $P < 0.008$].

3.2 Role of Vitamin D in Musculoskeletal Health

Knowledge of vitamin D is most extensive in relation to its importance in skeletal health. Rickets and osteomalacia in children, and osteomalacia in adults, are the most well known consequences of frank vitamin D deficiency.

3.2.1 Vitamin D Deficiency Rickets

While rickets is most commonly caused by vitamin D deficiency, it can be a secondary condition from disease of other organ systems, or from a very low calcium intake, certain medications or genetic conditions (142). This review focuses on ‘simple’ rickets, as a consequence of vitamin D deficiency.

3.2.1.1 Physical and clinical signs of rickets

Rickets is a failure to mineralise new bone tissue (142). Rickets can only occur in childhood, before the growth plates of bone (physes) are closed (143). Rickets is characterized by growth retardation and skeletal deformities, including cupping, splaying or fraying at the end of long bones (enlarged wrists are often seen), swollen costochondral junctions of the ribs (known as rachitic rosary) and either bowed legs or knock knees associated with weight-bearing, and a

waddling gait (142, 144) . Untreated rickets may result in deformity of the pelvis that can later (in women) result in prolonged obstructed labour (144).

Rickets is most often seen in young children aged 6-24 months, and is uncommon in newborns. Delayed closure of the fontanelle is an early indication of rickets in infants less than 12 months of age (145). Non-bone related signs of vitamin D deficiency in infants less than 6 months of age include symptomatic hypocalcaemia with convulsions or proximal myopathy (142). Most cases of radiologically proven rickets have serum 25-hydroxyvitamin D concentrations < 10 nmol/L and elevated alkaline phosphatase and parathyroid hormone concentrations (142).

3.2.1.2 Risk factors for rickets

Risk factors for rickets include: poor maternal vitamin D status, having dark skin, and breastfeeding for more than 6 months without vitamin D supplementation, living in polluted cities, covering up (clothing) for religious or social reasons, limited outdoor activity and increased use of sunscreen (146).

3.2.1.3 A short history of rickets

Early writings attest to the occurrence of rickets, but the first specific descriptions of rickets were made in the 17th century (147). The rise of rickets is synonymous with the industrial revolution. As Britain and other northern European and American countries became increasingly urbanised, so rickets became commonplace. The cities of the time were overcrowded and unplanned; little sunlight could penetrate the skies polluted with coal smoke, or pass into narrow streets or alleyways; the effects were often compounded by a poor diet (148). By the beginning of the 20th century rickets was widespread; In the Leiden, Netherlands, autopsies of infants who had died found clinical manifestations of rickets in more than 80 percent of infants examined (149).

A common early belief was that rickets was caused by dirty, unhygienic conditions. However, rickets was not confined to the poor (150), and Palm (151) reported that rickets was absent in children the tropics of India and China who were living in as equally squalid conditions as the poor of urban Britain, and recommended the use of sunbaths for the prevention and cure of rickets. While it had earlier (1868) been suggested that sunlight and cod liver oil were antirachitic (152), it was not until much later that this would be proven correct, and it was efforts into discovering the cause and treatment of rickets that contributed to the discovery of vitamin D (147). Animal experiments (153-155) determined that a property of certain foods, especially cod liver oil,

could alleviate rickets. The oxidation of cod liver oil determined that this antirachitic substance was not vitamin A, but a new vitamin: vitamin D (156). Ultraviolet light was also proven to cure rickets (157, 158). In 1927, pure vitamin D_2 (ergocalciferol) was obtained from the irradiation of the plant-based ergosterol. By the 1930s, synthetically produced Vitamin D_2 was widely used as a fortificant in milk and other foods (16). With a well-recognized means for treatment and prevention, rickets in Western countries, was effectively controlled.

3.2.1.4 Rickets today

By the late 20th century, rickets was, to many, little more than an historical curiosity. However, accumulating reports of rickets, particularly in infants who are dark-skinned and/or from immigrant families (159-166), have led to calls for greater vigilance (167-169) and for increased fortification of foods with vitamin D (170) or vitamin D supplementation (200IU/d) in all breastfed infants from 2 months of age (171), though it has been argued that 200IU/d may be too small a dose (172).

3.2.2 Osteomalacia

3.2.2.1 What is osteomalacia?

Vitamin D deficiency can cause osteomalacia (or 'soft' bones), a disease that can occur at all ages. Histologically similar to rickets, osteomalacia results from a failure to mineralize new bone as it is formed during bone remodelling, which leads to a mineral deficit in the bony tissue itself. Bone volume does not change, but the newly formed osteoid, or bone tissue, is soft instead of rigid.

3.2.2.2 Risk factors for osteomalacia

Persons at increased risk for osteomalacia include home-bound or institution dwelling persons, the elderly, dark-skinned persons living at northern or southern latitudes, or persons whose dress habits limit sun exposure to a great extent. Renal and gastrointestinal disease states can also result in osteomalacia (9).

3.2.2.3 The consequences of osteomalacia

Osteomalacia causes bone pain, impaired muscle function, and increased risk of fracture (144). A common feature of osteomalacia is osteopenia (bone thinning) which can be confused with osteoporosis and biochemical tests are therefore necessary to confirm diagnosis (144).

Osteomalacia is a known cause of persistent, non-specific musculoskeletal pain. In a Saudi Arabian study (173), of 360 patients presenting with chronic lower back pain with no obvious cause, 83% had 25-hydroxyvitamin D concentrations <22.5 nmol/L. After 3 months of high dose supplementation (5000-10,000IU/d) these symptoms had improved in all patients with initial serum 25-hydroxyvitamin D concentrations <22.5 nmol/L (n=299), and in 42 of the 61 patients (69%). Unfortunately this study had no control group. Some researchers argue that all persons presenting with persistent, non-specific musculoskeletal pain should be screened for vitamin D deficiency (174).

3.2.3 Hypovitaminosis myopathy

Muscle fatigue or weakness is also caused by vitamin D deficiency (175, 176), and is a common symptom of osteomalacia (177, 178). The weakness is primarily in the proximal muscle groups (179); sufferers complain of a feeling of heaviness in the legs, difficulty climbing stairs and rising from a chair (20). Aging is commonly accompanied by a loss of muscle mass and strength, which can lead to an inability to perform daily tasks without assistance, and an increased risk of falling which is especially problematic if the individual has propensity to fracture (section 3.2) due to osteomalacia or osteoporosis. Vitamin D treatment improves muscle strength in patients with osteomalacia (180, 181). However, this 'hypovitaminosis myopathy' is present in patients with no biochemical signs of bone disease (179). Relationships between serum 25-hydroxyvitamin D concentrations and muscle function, mobility or falls in the elderly have been reported (179, 182-184). Vitamin D status may also be lower purely *as a result* of reduced mobility, as elderly who are frailer may have less opportunity for sun exposure, and limited appetite for consuming vitamin D rich foods. Intervention studies are therefore necessary to determine a casual negative relationship between vitamin D status and either muscle function or falls. A number of studies have measured fracture (from falls) as an outcome, and are discussed in the following section (3.2.4). Only a few intervention trials have investigated the effects of vitamin D on muscle function, mobility or falls *per se* (185-188).

A well-designed multi-centre Australasian study randomized 243 frail elderly to receive a single very high dose (7500µg) of vitamin D or placebo, and 10 weeks of high-intensity home-based quadriceps resistance exercise or frequency-matched visits. The single dose was shown to improve serum 25-hydroxyvitamin D concentrations, but there was no effect of either intervention on physical health or falls, even in those with serum 25 hydroxyvitamin D concentrations < 31 nmol/L

at baseline (187). It may be that in this elderly cohort, calcium supplementation was necessary in conjunction with vitamin D to improve muscle function. Another 12 week RCT of 122 elderly women in long-stay geriatric care reported a 49% (95% CI, 14–71%; $p < 0.01$) reduction in the number of falls (after controlling for age, number of falls in the 6 weeks prior to the study, and baseline 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D serum concentrations) in participants supplemented daily with 1200 mg calcium and 20µg vitamin D3, compared to a group supplemented with calcium only (186). Musculoskeletal function (summed score of knee flexor and extensor strength, grip strength, and the 'timed Up & Go' test) also improved significantly in the Ca-Vitamin D group. Pfeifer (185), reported similar findings from a RCT of elderly German women with baseline serum 25-hydroxyvitamin D concentrations < 50 nmol/L. Starting in winter, women were supplemented for eight weeks with either 1200 mg/d calcium plus 20µg/d of vitamin D or calcium only. One year after the study began, researchers found a significant decrease in falls and reduction in body sway.

