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VITAMIN D STATUS: DETERMINANTS, OPTIMAL LEVELS, AND SUPPLEMENTATION

Catherine Jane Bacon

A thesis presented in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Auckland, 2009.
ABSTRACT

Vitamin D deficiency, indicated by the circulating metabolite 25-hydroxyvitamin D [25(OH)D], can lead to osteomalacia, increased fracture risk in the elderly, and may increase the risk of other medical conditions. However, optimal levels of 25(OH)D are uncertain, with some cross-sectional studies suggesting optimal levels of >75 nmol/L. This thesis assessed optimal levels of vitamin D and strategies for its supplementation.

In a trial of high-dose vitamin D₃ regimens in frail elderly, data suggest that 25(OH)D levels of 40 – 50 nmol/L may be sufficient. In the same study, calcium intake appeared to modify the relationship between 25(OH)D and PTH and subsequent estimates of optimal 25(OH)D based on these data may be lower when calcium intake is >1552 mg/day. It was also noted that large loading doses (500 000 IU) rapidly normalise 25(OH)D levels, whilst monthly 50 000 IU doses were also effective but took 3 – 5 months to reach plateau.

An analysis of adverse events recorded for a 5-year calcium trial in postmenopausal women showed that whilst season-adjusted baseline 25(OH)D levels <50 nmol/L increased the risk of stroke and a composite event (stroke, myocardial infarction or sudden death) compared to levels ≥50 nmol/L, these effects disappeared when adjustment for baseline confounders was made.

Data from two studies indicate that vitamin D deficiency or insufficiency is prevalent amongst urban Chinese women of childbearing age, and that in the Auckland region young Maori and Pacific women and children and adults of Middle Eastern, Southern Asian and African ethnicity are over-represented in the group of people identified as vitamin D deficient or insufficient. In a final study of middle-aged and older New Zealand men, more than half (55%) reported use of dietary supplements which may make a contribution to vitamin D status.

In conclusion, data here suggest that 25(OH)D levels of 50 nmol/L may be satisfactory for bone health, that large loading doses of vitamin D₃ are safe with respect to hypercalcaemia and effective, and that a number of non-elderly populations are at high risk of having insufficient vitamin D status.
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Secondly I would like to thank Dr Mark Bolland for his assistance and feedback on various sections of this thesis. He provided helpful feedback on oral and written reports of data reported in Chapter Five. Dr Bolland also conceived the idea for a prospective investigation of the relationship between baseline 25(OH)D levels and adverse events in a large calcium intervention completed within this research group (Chapter Six) the idea of completing an additional analysis of dietary supplement use by men enrolled in another calcium intervention (Chapter Eight). For Chapter Six, I’d also like to acknowledge Dr Bolland’s help with the statistical analyses. For Chapter Seven, Dr Bolland has completed a parallel investigation using the same data set and incorporating some analysis, which was completed by myself, and is also included in this thesis.

My appreciation also goes to staff in the Clinical Bone Group at the University of Auckland, including Dr Anne Horne, Dr Andrew Grey, Ruth Ames, Barbara Mason and Diana Wattie who helped me with setting up and running the research reported in Chapter Five, provided feedback on oral reports of these data, and made me feel very welcome in our basement premises. Particular acknowledgement is extended to Dr Horne for her feedback on Chapter Five, and to Ruth Ames and Barbara Mason for the collection and management of data reported in Chapters Six and Eight. I would also especially like to thank our departmental statistician, Greg Gamble, for his advice and assistance with analyses for Chapters Three to Six.

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My own contribution to Chapter Six entailed formulating an analysis plan to address the research questions described therein, and then writing this chapter.

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CHAPTER 1: INTRODUCTION

Vitamin D is a seco-steroid and the precursor to metabolites with endocrine actions in many cells. Like other steroid hormones, it binds to a nuclear receptor and acts via activation or repression of DNA transcription [2, 3]. Vitamin D is also known to have more rapid, non-genomic actions [4, 5]. Vitamin D is produced in the skin in response to ultraviolet (UV) radiation in the B wavelength range (290 – 300 nm). Relatively small quantities may also be obtained from a limited number of foods, notably oily fish and fortified products, and larger quantities from over-the-counter or prescription supplements. Subsequent metabolism to its active metabolites, 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D], takes place in the liver and kidneys, respectively. Renal production of 1,25(OH)2D is tightly regulated by parathyroid hormone (PTH) in response to circulating calcium, and consequently assays for 25(OH)D are used to determine vitamin D status.

The classical role of the most active vitamin D metabolite, 1,25(OH)2D, and its less active precursor, 25(OH)D, is to raise circulating calcium levels by enhancing intestinal calcium absorption [6]. Vitamin D metabolites also regulate both calcium and phosphate through actions within bone cells and renal tubules [2, 3, 7]. In addition, autocrine production of 1,25(OH)2D has been implicated with effects in many other cells which contain vitamin D receptors, including parathyroid, epidermal, digestive tract, gonadal and white blood cells [8, 9].