It appears that low vitamin D status may play a role in loss of muscle strength and function, and that supplementation with vitamin D, at least in conjunction with calcium, can ameliorate these conditions. More evidence is necessary to confirm and elucidate this relationship. There is some debate as to whether there is a direct effect of vitamin D (calcitriol) on muscle (20), whether it is caused by elevated PTH (which is influenced directly by serum calcium) (189), or a combination of the two (190).

3.2.4 Osteoporosis

Osteoporosis is a disorder characterised by decreased bone mass and density, thus leading to bone fragility and increased susceptibility to fracture (191). The bone that remains is of normal quality (29). More than 200 million women worldwide have osteoporosis and the annual incidence of worldwide osteoporotic hip fracture is expected to rise to 6.26 million by the year 2050 (192). While osteoporosis generally affects the elderly, bone accrual in early life and factors that affect bone remodelling throughout adulthood are thought to influence the risk of developing osteoporosis (193, 194). For example, serum vitamin D has been shown to be significantly correlated with accrual of bone density at the lumbar spine and femoral neck in Finnish girls aged 9-15yrs (195) and positively correlated with bone mineral in young Finnish men (196). Pre-pubertal Caucasian girls who were supplemented with vitamin D during infancy were found to have greater BMD at the radius and hip, than those who were unsupplemented (197). It is thought that accrual of more bone

in childhood results in more successful attainment of peak bone mass in early adulthood, and thus a decreased risk of osteoporosis in later life (198).

The aetiology of osteoporosis is multifactorial, with vitamin D status one factor in its affect on bone remodelling. Low vitamin D status reduces the efficiency of dietary calcium, and a consequent decline in serum calcium concentrations. This prompts an increase in the secretion of PTH, which promotes bone resorption to restore circulating calcium. If vitamin D inadequacy is prolonged, there is a chronic imbalance between bone formation and resorption, with reduced bone mass and an increase in the risk of osteoporosis and fracture risk. Furthermore, if risk of falling is increased via vitamin D compromised muscle function, then so too is the risk of fracture increased.

The importance of adequate vitamin D status for the prevention and treatment of osteoporosis has been established from several intervention trials of elderly, where 20µg/d vitamin D_3 reduced the risk of fracture (199). The exact serum concentration of 25-hydroxyvitamin D or intake of vitamin D that confers maximum protection against adverse bone turnover, bone loss, or osteoporotic fracture is still to be established. Studies that have attempted to discern cut-offs for 25-hydroxvitamin D for prevention of osteoporosis, and recommendations for intake, are discussed in sections 4.1 and 6 respectively

4 Defining vitamin D sufficiency

Serum 25-hydroxyvitamin D concentration is considered the best indicator of vitamin D status as it reflects both synthesis in the skin and that which is absorbed from the diet, a process which is not regulated (200) (see section 2.2). While population-based reference ranges for 25-hydroxyvitamin D have been used for defining vitamin D status, it is more appropriate to define vitamin D status using clinical or functional markers of heath risk, because vitamin D status varies so much between countries and populations (being heavily dependent on UV exposure, vitamin D intake, and other factors described in section 5). However, a universal, interpretive criteria to define the minimum concentration of 25-hydroxyvitamin D associated with maximal risk reduction, has not yet been established.

Estimated cut-offs for 25-hydroxyvitamin D concentration are generally based on studies that include descriptive case control and cohort studies, randomized controlled trials and small metabolic studies. Some aspects of these studies, which have resulted in differing expert estimates for the most appropriate cut-offs for 25-hydroxyvitamin D concentration, include: What aspect of health is considered (see section 3), what variables were measured to quantify bone or other health status, what assays are used to measure circulating 25-hydroxyvitamin D (201) (see section 2), and the characteristics of study participants (for example: age, health status and lifestyle factors including diet and physical activity).

In light of the sparse evidence as to what concentration of 25-hydroxyvitamin D might be associated with a lower risk of diseases such as cancer, the following review of 25-hydroxyvitamin D cut-off estimates focuses only on aspects of bone health.

4.1 Estimates of optimal 25-hydroxyvitamin D concentrations with regard to bone health

Cut-offs to indicate vitamin D insufficiency with regards to bone health have been based on the relationship between 25-hydroxyvitamin D and maximizing calcium absorption (202), minimizing the loss of BMD (203), and reducing the risk of falls (204) and fractures (205, 206) in the elderly, but the majority of evidence is based largely on the relationship between parathyroid hormone and 25-hydroxyvitamin D concentrations (207). Studies used as evidence for setting a 25-hydroxyvitamin D cut-off for vitamin D insufficiency in adults (with regards to bone health and/or calciotropic function) are summarized in Table 1.9.

4.2 PTH suppression and calcium absorption

As described in section 2.4, PTH rises in response to a drop in serum calcium. Low serum calcium may be a result of low vitamin D status and impaired calcium absorption. Therefore, there is an indirect inverse relationship between circulating PTH and 25-hydroxyvitamin D concentrations (207-211). In the absence of studies that have directly measured the relationship between vitamin

Table 1.9 Evidence for setting a 25-hydroxyvitamin D cut-off for vitamin D insufficiency in adults- biomarkers of bone health and/or calciotropic function

Author; Yr	Type of Study	Participant characteristics	25(OH) D assay method	Functional/ clinical marker	Main findings	Inferred 25(OH)D cut-off (nmol/L)
Need, 2000 (211)	Descriptive	PM f, n=496, no fracture	CPB	PTH	*Corr. with 25(OH)D* -0.28, p<0.001	> 40
Gallagher, 1998 (208)	Descriptive	m&f n=735, 65-87yrs	CPB	PTH	*Corr. with 25(OH)D* m: -0.25, p<0.001 f: -0.34, p<0.001	> 40
Vieth, 2003 (207)	Descriptive	n=1741, 19-97y, outpatients	RIA	PTH	No plateau in PTH as 25(OH)D increases.	-
Lips, 2001 (212)	Descriptive (BL from intervention)	f: 7564 women with osteoporosis	RIA	PTH	*Corr. with 25(OH)D:* -0.25, p<0.001	-
Chapuy, 1997 (213)	Descriptive. SUVIMAX France	m&f: n=1569 35-65yrs	RIA	PTH	*Corr. with 25(OH)D:* PTH = 35.5 + 32.5 exp[(-0.0439 x 25(OH)D] P<0.01 Threshold at 78 nmol/L	> 78
Thomas, 1998 (214)	Descriptive	Medical inpatients m&f: n=290 mean age 65yrs	CPB	PTH	Corr. with categories of deficiency/insufficiency P<0.001	> 37.5
Gloth, 1995 (215)	Descriptive cohort	m&f: n=244 <65yrs homebound; mobile controls n=128	-	PTH	*Corr. with 25(OH)D* -0.42, p<0.001 (both groups)	> 37.5
Krall, 1989 (216)	Descriptive cross-sectional	postmenopausal f: n=333	-	PTH	No seasonal variation on PTH when 25(OH)D > 95 nmol/L	> 90
Malabanan 1998 (217)	8 wk intervention, 50000IU/wk & 800 mg/d Ca	n=35, age $\bar{x}$= 67yrs 25(OH)D, between 62.5 & 25 nmol/L	RIA	PTH	PTH fell significantly with supplementation only for those with baseline 25(OH)D < 50 nmol/L (only n=7 >50 nmol/L)	> 50
Guillemant 1999 (218)	Longitudinal descriptive	m: n=175 age 13–17yrs	CPB	PTH	*Winter/ summer mean 25(OH)D: 21/59* PTH = 2.19 + 3.74 exp[0.0362 x 25(OH)D	> 83