Severe vitamin D deficiency disrupts bone mineralisation causing osteomalacia, or rickets in children [10]. Low levels of 25(OH)D that are not sufficiently depleted to result in osteomalacia or rickets, may still increase the risk of osteoporosis due to chronic elevation of PTH (secondary hyperparathyroidism) [8]. A vast body of literature exists, including interventional, prospective cohort and cross-sectional investigations with fracture risk and changes in bone mineral or bone turnover markers as outcomes. Recently, these have been systematically reviewed for a United States government report on the effectiveness and safety of vitamin D in relation to bone health [11, 12]. The authors concluded that fair evidence exists in older adults for a small benefit of vitamin D supplementation, when taken in combination with calcium, on fracture risk and bone mineral changes, and for an association of 25(OH)D with hip bone mineral density. Evidence for an association of 25(OH)D with fracture risk and all skeletal outcomes in other age groups was inconsistent [11, 12].
Added to the uncertainty over whether vitamin D interventions are beneficial, the optimal levels of 25(OH)D are a matter of ongoing debate. Even amongst experts, taking the entire body of literature into account, estimates vary from 50 – 80 nmol/L [13]. Various methodologies support these estimates. Some evidence comes from considering the relationship between 25(OH)D and 1,25(OH)\textsubscript{2}D or calcium absorption, which have produced estimates of optimal 25(OH)D ranging from 40 – 80 nmol/L [14-16]. However, such interpretation of these data is questionable due to regulatory processes which maintain 1,25(OH)\textsubscript{2}D with diminishing 25(OH)D and elevated PTH levels, and the confounding effects of age and renal function. Accordingly, another methodology entails the consideration of the relationship between 25(OH)D and PTH itself. Many studies have investigated a threshold for 25(OH)D, above which PTH plateaus at a minimal level. Estimates of optimal 25(OH)D based on these data do not elucidate the issue, since they vary from 25 – 122 nmol/L [17]. Moreover, an important limitation is that they are invariably based on cross-sectional data and there is substantial variability in PTH observed at particular levels of 25(OH)D [18, 19].

The potential consequences of a detrimental effect of low 25(OH)D levels on skeletal health are most severe in elderly, particularly men [20], when hip fractures are a common antecedent of death in the 3 – 6 months following fracture, and are also associated with an increased 4 – 5-year mortality risk [21, 22]. Fracture risk in elderly people related to low vitamin D status may be exacerbated by reduced muscle function and increased falls risk, as meta-analyses of vitamin D randomised, controlled interventions show a reduction in falls [23, 24], though not exclusively [25]. Consequently, most previous studies investigating optimal levels of 25(OH)D are of people within this demographic. However, low vitamin D status is not limited to the elderly and has been frequently reported in younger populations, particularly in people of Asian, Middle Eastern or African ethnicity, either living in these regions or migrated to Western countries [26-32].

Vitamin D status has also been linked to a multitude of non-skeletal health outcomes. These have been postulated to come about through local production of 1,25(OH)\textsubscript{2}D, in cells that possess both the enzyme for its production and a vitamin D receptor, acting in an autocrine manner that is not regulated via the classical calcium-PTH feedback loops [33, 34]. Suggested benefits of a high vitamin D status include a reduced risk of infections, autoimmune and chronic diseases such as heart disease, types 1 and 2 diabetes, multiple sclerosis, rheumatoid and osteoarthritis, respiratory infections, inflammatory bowel diseases and many types of cancer [33, 35, 36]. Levels of 25(OH)D are also associated with mortality after adjusting for confounders [37] and a meta-analysis of vitamin D interventions has confirmed a small benefit of vitamin D
supplementation on mortality [38]. However, apart from falls risk, very few data from interventional trials exist and most of the evidence comes from epidemiological and laboratory studies [39]. Furthermore, individuals with low vitamin D status are older, engage in less physical activity, are more overweight, have a lower dietary energy and associated nutrient intakes, and smoke more than their replete counterparts [40, 41]. They may also differ in other respects, notably in the frequency of pre-existing pathology associated with chronic disease risk and poorer health status. Such variables are likely to confound the results of all studies that are not randomised, controlled interventions.

Despite the equivocal evidence base for an effect of 25(OH)D on clinical outcomes or their diagnostic intermediates, another approach for estimating optimal levels of 25(OH)D is possible. This approach entails defining thresholds for various outcomes, including skeletal. Many vitamin D interventions, especially those with primary skeletal outcomes, have measured baseline and end-of-study levels of 25(OH)D. Consequently, levels associated with beneficial outcomes can be investigated. Similar comparisons of data from prospective cohort or cross-sectional studies can also be applied. A review, taking this approach concluded that optimal levels were at least 75 nmol/L, and may be higher, between 90 – 100 nmol/L, for some outcomes [42]. However, a more recent attempt to answer this question using a similar approach for skeletal outcomes was unable to obtain a clear answer due to the use of different assays and lack of a validated method for 25(OH)D assessment [11].

Though many studies have examined optimal levels of 25(OH)D by examining its cross-sectional relationship with physiological or clinical outcomes, few have examined this question using longitudinal data. A methodology that reduces inter-individual variability and is more clinically relevant entails seeking a threshold level of 25(OH)₂D at which beneficial effects associated with vitamin D supplementation are attenuated. Longitudinal studies examining this question in relation to calcium absorption and its physiological regulator 1,25(OH)₂D are limited by uncertainty over whether there is a threshold level of 25(OH)D at which additional vitamin D fails to elicit further increases in absorption. They are also limited by whether failure to invoke increases in 1,25(OH)₂D are due to its tight regulatory control rather than being indicative of sufficiency. Examination of this question using clinical outcomes is limited by doubts about whether circulating 25(OH)D is causally associated with these outcomes, and by whether vitamin D interventions are able to elicit any effect on them at all. Consequently, the first aim of this thesis will be to ascertain optimal 25(OH)D levels using longitudinal changes in PTH. Secondly, this thesis will identify determinants of 25(OH)D in two cohorts of young Chinese
women and in frail elderly. Data will also be analysed to establish factors that may influence the relationship between 25(OH)D and PTH. The third aim of this thesis will be to prospectively analyse the effect of baseline 25(OH)D on health-related outcomes. Fourthly, this thesis will examine the demographic composition of those with conservatively defined vitamin D insufficiency or deficiency. The final aim of this thesis will be to provide data relating to vitamin D supplementation. This aim will be two-fold. Firstly, the efficacy and safety of high-dose oral vitamin D regimens will be addressed and, in the penultimate chapter, it will describe the prevalence of vitamin D and other supplement use amongst middle-aged and older men.

Specific content of each chapter is summarised as follows:

In Chapter 2 (Literature Review) of this thesis, the molecular biology, metabolism and actions of vitamin D are more fully described with an emphasis on its role in maintaining calcium homeostasis. The various approaches for determining optimal levels of 25(OH)D are thoroughly reviewed, with a detailed consideration of factors shown to impact upon the observed relationship between 25(OH)D and PTH, namely methodologies applied in the existing body of literature, season of measurement, demographic factors, renal function, calcium intake, anthropometry and reproductive hormones. Issues relating to the efficacy and safety of vitamin D supplementation are then reviewed. Finally, determinants of vitamin D status are addressed in turn, with particular focus directed to the skin and sun exposure, seasonal variation, vitamin D intake and body composition.

In Chapters 3 (Effects of Vitamin D Supplementation on Parathyroid Hormone Levels in Premenopausal Chinese Women), 4 (Relationships Between 25-Hydroxyvitamin D and Parathyroid Hormone and Their Determinants in Two Cohorts of Premenopausal Chinese Women), and 5 (Pharmacodynamics of High Dose Regimens of Oral Vitamin D₃ in Frail Elderly), an optimal level of 25(OH)D is sought by examining its relationship with PTH. The studies described in Chapters 3 and 5 are primarily interventional studies of vitamin D, in Chapter 3 as a constituent of a milk product. Hence, longitudinal data are available to determine whether there was a threshold level of baseline 25(OH)D at which supplementation with vitamin D did not result in reductions in PTH.

In Chapter 3, analyses are undertaken in a cohort of young women, a demographic for which there is sparse data in relation to the question of an optimal vitamin D status. In Chapter 4, data from the cohort described in Chapter 3 are pooled with a similar cohort to increase the statistical power of the investigation. In this chapter, an optimal level of 25(OH)D is sought via analysis of
its cross-sectional relationship with PTH. Further, in this chapter determinants of 25(OH)D and PTH were analysed and the role of calcium intake and adiposity indices in modifying the relationship between them.

Osteoporosis that leads to fracture has the most serious consequences for frail elderly. Therefore, in Chapter 5, the question of an optimal vitamin D status is addressed in this important group, through analysis of changes in both PTH and a bone turnover marker (procollagen type I amino-terminal propeptide or P1NP) in relation to initial 25(OH)D levels. The study described in Chapter 5 also compares the efficacy and safety of three regimens of oral vitamin D₃ supplementation, using the only high-dose preparation available for subsidised prescription in New Zealand, which contains a dose of a size not readily available in other parts of the world.

In Chapter 6, the question of whether levels of 25(OH)D significantly predict a range of 5-year outcomes is addressed, using adverse events and other data collected as part of a calcium intervention in 1471 women living in the Auckland region. Because important differences between those with high compared to low vitamin D status potentially exist, the data have been analysed with a number of confounders adjusted for, including season of measurement, age, physical activity, body composition and nutritional factors in addition to separate consideration of the treatment and control groups.

In Chapter 7, analysis is of data from all tests for 25(OH)D requested within the Auckland region of New Zealand over an 15-month time period. Here, the purpose was to describe, demographically and ethnically, the population established to have levels of 25(OH)D conservatively regarded as being insufficient or deficient. We also compared the characteristics of those screened for and detected as vitamin D insufficient or deficient between hospital-and-community-based doctors.