Kinyamu, 1998 (219)	Cross-sectional	f: n=376 free-living 65-77yrs	CPB	PTH	*Corr. With 25(OH)D* -0.19, p<0.01 Calculation showed elderly would need to have 25(OH)D of 122 nmol/L for a 'normal' younger population PTH value	> 122
Khaw 1994 (220)	5 wk intervention. Single-dose 100000IU vit D	n= 189 free-living 63-76yrs	RIA	PTH	*Corr with 25(OH)D* -0.17, p<0.01	-
Jesudason, 2002 (221)	Cross-sectional	f: n=486 $\bar{x}$ age =63yrs	RIA	PTH Urinary bone resorption markers	*Corr. with 25(OH)D:* -0.224, p<0.01 -0.053 – 0.164, p<0.05	> 50 (PTH) > 60 (bone markers)
Ooms, 1995 (222)	Cross-sectional	f: n=330, elderly	CPB	PTH BMD hip	*Corr. with 25(OH)D:* -ve when 25(OH)D <25 nmol/L p=0.02 +ve when 25(OH)D <30 nmol/L p=0.001	> 30
Barger-Lux 2002 (223)	Longitudinal descriptive, post summer and 175 d later	m: n=30, seasonal outdoor workers	CPB	Ca absorption	With $\bar{x}$ [25(OH)D] of 122 nmol/L in late summer and 74 nmol/L in late winter, no significant seasonal change in calcium absorption fraction	> 75
Heaney, 2003 (202)	Randomized Crossover Intervention	PM f: n=34, 1 yr apart.	CPB	Ca absorption	Ca absorption 65% higher when mean [25(OH)D] = 86.5 nmol/L *vs.* 50 nmol/L	> 80 or 90
Zittermann, 1998 (224)	Descriptive cross-sectional	f: n=38 winter, n=38 summer age 24yrs, dietary Ca intakes $\bar{x}$= 1200 mg/d	RIA	Ca absorption Markers of bone resorption	[25(OH)D] correlated with Ca absorption, but no differences in markers of bone resorption or formation between summer (25(OH)D $\bar{x}$ =70 nmol/L and winter $\bar{x}$ =30 nmol/L	Bi-annual change: 30 – 70 in young women with 1200 mg/d Ca intake

			RIA	PTH Total ALP osteocalcin Urinary bone resorption markers Falls	Ca *vs.* Ca+vit D, $\bar{x}$ 25(OH)D 29 vs. 66 nmol/L -33% p=0.002 -8% p=0.037 NS N-Tx -12% p=0.038 & DPX -29% p<0.0001 -49% p=0.01	> 66
Bischoff, 2003 (186)	Double-blind RCT	f: n=122 age $\bar{x}$ = 85yrs				
Bischoff-Ferrari, 2004 (203)	Cross-sectional Descriptive NHANES III, 1988-94	n=13,432, US, 20+yrs	RIA	BMD hip	Significant difference in BMD across quintiles of 25(OH) D	90-100
Dawson-Hughes, 1991 (225)	12 mo intervention, 400IU /d vit D & 377mg Ca or calcium only	f: n=247, $\bar{x}$ age = 61yrs	CPB	PTH BMD spine	*Winter/ summer mean 25(OH)D, Pl vs. trtmnt* Ca: 61/81 Ca+VitD: 92/97 NS Winter bone loss was reduced in trtmnt group	> 90
Chapuy, 1992, 1994 (226, 227)	3yrs mo intervention, Ca 1200mg/d and vit D 800IU/d or placebo	f: n=3270 elderly nursing home residents	CPB	Hip fracture	Supplementation reduced risk of fracture OR=0.70 p<0.01	> 100
Dawson-Hughes, 1997 (228)	3yrs Intervention 500 mg Ca and 700IU vit D or placebo	m&f: n=389 65yrs+ free-living	CPB	Total BMD Fracture	Data at Year 2&3 , PL vs. Trtmnt: mean change: -0.14 vs. +0.23 p<0.001 RR= 0.40p=0.03	>112
Trivedi, 2003 (229)	RCT 4-monthly dose 100,000IU vit D	m&f: n= 2686, 65-85yrs free-living (n=189 25(OH)D subset)	CPB	Fracture	Supplementation reduced risk of first fracture RR=0.78, p=0.04	> 55
Larson, 2004 (230)	Intervention, 1000mg/d Ca & 400IU/d vit D, or control , for 24 mo.	m&f: random subset of large study, n=104, 66yrs+	RIA	osteoporotic fracture	$\bar{x}$ 25(OH)D raised from 37 to 47 f: RR=0.79, p<0.025 m: ns	> 50

All concentrations for 25-hydroxyvitamin D or 25(OH)D are given in nmol/L

D and bone resorption, elevated PTH concentrations have been used as a surrogate for bone resorption (see section 4.1), which can result in an increased risk of fracture (231). While there is some disagreement as to whether a plateau in PTH is reached should 25-hydroxyvitamin D be raised sufficiently high enough (207), it is generally agreed that an optimum 25-hydroxyvitamin D concentration is that which keeps circulating PTH at a minimum (232).

Large descriptive cross-sectional studies have regressed 25-hydroxyvitamin D concentrations against PTH concentrations in order to determine the concentration of 25-hydroxyvitamin D above which PTH is thought to plateau (208, 211-215, 218, 221, 222). 25-hydroxyvitamin D concentrations associated with a maximally suppressed PTH concentration have been reported to be from 30 nmol/L (222) to 90 nmol/L (216).

Another way to estimate the lowest 25-hydroxyvitamin D concentration is to measure the response of circulating PTH to vitamin D intervention (233). For example, Malabanan (217) and colleagues reported that a weekly dose of 50 000IU vitamin D (and 800 mg/d calcium) over 8 weeks produced a 35% decrease in PTH in persons with baseline serum 25-hydroxyvitamin D concentrations between 27.5 and 40 nmol/L (n=11), and a 26% decrease in PTH in persons with baseline serum 25-hydroxyvitamin D concentration between 40 and 50 nmol/L (n=17). There was no significant drop in PTH in those with baseline serum 25-hydroxyvitamin D concentrations between 50 and 62.5 nmol/L.

A third means of determining an adequate circulating 25-hydroxyvitamin D concentration is to determine the 25-hydroxyvitamin D concentrations at which seasonal variations in plasma PTH are eliminated. Krall (216) reported results from a cross-sectional study of 333 healthy, white post-menopausal women living in Massachusetts, with low median calcium (408 mg a day) and vitamin D intakes ~112IU/d. Women studied between August and October (end of summer), had the lowest circulating PTH and highest 25-hydroxyvitamin D concentrations ($\bar{x}$ = 93 nmol/L), and women studied between March and May (end of winter) had the highest PTH and lowest 25-hydroxyvitamin D concentrations ($\bar{x}$ = 63 nmol/L). However, the mean PTH and 25-hydroxyvitamin D levels did not vary with season in women with a vitamin D intakes >220IU /d, an intake which the authors associated with a serum 25-hydroxyvitamin D concentration of 95 nmol/L, between March and May. This data is limited by its cross-sectional nature.

It can be seen from Table 1.9 that estimates for the inflection point where there is no longer a relationship between PTH and 25-hydroxyvitamin D, do vary by study. An informal consensus of

investigators decided on a value of 75 nmol/L (234). It must be stressed that the majority of studies have investigated this relationship amongst older participants, where secondary hyperparathyroidism has been associated with osteoporotic fracture (233). Less is known about the relationship between PTH and 25-hydroxyvitamin D in younger groups, indeed whether maximal suppression of PTH in young adults or children is a desirable goal.

A direct clinical indicator of vitamin D status is absorption of calcium from the diet, though few studies have investigated this relationship. Barger-Lux and Heaney (223) reported results from a study where calcium absorption fraction in 30 men following a season of extended outdoor activity was found to not have changed 6 months later. In late summer the median 25-hydroxyvitamin D concentrations of the group was 122 nmol/L, which dropped to 74 nmol/L in winter. It appears that vitamin D status was high enough that calcium absorption was not compromised. Heaney (202) and colleagues also reported result from quantified absorption of an oral 500 mg calcium oral load, using pharmokinetic methods, among postmenopausal women at lower levels of vitamin D repletion: [mean concentrations 86.5 nmol/L (n=24) and 50 nmol/L (n=24)]. By measuring the area under the curve of serum calcium profiles, the authors found calcium absorption was 65% higher in participants with 25-hydroxyvitamin D concentrations averaging 86.5 nmol/L than those averaging 50 nmol/L. These results suggest that 25-hydroxyvitamin D concentrations of approximately 75 nmol/L may be necessary to maximise calcium absorption.

Further, while the amount of absorbed calcium has some relationship with bone health, using calcium absorption to define vitamin D status for optimal bone health is complicated by differences in calcium intake and variables associated with age. Zitterman *et al* measured fractional calcium absorption in young German women with a high average daily calcium intake of 1200 mg, in summer and winter (n=38 each season; 25-hydroxyvitamin D concentrations $\bar{x}$=30 nmol/L in winter and $\bar{x}$=70nmol/L in summer). The researchers found that while serum 25-hydroxyvitamin D concentrations correlated with both season and calcium absorption, there was no significant association between season and biomarkers of bone formation and resorption (224). Bischoff *et al*, who supplemented elderly living in long stay geriatric care with 1200 mg/d calcium, found that markers of bone resorption fell – but only in those given 800IU vitamin D_3, raising median 25-hydroxyvitamin D concentrations to 65.5 nmol/L (186). While measures of calcium absorption were not reported, it is apparent that the improved vitamin D status improved calcium absorption. If one is to define vitamin D status (with regards to bone health), based on the association between 25-

hydroxyvitamin D concentration and calcium absorption, findings must be considered within the context of other factors such as calcium intake, hormonal status and age, before claiming a risk to bone health. The most easily defendable evidence for defining optimal 25-hydroxyvitamin concentrations comes from studies that measure bone mass, or fracture rates.

4.3 Bone Mass and Fracture

The effect of vitamin D status solely on the risk of falling (discussed in section 3.2.3) may be a confounding factor in osteoporosis studies that use the fracture (clinical outcome of osteoporosis) as the measured endpoint or variable. In the following section of the review no attempt is made to separate this effect from the effect of vitamin D on fracture. However, a number of studies have examined the effect of vitamin D status or supplementation on indices of bone mass. Studies investigating associations between 25-hydroxyvitamin D and low bone mass and/or fracture risk are discussed below.

While a number of intervention studies have investigated the effects of vitamin D supplements (with or without calcium) on incidence of fracture or bone mass, few studies have quantified these effects as a means to discerning appropriate circulating 25-hydroxyvitamin D concentrations (203, 222, 225-230). The largest of these studies reported data from 13432 participants aged 30yrs and older, in the NHANES II survey – a National Survey of the contiguous states of the US from 1988-94. Dual energy x-ray absorptiometry was used to estimate bone mineral density at the hip, which was found to vary significantly by 25-hydroxyvitamin D concentration. In older white Americans, the steep positive slope of BMD against serum 25-hydroxyvitamin D concentration appeared to plateau somewhere between 90 and 100 nmol/L. This data is concordant with results from a 12 months intervention reported by Dawson-Hughes (225), where a small vitamin D supplement (400IU) together with 377 mg calcium was shown to reduce seasonal bone loss from the spine in women, compared to women receiving calcium alone. The treatment group also experienced a smaller seasonal difference in 25-hydroxyvitamin D (mean in winter 92 nmol/L, summer 97 nmol/L). These results suggest that increasing 25-hydroxyvitamin D concentrations to at least 90 nmol/L can result in significant reduction in seasonal bone loss, which may result in a reduced risk of fracture. Conversely, baseline data from an intervention in elderly but mobile Dutch women is suggestive of a relationship between bone mineral density at the femoral neck (hip) only when 25-hydroxyvitamin D concentrations were <30 nmol/L (222).

Ultimately, fracture is the most important endpoint in vitamin D studies, because it is fractures alone (and perhaps reduced muscle function) that are the functional outcomes of inadequate vitamin D status in adults. Several studies have shown combined supplementation with vitamin D and calcium improves BMD and reduces fractures. For example, Dawson-Hughes (225) showed daily supplementation with 10µg vitamin D3 and 377 mg calcium significantly increased BMD at the spine and decreased the risk of vertebral fractures in postmenopausal women. In a French study, supplementing elderly women with 20µg/d vitamin D3 and 1200 mg/d calcium decreased risk of hip and other non-vertebral fractures and increased proximal femur BMD (227). However, the level of 25-hydroxyvitamin D associated with a decreased risk has not been well quantified. In a 5 yr RCT of 2686 participants aged 65-85y, supplemented with 100,000IU D3 every four months or placebo, Trivedi *et al* (229) showed that fracture risk was reduced by 33%. This dose of vitamin D in the same population had previously been shown to raise serum 25-hydroxyvitamin D from a mean of 35 nmol/L to 55 nmol/L over 6 weeks (220). The inconsistency of results amongst these studies may in part reflect differences in calcium intake and other differing factors between populations. It has been suggested that 25-hydroxyvitamin D requirements will therefore differ between groups and populations. Some discrepancies in the serum 25-hydroxyvitamin D concentration associated with risk reduction were addressed in a meta-analysis by Vieth (29), who considered the baseline 25-hydroxyvitamin D of participants, the resulting concentrations associated with a successful reduction in risk of fracture, and standardized these values adjusting for differences in vitamin D determination assays. Vieth has shown that all of the placebo controlled clinical trials that were successful in reducing fracture incidence, raised mean circulating 25-hydroxyvitamin D concentrations by at least 73 nmol/L, yielding a 30% reduction in hip or non vertebral fracture.

Other reviews of criteria for defining vitamin D status based on bone health are summarised in Table 1.10, and estimates for optimal vitamin D status vary from 50 to 80; depending on variable is measured in association with 25-hydroxyvitamin D concentrations, differences in the assays used for vitamin D determination, and other population differences such as calcium intake and age. The majority of evidence has come from older populations; more evidence is necessary from studies of younger populations. While fracture is not an appropriate endpoint for studies of vitamin D status in younger populations, more improved techniques in assessing bone structure and metabolism will aid research in this area. At present, it seems likely that for the general population an optimal

circulating 25-hydroxyvitamin D concentration for bone health is at least 50 nmol/L in, but may be higher (e.g. 80 nmol/L) in older groups.

Table 1.10 Reviews of criteria for defining stages of vitamin D status related to bone health in adults by 25-hydroxyvitamin D concentration

Review (Author, yr)	Vitamin D status by circulating 25-hydroxyvitamin D concentration in nmol/L	
Lips 2001 & 2004 (233, 235, 236)	Severe Vitamin D deficiency	< 12.5
	Moderate Vitamin D deficiency	12.5 – 25
	Mild Vitamin D deficiency	25 – 50
	Vitamin D replete	**> 50**
Vieth 2004 & 2005 (29, 237)	**Osteoporosis prevention**	**70 – 80**
Hollis, 2005 (238)	Deficiency	< 80
	Repletion	**80 – 250**
	Toxicity	> 250
Heaney, 2004 & 2005 (17, 232)	Osteomalacia (risk of)	< 25
	Osteoporosis (risk of)	25-80
	Normal / Optimal	**> 80**

5 Factors associated with 25-hydroxyvitamin D concentrations

As described in section 1, vitamin D_3 is synthesised through the action of ultraviolet B (UVB) light on 7-dehydrocholesterol, which is distributed throughout the epidermis and dermis in the skin of animals (3, 4). While diet can be a valuable source of vitamin D, in most free-living individuals exposure of the skin to sunlight is the largest contributor to serum 25-hydroxyvitamin D concentrations (16). Sun exposure is dependent upon a number of factors including environmental conditions, genetics, age, cultural practices and personal habits, any or all of which may affect the intensity or effectiveness of ultra-violet B light on dermal vitamin D synthesis.

5.1 Environmental conditions: Season, Climate and Latitude

The ultraviolet (UV) light index, developed by the National Weather Service (NWS) and the United States (US) Environmental Protection Agency (EPA) (www.epa.gov), represents the amount of UV radiation reaching the earth's surface at any given time that has the potential to harm the skin. Factors used in its calculation include atmospheric ozone, angle of the sun, ground elevation, and cloud cover. The UV index is reported as exposure categories of Global Solar UV Index. Close to the equator the angle of the sun is acute, and exposure of the skin to sunlight would be expected to permit dermal vitamin D synthesis to occur throughout the year. At greater latitudes sunlight reaches the earth at a more oblique angle thereby reducing the potential for vitamin D synthesis; an effect that becomes more pronounced as latitude increases, particularly in winter months (16, 21, 239, 240). A seasonal variation in serum 25-hydroxyvitamin D concentration is thoroughly described (1, 2, 209, 240-242). Climate also would appear to have an independent effect, due to cold temperature requiring more clothing.

Other environmental conditions with the potential to negatively affect dermal vitamin D synthesis include air pollution and urban living associated with an indoor lifestyle.

5.2 Tanning, Sun Avoidance, Sunscreen and Clothing

Personal habits, such as sunscreen use (243, 244), being homebound, or deliberately avoiding the sun due to either a preference for fair skin or in order to reduce the risk of developing skin

cancer, reduce dermal synthesis of vitamin D. Cultural practices such as complete clothing cover effectively minimise sun exposure (245), and low vitamin D concentrations in veiled women are well described (246, 247). Furthermore, tanners have been shown to have higher 25-hydroxyvitamin D concentrations than non-tanners (248).