In Chapter 8, the prevalence of vitamin D and other dietary supplement use in middle-aged men is investigated. In addition, this survey aimed to establish the amount of money spent on supplements, reasons for use and sources of information. Finally, the study aimed to assess the possibility that consumption of lipid-soluble vitamins may have reached risky levels when combined with dietary food intake.

A summary of the conclusions from these studies and an evaluation of the contribution of these data to the body of knowledge in the field appear in Chapter 9.
CHAPTER 2: LITERATURE REVIEW

2.1 Physiology of Vitamin D and the Regulation of Calcium Homeostasis

2.1.1 Vitamin D, Parathyroid Hormone & Calcium Homeostasis

The major role of vitamin D in the human body is in the regulation of blood calcium levels. Notwithstanding, it also plays an important role in phosphate homeostasis and in the regulation of various bone factors. Since its receptor is expressed in a wide variety of other tissues, other direct actions are likely, although the physiological importances of these are unclear [7, 43]. Extracellular calcium is maintained between 2.1 and 2.6 mmol/L (8.5–10.5 mg/dL and deviations outside these parameters result in critical changes in physiological functioning [6]. Hypocalcaemia, which results in sensory and muscular hyperexcitability or respiratory tetany in extreme cases, is caused by a calcium-mediated increase in neuronal permeability to sodium [6]. In contrast, hypercalcaemia results in muscle weakness and lethargy, itching, reduced gastrointestinal motility, renal stones, and headaches [44]. Only about half of the plasma calcium exists in free ionized form. The rest is bound: predominantly to protein, with a small amount (around 4%) bound to anions such as bicarbonate and phosphate [44]. If plasma protein levels rise, then more of the total calcium is bound and thus not physiologically active, and vice versa. Consequently, in routine clinical biochemistry, it is usual to adjust plasma calcium for albumin content since albumin is responsible for around 80% of plasma protein calcium transport [45].

Bone, which contains 99% of body calcium, provides a vast reservoir of this mineral. Bone forming cells (osteoblasts) ultimately control the deposition of inorganic calcium in the form of hydroxyapatite crystals, whilst bone resorbing cells (osteoclasts) control its catabolism to calcium. Three hormones regulate calcium balance. Parathyroid hormone (PTH) and hormonally active vitamin D metabolites have the net effect of raising blood calcium levels through action on bone metabolism, distal tubular renal reabsorption and intestinal absorption. Calcitonin has been shown to reduce artificially induced hypercalcaemia in dogs [46] and, in supraphysiological doses, to lower plasma calcium in young rats [47]. Despite this, there is no evidence that it has a role in the normal calcium homeostasis of adult humans [46, 47].

Because calcium phosphate has a low solubility, excess precipitation occurs when the levels of either phosphate or calcium rise. Consequently, independent alterations in phosphate inversely affect calcium levels and their homeostasis is closely linked. Both PTH and vitamin D
metabolites regulate phosphate in addition to calcium. Vitamin D enhances intestinal phosphate absorption and may promote renal reabsorption [6, 7]. PTH, conversely, prevents renal phosphate reabsorption, thereby increasing its urinary excretion [6].

2.1.2 Parathyroid Hormone Structure, Regulation & Action
Parathyroid hormone is an 84 amino acid polypeptide synthesised and released from the chief cells of the four parathyroid glands located on the dorsal aspect of the thyroid gland. It is secreted in a pulsatile manner, about 7-8 pulses per hour [10], with a normal circulating level of between 1.1 and 6.8 pmol/L (10–65 ng/L) [48]. It is synthesised from a longer polypeptide chain primarily in response to reduced extracellular calcium but also to raised extracellular phosphate [49]. Calcium binds to a G-protein coupled receptor (the calcium sensing receptor) on the chief cell membranes. The cellular response is amplified through adenylyl cyclase which when activated converts intracellular adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) [6]. The consequent inhibition of PTH synthesis and release [49], is mediated through the production of protein kinase A and phosphorylation of other intracellular proteins [6]. Hence, more calcium binding results in less PTH secretion and, conversely, reduced bound calcium increases PTH secretion. Since PTH also ultimately raises extracellular calcium, calcium and PTH form part of a classical endocrine negative feedback loop whereby their levels are normally self-regulating.

The mechanism of the hypercalcaemic action of PTH is threefold. Firstly, PTH mobilises calcium from bone. Surprisingly, the osteoclasts responsible for bone resorption do not contain PTH receptors. Instead, bone resorption occurs indirectly through osteoblasts, which do have PTH receptors, and paracrine factors, including an osteoclast differentiation factor known as receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin (OPG) [6]. When bone cell exposure to elevated PTH is continuous, the production of RANKL on the cell membrane of osteoblasts is stimulated [50]. RANKL, in turn, interacts with a receptor on the surface of preosteoclasts, resulting in accelerated production of mature osteoclasts and bone resorption [9, 51]. Production of OPG, which blocks the interaction of RANKL with its preosteoclastic receptor, is also reduced [50]. Paradoxically, during intermittent administration of PTH, or its synthetic polypeptide fragments, osteoblastic bone formation dominates [51-54]. This is probably due to raised levels of OPG [51, 55].