5.3 Ethnicity/ Skin colour

A genetic influence on dermal vitamin D synthesis is natural skin pigmentation, with melanin acting as a natural sunscreen (16). Thus, darker skinned people require a longer period of UV exposure to produce an equivalent amount of vitamin D than fairer skinned people (249).

A lower vitamin D blood level in African-Americans than Americans of European descent is well described (138, 250, 251). In New Zealand, Maori and Pacific Peoples have been shown to have lower serum 25-hydroxyvitamin D concentrations than those of European descent (1, 2, 252). Of children presenting with rickets in New Zealand, many are of races with greater skin pigmentation (162).

5.4 Obesity

Obesity has been shown to be associated with lower vitamin D status in New Zealand adults and children, independently of age, sex, ethnicity or season (1, 2). Lower serum 25-hydroxyvitamin D concentrations have been reported elsewhere in obese compared to non-obese subjects (253, 254).

It appears that, as vitamin D is fat soluble, some of it is stored in the adipose tissue. An inverse correlation between body fat content and serum 25-hydroxyvitamin D concentrations in healthy women exists (255). Abdominal fat from obese patients receiving gastric surgery was reported to contain 4-400 ng/g vitamin D_2 and vitamin D_3 (16). In the obese, adipose tissue may serve as an 'irreversible sink' for vitamin D (16, 256). Wortsman and colleagues (256) gave non-obese and obese subjects a 50 000IU oral dose of vitamin D_2 or exposed them to simulated sunlight using a tanning bed and reported that the obese subjects had no more than 50% increase in 25-hydroxyvitamin D concentrations compared with non-obese individuals. There was no significant difference in either skin content of 7-dehydrocholesterol or its percentage conversion to previtamin D_3 or vitamin D_3. This suggests obesity affects the release of vitamin D into the circulation, rather

than the capacity of the skin to produce vitamin D_3, as there are differences between skin surface area in the obese and non-obese.

5.5 Age

Prevalence of low vitamin D status has been shown to be high in elderly populations in New Zealand (2, 257, 258) and elsewhere (259, 260). Elderly with hip (261, 262) or femur (263) fractures have been shown to have high prevalence of vitamin D deficiency or insufficiency.

While ageing does not decrease the efficiency of the intestine to absorb dietary vitamin D (264), skin synthesis of vitamin D becomes less efficient as people get older (265), with decreasing concentrations of 7-dehydrocholesterol (vitamin D precursor) in the skin (266). However, it has been suggested that the lower vitamin D status of older age groups is not inevitable, and is predominantly due to greater sun avoidance, and homebound or institutionalized living (267).

5.6 Diet

There are few good natural dietary sources of vitamin D. Naturally occurring forms of vitamin D are present in foods predominantly of animal origin, such as eggs, liver and fatty fish. Natural food sources of vitamin D are illustrated in Table 1.11. The information shown in Table 1.11 comes from the United States Department of Agriculture, as the New Zealand Food composition database does not include the vitamin D content of foods.

Table 1.11 Natural food sources of vitamin D (268)

Food	Serving size	Amount per serve (IU)	Amount per serve (µg)
Sardines	50g (canned in oil, drained)	250	6.3
Salmon	100g (cooked)	360	9.0
Tuna	85g (canned in oil)	200	5.0
Beef liver	100g (cooked)	15	0.4
Eggs	1 whole (vitamin D is in yolk)	20	0.5

Some foods are fortified with vitamin D. Foods commonly fortified with vitamin D in the world include breakfast cereals, edible oil table spreads and margarines, milks and dairy products such as yoghurt. Permissions or allowances for fortification with vitamin D vary considerably between countries, as does the enthusiasm of industry to fortify certain products.

In countries with vitamin D fortification such as the US and Canada, or in countries with high fatty fish or sea mammal consumption (such as Japan or Alaska, (131, 269-271)), dietary vitamin D intake may make a significant contribution to circulating 25-hydroxyvitamin D concentrations (272). Fish contributes 6.4µg/d and 1.5-1.8µg/d to Japanese and Norwegian vitamin D intakes, respectively (273). Circulating 25-hydroxyvitamin D were significantly higher in participants who consumed oily fish, than those who didn't ($p<0.0001$) in a cohort of British adults (274) and fish consumption was positively associated with serum 25-hydroxyvitamin D in elderly Japanese women (275).

Because vitamin D is not ubiquitous in the food supply, certain dietary patterns and behaviours are associated with an increased likelihood of meeting recommendations for intake. For example, in the US, where many fluid milks are fortified, high milk consumption is a predictor for meeting the vitamin D requirement (276, 277). Intakes of vitamin D are 2-3µg higher in countries, such as the US, Canada and Australia, where there is mandatory fortification of staple foods (margarine and milk) and optional fortification of other food classes is permitted (8, 278).

5.7 Supplement Use

A number of preparations are available that contain vitamin D. A widely available vitamin D supplement is cod liver oil. Many multivitamin/mineral preparations and calcium supplements contain small doses of vitamin D (amount varies between 100-400IU (279)). The vitamin D present in supplements can be present as both vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The supplemental form of vitamin D determines the extent of effect on 25-hydroxyvitamin D concentration, with greater increases resulting from supplementation with Vitamin D_3, compared to D2 (6, 7, 280). Armas *et al* (280) determined that there is at least a 3-fold difference in potency between Vitamin D_2 and Vitamin D_3, with the latter having the greater potency. A lower binding affinity of D_2 metabolites to vitamin D-binding protein, resulting in shorter circulating half-lives, appears to be the cause of these differences (281).

Vitamin D supplements are associated with significantly higher vitamin D intakes (282) and circulating 25-hydroxyvitamin D concentrations, compared to those who do not use them (274, 283, 284). The differences are more pronounced in winter months (284) and in those who have inadequate sun exposure (283). Even in countries where fortification is mandatory, dietary intake of vitamin D may still be insufficient (285). Consequently supplements are needed to ensure adequate

vitamin D status in those who have a high risk of insufficiency, to prevent poor bone health and other potential adverse affects.

6 What level of intake of vitamin D is necessary to achieve optimal status?

Due to the rise in estimates for optimal 25-hydroxyvitamin D researchers have recommended that intakes also increase, in order to achieve these optimal intakes. Consequently, many researchers consider the current recommendations to be inadequate. Carrying out research in the effect of doses above 2000IU have been hampered by early estimates of what constitutes a toxic intake vitamin D. Vitamin D toxicity is discussed below.

6.1 Dose-response Studies

In order to estimate the intake necessary to achieve a given vitamin D status, the relationship between vitamin D intake and circulating 25-hydroxyvitamin D concentrations needs to be clarified. Response of serum 25-hydroxyvitamin D concentrations to different vitamin D dosing regimens can be seen from intervention trials, summarized in table 1.12.

To gain an estimate of the dose-response relationship several studies were combined, nonetheless in order to account for the varying environmental factors that affect vitamin D status the studies had to meet several criteria. Having a placebo/control group was necessary to ensure total end difference in concentrations could be determined. The supplemental form administered had to be vitamin D_3, due to the differences in biopotency between vitamin D_2 and D_3, and serum concentrations either had to a reach a plateau, or study duration needed to be long enough to ensure this was met. Of the studies used, the shortest duration was 12 wk, which is greater than the exclusion criteria of 4 wk used in an earlier review (286). No attempt was made to utilise the method used to analyse 25-hydroxyvitamin D as a criteria for study selection, as this would greatly reduce the amount of data available. Additionally, it was assumed that the differences amongst group means in different publications reflect true differences. By plotting dose against change in 25-hydroxyvitamin D, the overall slope was 0.5 nmol/L, meaning the average increment in serum

Table 1.12 Response of serum 25(OH)D to varying doses in intervention trials

Author	Year	Design	Location	Assay	n	Sex (M/F)	Age[1]	Weight (kg)[1]	BMI[1]	Duratio	Baseline Month	Vehicle	Vit D form	Dose	Base 25(OH)D[1]	Final 25(OH)D[1]	Change[1]	Difference between control treatment	units	Notes
Ooms	1995	Parallel	Amsterdam	Nichols	348	Female	80.6 ± 5.5	71.5 ± 11.7	28.6 ± 4.0	2 yr		Supplement	D3	0	26	23	-3		nmol/L	
							80.1 ± 5.6	70.6 ± 10.9	28.1 ± 4.1					400 IU	27	62	35	38		
Barger-Lux	1998		Omaha	HPLC	13	Male	28 ± 4	81.9 ± 12.9	25.7 ± 3.2	8 wk	January	Supplement	D3	25 µg	67±25		28.6 ± 5.3		nmol/L	
			41□N		10									250 µg			146.1 ± 12			
					14									1250 µg			643 ± 42.7			
Hunter	2000	Parallel	UK	Incstar	64	Female	59.2 ± 5.8	62.9 ± 9.1	24.1 ± 3.2	6 mo		Supplement	D3	0	28.1±11.3	32.3	4.2		nmol/L	
			50-60¼N					62.4 ± 10.2	24.1 ± 3.7					800 IU	28.3±12	44.3	16.1	11.9		
Heaney	2003	Parallel	Omaha,	Nichols	67	Male	38.7 ± 11.2	84.8 ± 11.1	26.2 ± 2.4	120-160	Late	Supplement	D3	0	70.2	58.8	-11.4		nmol/L	Changes are calculated
			41□N	Methods							October			20.9 µg	72.05	84.1	12	32.4		from exponential
														137.5 µ	69.3	161.2	91.9	103.3		models. Actual values
														275 µg	65.6	225	159.4	170.8		only in figure.
Tangpricha	2003	Parallel	Boston	Chen	12		29 ± 9.0			12 wk	Mid March	Orange juice	D3	0	50 ± 10	73 ± 8	22.5 ± 5.0		nmol/L	Plateaued after 4wk & showe
			42□N		14									1000 IU	37 ± 8	94 ± 20	57 ± 7	34.5		gradual increase above 85 nom
Veith	2003	Parallel	Toronto,	DiaSoran	33	10/23	41.6	67.8		5 mo	Jan-Feb	Supplement	D3	25 µg	43.3 ± 16.8	68.7 ± 16.9	25.4		nmol/L	Only 15 in each group complet
			43□N		28	10/18	39.9	66.4						100 µg	37.9 ± 13.4	96.4 ± 14.6	58.5			5 mo
Meier	2004	Prospectiv	Germany.	Instar	43	14/29	57.9 ± 11.3	71.6 ± 15.7	26.2 ± 4.8	6 mo	July	Supplement	D3	0	24.6 ± 9.4	20.5 ± 8.5	-4.1		nmol/L	
			50□N				55.2 ± 10.9	72.5 ± 15.7	26.1 ± 4.4					500 IU	19.5 ± 10	35.1 ± 8.1	15.6	19.7		
Natri	2006	Parallel	Helsinki.	DiaSorin	9		29	66.7	23.1		Feb-March	Control		0			-0.3 ± 4.0			Excluded data regarding consum
			60□N		11		31.1	61.2	22.1			Supplement		10 µg			19.5 ± 10	19.8		of vitamin D fortified bread
Viljakainen	2006	Parallel	Helsinki	HPLC	212	Female	11.4 ± 0.4	42.4		12 mo	Sept-Oct/	Supplement	D3	0	47.8 ± 18.2		-5.0 ± 11.9		nmol/L	Only changes in serum 25(OH)
			60□N								Feb-March			5 µg	46.3 ± 17.4		5.4 ± 15.3	10.4		provided, no information on fi
														10 µg	46.7 ± 16.2		12.1 ± 13	17.1		concentrations
Viljakainen	2006	Parallel	Helsinki	HPLC	12	Female	71 ± 4	67.2 ± 11.4		12 wk				0	52.2 ± 19.9		-8.3 ± 13.2			
			60□N		13		71 ± 5	68.9 ± 12.2						5 µg	46.0 ± 14.3		10.9 ± 8.5	19.2	nmol/L	
					11		72 ± 3	66.6 ± 12.8						10 µg	46.5 ± 10.2		14.4 ± 6.9	22.7		
					13		71 ± 4	71.2 ± 12.5						20 µg	44.1 ± 13.5		23.7 ± 11	32		
Talwar	2007	Parallel	Long	DiaSorin	208	Female	61.2 ± 6.3	79.2 ± 12.6	30 ± 4	3 mo		Supplement	D3	0	43.2 ± 16.8	39.1 ± 18.2	-4.1		nmol/L	Supplementation was increased
			Island				59.9 ± 6.2	78.0 ± 13.6	29 ± 4					20 µg	46.9 ± 20.6	71.4 ± 21.5	24.5	28.6		50 µg/d in the treatment grou
			44□N							2 yr 3 mo				0		41.6 ± 18.1	-1.6			
														50 µg		87.3 ± 27.0	40.4	42		

[1] values are mean ± SD

* Participants initially observed for 12 mo, followed by supplementation for 6 mo

25-hydroxyvitamin D for every microgram (40IU) of vitamin D_3 is 0.5 nmol/L. However, studies using very high doses of vitamin D – not relevant for the general population – had a significant impact on the slope of the line. The dose-response was recalculated based on studies using doses ≤25µg, and the resultant slope was 0.7 nmol/L/µg D_3 **(Figure 2)**. This was identical to the dose-response reported by Heaney *et al* (287). The dose-response selection suffers from an absence of studies intakes of vitamin D between 25-50µg/d. A recent dose-response study carried out by Talwar *et al* (288) produced higher dose-response slopes: 1.1 nmol/L/µg for a dose of 20µg/d and 0.76 nmol/L/µg with a 50µg/d dose.

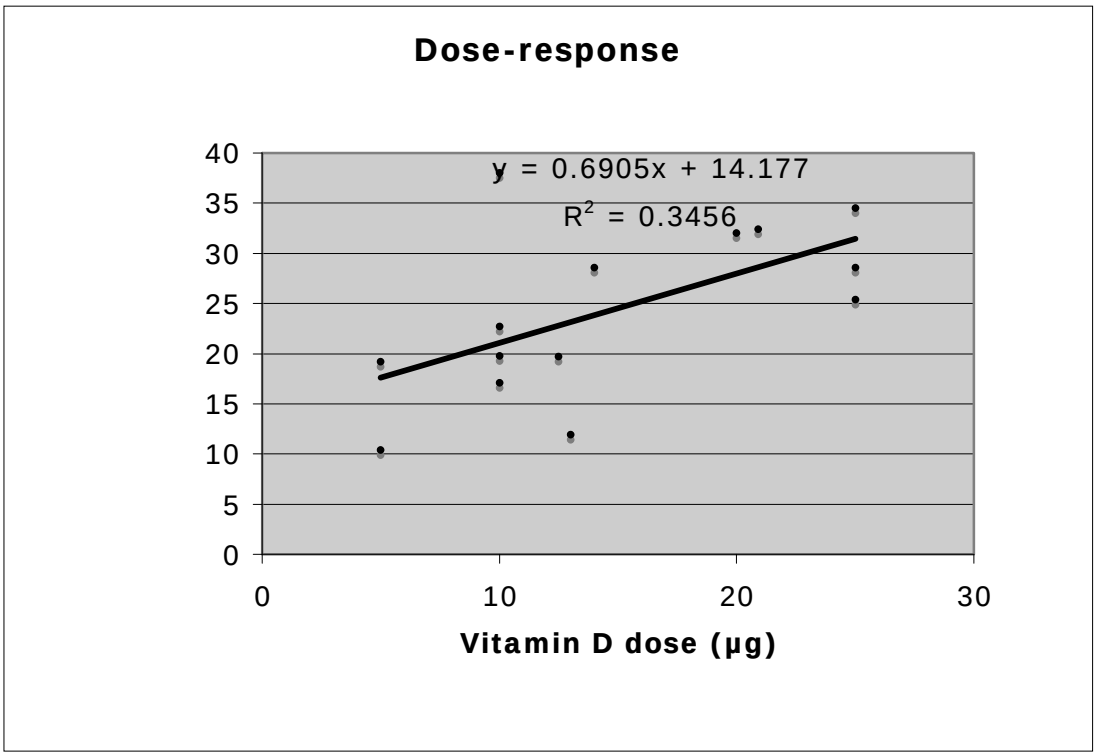

Figure 2: Response of serum 25-hydroxyvitamin D to supplemental vitamin D at levels ≤ 25µg/d. Limited to studies using 0-25µg/d vitamin D_3.

6.2 Toxicity

Of all the vitamins, large amounts of vitamin D are most likely to cause harm. Supplement overdose or less frequently accidental over-fortification of milk are the main causes of vitamin D toxicity. Case reports suggest that several reasons exist for consumption of high doses: industrial mistakes involving improper dosing with a vitamin D supplement (289), fortification error (290), mislabeling of ingredients (291), intentional poisoning (292), or iatrogenic intoxication (293).