Secondly, PTH stimulates calcium reabsorption in the distal tubule of the kidneys. PTH has a negligible or even inhibitory effect on proximal tubule calcium reabsorption, which constitutes
about 60% of the filtered load and occurs in parallel with Na\(^+\) transport [44, 56]. Almost all of the non-protein-bound calcium is filtered, and normally about 2.5% of this is excreted [44]. Despite its reabsorptive action in the distal tubule, elevated PTH tends to increase urinary calcium excretion because of the increased filtered load that results from hypercalcaemia due to its bone and intestinal effects [44, 56]. However, urinary calcium is not necessarily proportional to the level of circulating calcium as it is affected by many other renal, hormonal factors as well as some medical conditions and drugs [57]. PTH also inhibits the expression levels of two different sodium-phosphate co-transporters in the kidney proximal tubule, which reduces phosphate reabsorption. The integrated effect of PTH on bone and kidney cells explains the gradual bone mineral loss which characterises chronic hyperparathyroidism [8].

The third hypercalcaemic action of PTH is to indirectly enhance intestinal absorption of calcium. In hypocalcaemic conditions, PTH causes increased production of the enzyme 1\(\alpha\)-hydroxylase which catalyses the hydroxylation of 25-hydroxyvitamin D (25(OH)D) to its more active form 1\(\alpha\),25-dihydroxyvitamin D (1,25(OH)\(_2\)D) [7]. The active vitamin D metabolite acts to increase calcium absorption in the small intestine which is thought to be mainly in response to increased production of a calcium binding protein (calbindin D\(_{9k}\)) through a classical steroid hormone gene transcription mechanism [7].

PTH may also reduce 1,25(OH)\(_2\)D breakdown via an inhibition of the enzyme 24-hydroxylase in the renal proximal tubule [56], although it may have the opposite effect in the distal tubule, resulting in increased deactivation of all vitamin D metabolites [7]. 1,25-Dihydroxyvitamin D in turn, negatively feeds back on the gene expression of both PTH and 1\(\alpha\)-hydroxylase to limit its own production [7, 44, 58, 59].

PTH is broken down in the liver and kidney and has a half-life of less than 20 minutes [6]. The initial steps in its catabolism entail cleavage into large polypeptide fragments. Since only the first 34 amino acids from the amino terminal end (of the original 84) constitute the active portion of PTH there was formerly some difficulty in the interpretation of PTH radioimmunoassay as fragments of the hormone, which were included in the assay, might or might not retain biological activity [44]. Fragments of PTH are now generally assumed to lack the active domain [60] and only intact assays are used.

Clinical manifestations of primary hyperparathyroidism and the rarer hypoparathyroidism directly parallel the respective symptoms of hypercalcaemia and hypocalcaemia previously
outlined (refer to Chapter 2.1.1 Vitamin D, Parathyroid Hormone & Calcium Homeostasis, pg. 7). Primary hyperparathyroidism is a common condition in the elderly and is often picked up through raised calcium identified during routine blood screening and then confirmed by serum PTH measurement [6]. It may result from a parathyroid adenoma, generalised hyperplasia of the glands, or rarely by carcinoma [10]. Secondary hyperparathyroidism results from low plasma calcium, which normally results from solar/nutritional vitamin D deficiency, gastro-intestinal and hepatic disorders, or chronic renal failure [10].

2.1.3 Vitamin D Synthesis & Metabolism

Vitamin D is a generic term applied to the two precursors of metabolically active sterol hormones in the broader vitamin D group: vitamin D₃ (cholecalciferol) and vitamin D₂ (ergocalciferol). Steroids are, or are derived from, compounds containing a characteristic tetracyclic skeleton of cyclopenta[α]phenanthrene [61] (Figure 2.1). Strictly speaking, the vitamin Ds are seco-B-steroids, meaning that the second or “B” hexagonal carbon ring has been opened. In vitamin D, this involves a bond rupture between carbons 9 and 10 and subsequent rotation about its carbon 6 and 7 single bond to a more stable trans conformation [61, 62]. Vitamin D₃ is produced naturally in response to solar radiation from 7-dehydrocholesterol (7DHC), also known as provitamin D₃, which is synthesised from cholesterol and present in the skin of humans and most other vertebrates [62]. It is also ingested, usually in small quantities, in a limited number of foods such as oily fish and some livers. Vitamin D₂ is the plant-derived form of the vitamin, synthesised naturally or artificially by irradiation of plant ergosterol [8]. The metabolism of the two forms is very similar and until recently they have been considered to be equally bioactive in humans [63]. Consequently, throughout the remainder of this thesis, the subscript to indicate the D₃ form of vitamin D metabolites has been dropped, except when referring to D₃ specifically.

Conversion of 7DHC to vitamin D₃ is a two step process which takes place in the epidermis of the skin and is not thought to require any other activating peptides [62]. The first step produces an intermediate (previtamin D₃). It entails a carbon 9-10 lysis using energy absorbed from ultraviolet (UV) light in the B (UVB) or long-wave C (UVC) spectra. This conversion is followed over the next three days by spontaneous, thermally dependent isomerisation to vitamin D₃, which is enhanced by active translocation of vitamin D₃ from the epidermal production sites to the capillaries deeper in the dermis [64]. Skin production is to some extent self-regulating. During prolonged exposure to UV both previtamin D₃ and vitamin D₃ undergo isometric conversion to biologically inert products [65-67].
Both the intensity and the specificity of wavelength of UV radiation are important in determining vitamin D production in the skin. Epidermal conversion of 7DHC to previtamin D₃ occurs with wavelengths between 260 and 315 nm: the optimal range of its action spectrum is between 295 and 300 nm [68] (Figure 2.2). Atmospheric ozone blocks UV wavelengths shorter than 290 nm and the spectral irradiance (W/cm²) of UV light hitting the Earth’s surface increases logarithmically up to a plateau at around 330 nm [68]. Natural solar radiation that initiates this conversion is therefore UVB in the range 290 – 315 nm [69], with a theoretical ideal at slightly longer wavelengths than the peak of the action spectrum due to the rapidly increasing level of spectral irradiance at wavelengths in this region [68] (Figure 2.2). Under normal circumstances therefore, vitamin D production takes place within a solar radiation range that is very close to defined ranges of the UVB spectrum (280 or 290 – 315 or 320 nm) [7, 70-72], although there is minimal stimulation of epidermal 7DHC production at the highest extent of this range.

Figure 2.1 The Characteristic Tetracyclic Skeleton of Cyclopental[α]phenanthrene That All Steroids Possess or From Which They are Derived. Carbons in the ring are numbered as shown. Adapted from Leigh et al. 1998 pp. 122 [61].
Activation of vitamin D to 25(OH)D occurs in the mitochondria of hepatic parenchymal cells and is catalysed by 25-hydroxylase [7]. Further hydroxylation to the more active 1,25(OH)₂D takes place in proximal tubule cells of the kidney via mitochondrial 25-hydroxyvitamin D 1α-hydroxylase [7]. A summary of the metabolic pathway for the production of 1,25(OH)₂D₃ is shown in Figure 2.3 With the exception of pregnancy, during which it is produced by the placenta, 1,25(OH)₂D synthesis was thought to occur solely in the renal cells [62, 73]. However, anephric patients do have measurable levels of 1,25(OH)₂D₃ and both extrarenal expression of 25-hydroxyvitamin D 1α-hydroxylase and mediation of the reaction by other enzymes and regulatory processes have been postulated [7, 74, 75]. Malignant and non-malignant cells, notably of the colon, have been shown to possess 25-hydroxyvitamin D 1α-hydroxylase [76-78]. Production of 1,25(OH)₂D has also been demonstrated in keratinocytes [79], lung [80] and prostate [81] cancer cell lines, and is assumed to be autocrine in nature: with local production followed by rapid side-chain hydroxylation to inactive metabolites [78]. Vitamin D metabolites are transported in the blood bound to albumin-like vitamin D binding protein, to which 25(OH)D has the highest affinity. Since vitamin D binding protein itself is normally only about 5% saturated, almost all the 25(OH)D and more than 99% of 1,25(OH)₂D is bound to either vitamin D binding protein or albumin [3, 7, 82].
The enzyme vitamin D 24-hydroxylase enables the deactivation of both 25(OH)D and 1,25(OH)\(_2\)D to 24,25-dihydroxyvitamin D (24,25(OH)\(_2\)D) and 1,24,25-trihydroxyvitamin D respectively [7, 44, 83]. Divergent pathways that convert 25(OH)D\(_3\) to other forms have also been shown [84, 85]. Turnover of 25(OH)D is slow; toxicity may take as long as 2 months to reverse [10]. The rate of turnover is likely to depend on a number of factors including calcium intake and residual PTH levels [86], dietary fibre intake [87] and the size of the vitamin D pool [88]. The last of these is thought to be dependent upon adiposity since vitamin D is lipid soluble and is likely to disperse throughout the body fat compartments [89, 90].

Measurements of the half-life of 25(OH)D\(_3\) have been made using tritium-labeled hydrogen radioisotopes which are normally placed on carbons 1 and 2 or 26 and 27 and administered intravenously or orally. Estimations have varied from 12 to 27 days [84] and depend on the amount of dietary fibre [87] and whether initial levels are taken into account [91]. Using an analytical method whereby carbons not involved in hydroxylation were labeled using non-ionising deuterium and measured via mass spectrometry, Vicchio et al. [84], observed a 25(OH)D half-life of only 10.4 days in four men. They hypothesised that previous estimates of half-life were overestimated due to a deceleration of oxidation of isotopically labeled sites. Because of a high risk of hypercalcaemia with administration of hydroxy or dihydroxy vitamin D metabolites, vitamin D itself is the preferred first-line therapy for vitamin D deficiency [7].