Levels of vitamin D in these situations have involved intakes as high as 42 mg (1.7 millionIU) per day. Symptoms include acute renal failure and nephrocalcinosis. Apart from acute toxicity, there is the possibility of chronic toxicity occurring at levels that could be induced by consumption of supplements, fortified foods, and/or foods naturally high in vitamin D.

The purpose of vitamin D fortification and supplementation is to correct an environmental, rather than a classical nutritional deficit, which means that considerations regarding a toxic intake have to take environmental factors into consideration. There has never been a reported case of vitamin D intoxication due to excessive exposure to sunlight. Although large doses of UVB may result in the synthesis of large amounts of previtamin D_3, much of this product is degraded into inert photo-products (5). Nevertheless, total-body sun exposure provides the equivalent of a 250µg oral dose of vitamin D3 (286, 294, 295), which would cause high values for serum 25-hydoxyvitamin D, and in combination with high supplemental intakes may result in toxicity (286).

6.3 Tolerable Upper Level of intake

The Food and Nutrition Board reviewed hypervitaminosis D, of which the major symptom is hypercalcaemia (>2.75 mmol/L) (296). An elevated concentration of 25-hydoxyvitamin D in excess of 400 nmol/L is considered to be the best indicator of hypercalcaemia due to vitamin D toxicity. The Tolerable-Upper Level (UL) of vitamin D for adults was based on an intake of vitamin D that did not put calcium into the hypercalcaemia range and an uncertainty factor which lead to the UL being set at 50µg/d (2000IU). By definition, a UL poses zero risk of adverse effects and the UL for pregnancy was set at the same level as for non-pregnant and lactating women because pregnant women who received supplements of 25-50µg per day (1000-2000IU) showed no adverse effect (297, 298).

Since setting of the UL in 1997, there have been several studies indicating that the UL for adults was set too low, and that intakes of vitamin D above 50µg/d (2000IU) do not induce hypercalcaemia (286, 287, 293), furthermore a risk assessment comparing well-designed trials suggests that vitamin D is not toxic at levels of intake substantially higher than the current UL (299). In a dose-response study, Heaney and co-workers gave up to 250µg (10000IU) vitamin D3 per day to healthy young men for 160 days and found no hypercalcaemia (287).

7 International Experience with Vitamin D

7.1 New Zealand and Australia

The addition of vitamin D to table edible oil spreads and table margarine is mandated in Australia from 5.5 to 16µg/100g. In New Zealand there is no mandatory margarine fortification with vitamin D. However, since 1996 voluntary fortification of margarine, fat spreads and their reduced fat counterparts has been permitted at a maximum claim of 10µg/100g. Further, as most margarine in New Zealand originates from Australia many are fortified. In New Zealand, vitamin D may also be added on a voluntary basis to a range of milk and milk products, some of their legume and cereal equivalents, as well as formulated beverages. The maximum allowable claim per reference quantity for these products ranges from 10 to 25% of the RDI. Vitamin D is also permitted at higher fortification levels in formulated supplementary foods and formulated meal replacements where the maximum claim per reference quantity is up to 50% of the RDI. Current permissions for food voluntarily fortified with vitamin D are given in Table 1.13. Currently, there is no population representative intake data for vitamin D for New Zealanders. The range of vitamin D intakes reported in the CSIRO National Dietary Survey (Baghurst, 1999 in Nowson and Margerison, 2002 (278)) indicates that very few adults reach 5 µg/d. Indeed the highest decile of intake was only 5.55 µg/d, reported in men aged 18-29yrs, and the mean intakes of adult Australian men and women were 2-3µg/d (278). There is no representative data for biochemical vitamin D status of Australians. Mean serum 25-hydroxyvitamin D concentrations, measured in the New Zealand 1997 National Nutrition Survey, were 47 and 52 nmol/L in men and women, respectively (2). Furthermore, based on a cut-off of ≤50 nmol/L the results of this survey indicate that 48% of New Zealand adults were vitamin D insufficient. Results from the Children's National Nutritional Survey 2002 also revealed that mean serum hydroxy concentrations for boys and girls are the same – 47 and 52 nmol/L, for each (1).

The New Zealand Ministry of Health does not recommend routine vitamin D supplementation during pregnancy, lactation, or infancy. However, it is suggested that women (and their infants) who have dark skin, who are housebound, or who do not expose themselves to sunlight for cultural or religious reasons may need a 10µg/d vitamin D supplement under supervision of the lead maternity caregiver. There are likewise no recommendations for routine supplementation in healthy adults although the Food and Nutrition Guidelines for Healthy Adults indicate that vitamin D may be necessary for the institutionalised or the elderly (300).

Table 1.13 Voluntary Vitamin D fortification permissions: FSANZ Food Standards 1.3.2

Food	Reference Quantity	Maximum Claim Per Reference Quantity (proportion RDI)	Maximum Permitted Quantity of Vitamin D per Reference Quantity
Dairy products			
Dried milks	200 mL	2.5 µg (25%)	3.0 µg
Modified milks and skim milk	200 mL	1.0 µg (10%)	1.6 µg
Cheese and cheese products	25 g	1.0 µg (10%)	1.6 µg
Yoghurts (with or without other foods)	150 g	1.0 µg (10%)	1.6 µg
Dairy desserts containing no less than 3.1% m/m milk protein	150 g	1.0 µg (10%)	1.6 µg
Butter	10 g	1.0 µg (10%)	1.6 µg
Edible oils and spreads			
Edible oil spreads and margarine:	10 g	1.0 µg (10%)	1.6 µg
Analogues derived from legumes			
Beverages containing no less than 3% m/m protein derived from legumes	200 mL	1.0 µg (10%)	1.6 µg
Analogues of yoghurt and dairy desserts containing no less than 3.1% m/m protein derived from legumes	150g	1.0 µg (10%)	1.6 µg
Analogues of cheese containing no less than 15% m/m protein derived from legumes	25g	1.0 µg (10%)	1.6 µg
Analogues derived from cereals			
Beverages containing no less than 0.3% m/m protein derived from cereals	200 mL	1.0 µg (10%)	1.6 µg
Formulated Beverages			
	600 mL	2.5 µg (25%)	

7.2 Canada

The addition of vitamin D to milk and margarine, and certain vegetarian foods is mandated in Canada. For fluid milk vitamin D is added to provide 300- 400IU (7.5-10µg) in a reasonable daily intake of 852 mL or 35-47IU (0.9-1.2µg) per 100 mL (301). Evaporated milk, powdered milk, and goat's milk must also be fortified with vitamin D, as must calcium-fortified milks of plant origin (particularly soy), to yield a vitamin D content similar to that of cow's milk. All margarines in Canada are fortified with vitamin D (13µg/100 g) (8). Other foods for which vitamin D addition is permitted are meal replacements, nutritional supplements, and formulated liquid diets. The amounts of vitamin D added depends on the energy content and intended use of the product; for example, for formulated liquid diets, the amount may be no less than 2.5µg and no more than 10µg per 1000 kcal, as long as the intended total energy intake is < 2500 kcal. Currently, with the exception of some egg products, no other fortification with vitamin D is permitted in food. Fortified milk may be used in food manufacturing (e.g., yoghurt), but in practice, milk that is used for baking and for making milk products such as soft and hard cheese is not fortified. There is concern in Canada that with the decline of milk consumption vitamin D intakes may also be falling. A change in government regulation to permit fortification of a wider range of food-stuffs has been advocated by some commentators.

Mean (SE) vitamin D intakes in the 2004 Canadian Community Health Survey (n=35000) were 6.01 ± 0.11µg/d. Fortified milk and margarine products accounted for nearly 80% of this intake. There is no representative data, either national or regional, on the serum 25 OH D concentrations of Canadians. Nevertheless, data from convenience samples suggest that the vitamin D status of Canadians is somewhat better than New Zealanders despite its colder climate and higher latitude.

The current Adequate Intake for Vitamin D used in Canada is 15µg/d for those greater than 70yrs, 10µg/d for those aged between 50-70yrs, and 5µg/d for the rest of the population (296). Health Canada indicates that for some population subgroups it is impossible, or very difficult, to meet these AIs and recommends the use of a supplement. Because breast milk is usually a poor source of vitamin D, Health Canada recommends that all breastfed, healthy term infants in Canada receive a daily vitamin D supplement of 10µg (302). They indicate, “supplementation should begin at birth and continue until the infant's diet includes at least 10µg (400IU) per day of vitamin D from other dietary sources or until the breastfed infant reaches one year of age.” Likewise, Health

Canada recommends that everyone over the age of 50 should take a daily vitamin D supplement of 10µg in addition to following Canada's Food Guide.