Despite the number of studies investigating the pharmacokinetics of 25(OH)D, there is far less data establishing the time-course of its response to vitamin D supplementation. One such study noted that a peak 25(OH)D was attained in 13 – 21 days following a single 300 000 IU (7.5 mg) vitamin D\(_3\) dose [92]. The half-life for 25(OH)D in this study was 90 days. Other authors have demonstrated that steady state levels of 25(OH)D are approached at around 60 days in response to daily 1000 IU (25 \(\mu\)g) vitamin D\(_3\) doses and at around 100 days in response to daily 5000 IU doses [93]. To date, no studies have investigated vitamin D pharmacodynamics, in terms of the time-course of response of PTH, or other metabolite indicative of its physiological activity, after supplementation.
Figure 2.3 Metabolic Pathway of Vitamin D and Its Metabolites.
Adapted from Zhu and Okumura, 1995 [62].
2.1.4 Actions of Vitamin D and Its Metabolites

Vitamin D action is thought to occur through genomic mechanisms involving a classical steroid-hormone nuclear receptor and through rapid non-genomic mechanisms which act independently of this receptor. Classic nuclear vitamin D receptors are expressed in a wide variety of tissues, notably in the small intestine and bone cells, but also for example in parathyroid chief cells, epidermal keratinocytes, activated B and T lymphocytes, mononuclear cells, and cells in the stomach, pancreas, colon, muscle, brain, breast, pituitary and gonads [7-9, 94]. A range of different genotypes of vitamin D receptor are known to exist and though it has been suggested that receptor phenotype might affect vitamin D metabolism or action, the results of studies remain equivocal [95]. A recent meta-analysis failed to show any difference in bone mineral density between five polymorphisms, although one was associated with a reduction in vertebral fracture risk of borderline statistical significance [96]. Vitamin D-mediated rapid responses that occur independently of the vitamin D receptor have been studied in cells in a range of organ systems including digestive, skeletal, epithelial, muscular, immune, and parathyroid cells [4, 5].

Both 1,25(OH)₂D and 25(OH)D bind to the vitamin D receptor, although the receptor affinity for 1,25(OH)₂D is 1000 times higher than that for 25(OH)D [7]. However, since the serum concentration of 25(OH)D is in the order of 1000-fold higher [7, 97], its contribution to receptor activation cannot be overlooked. Investigating the functional importance of distinct vitamin D metabolites on calcium absorption, Heaney et al. [98] have estimated that 25(OH)D explains 14% of the total vitamin D-related absorptive activity. Notably, one of the effects of raised 25(OH)D might be to displace 1,25(OH)₂D from its binding protein, thus increasing activity with no increase in total serum levels [98].

Ligand-receptor complexes up-regulate the expression of genes bearing vitamin D response elements in their promoter regions [95, 99]. Some responses appear to occur more rapidly than can be explained by genomic mechanisms. These rapid responses to vitamin D metabolites have been studied in a range of cells including those in the digestive tract, liver, muscle and bone [4, 5]. Both 1,25(OH)₂D₃ and genomically inactive 24,25(OH)₂D₃ have been shown to elicit rapid effects and the existence of two independent membrane receptors has been postulated [5].

The primary physiological action of vitamin D is to increase calcium (and phosphate) absorption in the small intestine. Nuclear vitamin D receptors are found throughout the small intestine but are most concentrated in the duodenum, whilst most phosphate absorption is thought to take place in the distal small bowel [7]. In the intestine, vitamin D-receptor complexes regulate
calcium binding protein calbindin D<sub>9k</sub>, which facilitates calcium transport through the intestinal epithelial cells [3, 7], presumably by allowing high quantities of calcium in the cells whilst maintaining low free intracellular calcium concentrations to be maintained for normal cellular function [44]. The existence of rapid non-genomic calcium transport stimulated by vitamin D metabolites is also well established and known as transcalcaltachia [100].

In bone, vitamin D metabolites also act to increase or maintain blood calcium. Like PTH, the main effects on resorption are mediated through osteoblast cells and their precursors rather than osteoclasts directly. In supraphysiological doses, 1,25(OH)<sub>2</sub>D causes increased osteoblast production of RANKL and its receptors, which stimulates osteoclast differentiation and hence resorption [7, 9, 101]. Moreover, it is known to stimulate osteopontin, an osteoclast attachment protein which stimulates resorption [10, 94]. There is some evidence that vitamin D metabolites also have non-genomic effects on bone which may parallel genomic actions in osteoblasts [5]. Despite its accelerative effect on bone turnover, when dietary intake of calcium and phosphorus intake is sufficient, the overall action of vitamin D is likely to be osteogenic. This is mainly due to increased levels of calcium and phosphate available for mineralisation [7, 95]. Some recent work suggests that 1,25(OH)<sub>2</sub>D may also have a more direct role in protecting bone mineralisation. Suda et al. [101], have shown that at physiological doses, 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibits PTH induced expression of RANKL, thus inhibiting resorption. In addition, 1,25(OH)<sub>2</sub>D-stimulated calbindin D<sub>28k</sub> is present in osteoblasts and may be protective of osteoblastic apoptosis [99]. 1,25 Dihydroxyvitamin D (1,25(OH)<sub>2</sub>D) is also known to stimulate osteoblast production of bone matrix proteins such as osteocalcin and alkaline phosphatase [10, 94], although the functional significance of this is not clear [8].