7.3 Finland

Due to the widespread prevalence of vitamin D insufficiency in Finland (196, 303) the Ministry of Social Affairs and Health recommended that food manufacturers fortify fluid milks and margarines with vitamin D. As a result, from 2003 milk, butter, milk, yoghurt and milk substitutes (e.g. soy milk) have been fortified at 0.5µg per 100ml and margarines have been fortified and 10µg per 100g, respectively.

Prior to fortification vitamin D intakes have been reported to be between 2.9 and 4.3µg/d in 9-15yrs old Finnish girls (304); 5.8µg/d for adult men and 3.8µg/d for women (305); and median intake was 2.9µg/d in pregnant women (306). The recommended intake of Vitamin D in Finland is 10µg/d for children <2yrs of age, adults >64yrs and pregnant and breastfeeding women. For the remainder of the population, the recommended intake is 7.5µg/d (307).

The effect of this fortification has been examined in children and adult men. Two cohorts of children were examined in wintertime, one in 2001-2002 before fortification, and the other following initiation of fortification in 2003-2004 (308). Four-day food records were used to assess intake of vitamin D. Total daily intakes of vitamin D were significantly higher after fortification – 7.9µg/d (95% CI 6.3-9.6µg/d). Prior to this intakes were 4.4µg/d (95% CI 3.6-5.2µg/d) (308). However, both of these values are less than the recommended intake. Following fortification, male military conscripts were ingesting a mean of 7µg/d vitamin D, from milk and margarine (309). The prevalence of vitamin D insufficiency (serum 25-hydroxy vitamin D <40 nmol/L) also fell by 50% following fortification, from 78% in January 2003 to 35% in January 2004. These measurements were taken in January, as the midwinter values better reflect the long duration of vitamin D insufficiency during wintertime. This then means, at the end of wintertime it is likely that there will be a greater prevalence of vitamin D insufficiency in young Finnish men.

7.4 United Kingdom

Margarine fortification with vitamin D is mandated at a level of not less than 7.05µg and not more than 8.82µg per 100 g (310). The amount of added vitamin D is relatively low, because the purpose of the fortification is only to increase the vitamin D concentration of margarine to concentrations that occur naturally in butter. Some low fat milk and breakfast cereals as well as most dried milk powders are fortified with vitamin D on a voluntary basis. The fortification of low-fat and dairy spreads with vitamin D is not mandated, which is a concern as intakes of these has increased in recent years. Serum 25-hydroxyvitamin D concentrations in surveys of UK adults are similar to those reported for New Zealand adults, around 50 nmol/L (311), though the latitude of the UK (54ºN) is much higher than that of New Zealand (41ºS).

The UK Reference Nutrient Intake (RNI) is 10µg/d for men and women over 65yrs, 8.5µg/d for infants under 6 months, and 8.5µg/d for children from 6 months to 3yrs (312). There is no RNI for other groups. Mean vitamin D intakes for British adults (19-65yrs) were 3.7 ug/d for men and 2.8 ug/d for women (313). With the exception of infant preparations vitamin D is currently available without prescription as a dietary supplement only as part of cod liver oil or multivitamin products. The UK health departments recommend a daily dose of 10µg/d vitamin D for breastfed infants from 6 months or from 1 month if there is any doubt about the mother's vitamin status during pregnancy (312). The UK Health Department also recommends 10µg/d vitamin D for pregnant and breastfeeding women as part of a multivitamin supplement (310). Children of low income parents are entitled to free vitamin supplements from 6 months of age until their fourth birthday under the "Healthy Start" program. Likewise, low income women are entitled to free vitamins during pregnancy and up until their baby is one year old (314). The Food Standards Agency, in a recent report, concluded that most active older people with a healthy diet should not need to take extra vitamin D. But they said older people should consider taking extra vitamin D, 10 micrograms per day (or 400 units), if: they rarely get outdoors or are housebound, they always cover up all of their skin when outside, they don't eat meat or oily fish.

7.5 United States of America

In the United States it is permitted to add vitamin D to a wide range of foodstuffs including, for example, RTE breakfast cereals, yoghurt, milk, and calcium fortified orange juice (Table 1.14). The addition of vitamin D to eligible foods, with the exception of fortified milk, in the United States

is optional. Further, fluid milk in the United States, unlike Canada, is not required to have vitamin D added unless the label declares that it is fortified. In practice, however, > 90% of fluid milk in the market place is fortified. Vitamin D is permitted to be added to milk at up to 2.1µg/100 g, with 1.0µg/100g per day the usual amount added. In the US National Health and Nutrition Examination Survey III data, conducted in 1988-94, mean (SE) dietary intake of vitamin D was 5.9 ± 0.2µg/d, the majority coming from fortified foods (8). For example, of the 5.4µg/d vitamin D consumed by males 19-50yrs, 2.3µg/d of vitamin D came from fortified milk, 0.3µg/d from fortified breakfast cereals, 1µg/d from other fortified foods, and 1.9µg/d from natural sources. Mean serum 25-hydroxyvitamin D in NHANES, in the southern latitudes for the months November–March ranged from 60 nmol/l in women 80yrs or older to 79 nmol/ in men 12–19yrs; concentrations in the northern latitudes in the months April–October ranged from 62 nmol/l in women 80yrs or older to 90 nmol/l in men 12–19yrs. The mean serum 25-hydroxyvitamin D in the New Zealand NNS was 50 nmol/L, somewhat lower than in the US. One possible explanation for this difference is greater food fortification in the US but supplement use may also play a role.

Recommended intakes of vitamin D are identical to Canada. The American Association of Paediatrics recommends a daily supplement of 5µg vitamin D for breastfed infants beginning within the first 2 months of life unless they are weaned to receive at least 500 ml per day of vitamin D-fortified formula (171). The US Office of Dietary Supplements also indicates that people with limited sun exposure, dark-skinned people, and older people may also benefit from a supplement (268).

Table 1. 14 Lawful addition of vitamin D to foods in the United States (8).

TABLE 1 Lawful addition of vitamin D to foods in the United States (Adapted from Calvo et al, 2004)

Category of food	Fortification status	Maximal level allowed [1]	Surveyed products fortified with vitamin D	
			Estimate of fortified products	Usual Fortification Level
Cereal flours and related products				
Enriched Farina	Optional	8.75 µg/100 g	Few	
Ready-to-eat breakfast cereals	Optional	8.75 µg/100 g	Most	1 - 3.5 µg (10–35% DV)
Enriched rice	Optional	2.25 µg/100 g	None	None
Enriched corn meal products	Optional	2.25 µg/100 g	None	None
Enriched noodle products	Optional	2.25 µg/100 g	None	None
Enriched macaroni products	Optional	2.25 µg/100 g	Very few	1 µg/252 g (10% DV)
Milk				
Fluid milk	Optional	2.1 µg/100 g	All	10 µg/quart or 946 mL
Acidified milk	Optional	2.1 µg/100 g	All	10 µg/quart or 946 mL
Cultured milk	Optional	2.1 µg/100 g	All	10 µg/quart or 946 mL
Concentrated milk	Optional	2.1 µg/100 g	All	10 µg/quart or 946 mL
Nonfat dry milk fortified with A and D	Required	2.1 µg/100 g	All	10 µg/quart or 946 mL
Evaporated milk, fortified	Required	2.1 µg/100 g	All	10 µg/quart or 946 mL
Dry whole milk	Optional	2.1 µg/100 g	All	10 µg/quart or 946 mL
Milk products				
Yogurt	Optional	2.25 µg/100 g	Few	1 - 2 µg/RACC [2]
Low fat yogurt	Optional	2.25 µg/100 g	Few	1 - 2 µg/RACC [2]
Nonfat yogurt	Optional	2.25 µg/100 g	Few	1 - 2 µg/RACC [2]
Margarine	Optional	8.3 µg/100 g	Few	1 - 3.5 µg/RACC [2]
Calcium-fortified fruit juices and drinks [3]	Optional	5 µg/RACC	NA [4]	2.5 µg/RACC

1. Maximal level of vitamin D that can be added in accordance with 21 CFR 184.1 (b) (2) for the category of food.
2. RACC, reference amount customarily consumed or the US FDA regulatory serving size.
3. Vitamin D_3 may be added, at levels not to exceed 100 IU per serving, to 100% fruit juices, excluding fruit juices that are specially formulated or processed for infants, which are fortified with > 33% of the RDI of calcium per serving.
4. NA, not appropriate; it is premature to evaluate the number of products in the market place given that the regulation was approved in April 2003.

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