The existence of functionally important vitamin D receptors is well established in the other calcium regulating organs, namely the kidney and parathyroid glands [9, 59, 102]. Vitamin D metabolites have important regulatory effects on vitamin D 24-hydroxylase in the kidney [7, 99] and on PTH in the parathyroid gland [7, 59]. They may also have effects on calcium and phosphate renal reabsorption, although these remain unsubstantiated [7].

2.1.5 Regulation of Vitamin D Activity

The production of vitamin D is ultimately dependent upon reactions that take place in the skin. The rate of the initial conversion of 7-dehydrocholesterol (7DHC) to previtamin D is affected by the skin pigment melanin. Melanins are a biogenetically related group of complex, tyrosine-derived molecules of uncertain structure [103]. They are capable of powerful radiation
absorption [104]. They are produced by melanocytes in the strata basale, the innermost layer of the epidermis [105]. Melanins are then translocated in pigment granules termed melanosomes, to adjacent epidermal cells. These undergo migration towards the outer skin surface over about two weeks. Migration to superficial cell layers is relevant to previtamin D₃ production since the two innermost layers of the epidermis contain the highest concentration of 7DHC [64, 105]. The concentration of 7DHC is unlikely to limit production since previtamin D₃ levels plateau at around 10-15% of the original 7DHC concentration [65].

It has been estimated that about 1.6 million years ago, early hominids lost hair over most of their bodies and with it evolved melanin-pigmented skins [106]. These authors have suggested that selection pressure to avoid radiative destruction of folate led to the evolution of deeply pigmented skins in equatorial regions. At higher latitudes, selection pressure led to a reduction in pigmentation to ensure adequate vitamin D production. Whilst vitamin D production is therefore constrained by melanin concentration, epidermal reactions also prevent excess production. During prolonged UV exposure, rather than forming vitamin D₃, previtamin D₃ is converted to biologically inert isomers lumisterol₃ and tachysterol₃ [65].

There is minimal regulation of the liver enzyme 25-hydroxylase and therefore of 25(OH)D synthesis. This step in vitamin D metabolism is only limited in very advanced liver failure [7]. Production of 25(OH)D therefore depends mainly on vitamin D synthesis and dietary intake. As outlined previously, the alternate pathways of 25(OH)D hydroxylation on either carbons 1 or 24, determines its hormonal activity. Accordingly, these steps are the key regulatory points in vitamin D metabolism.

Gene expression of both vitamin D 1α-hydroxylase and 24-hydroxylase are tightly controlled by the hormones of calcium and phosphate homeostasis as well as by the concentrations of the ions themselves. Enzyme synthesis, of 1α-hydroxylase in particular, is also affected by the levels of other hormones. During hypocalcaemic conditions, up-regulation of 1α-hydroxylase by PTH accounts for the classical response, stimulation of 1,25(OH)₂D production, although there is some evidence that lowered calcium levels also contribute directly [7]. Reduced or elevated phosphate concentrations also respectively up- or down-regulate synthesis of the enzyme [7, 44]. When calcium levels are normal, 1,25(OH)₂D limits its own production by down-regulating 1α-hydroxylase. Calcitonin also increases synthesis of the enzyme by a relatively small amount compared to PTH [7]. During lactation, prolactin stimulates 1α-hydroxylase synthesis, allowing optimal absorption of calcium during milk production [6, 7]. There is some evidence that
oestrogen, other gonadal steroid hormones and growth hormone also stimulate $1,25(OH)_2D$
production [7, 44].

Vitamin D 24-hydroxylase results in deactivation of the vitamin D metabolites insofar as their
 genomic actions are concerned. Nonetheless, genomically inactive $24,25(OH)_2D$ may itself
initiate direct, rapid actions [5]. Up-regulation of 24-hydroxylase synthesis in the intestine and
kidneys by $1,25(OH)_2D$ primarily control mechanism for this enzyme [99]. PTH also inhibits 24-
hydroxylase synthesis in the renal proximal tubules (refer to Chapter 2.1.2 Parathyroid Hormone
Structure, Regulation & Action, pg. 8), however, the effects of high $1,25(OH)_2D$ appear to
dominate in primary hyperparathyroidism, hence catabolism of $25(OH)D$ is accelerated and the
turnover of vitamin D metabolites is very high [7]. In these conditions, inadequate
photosynthesis or intake of vitamin D becomes a more important issue.

The potential importance of the regulation of vitamin D metabolism by the vitamin D receptor
and binding protein cannot be overlooked. Recent results have shown that mice with inactivated
vitamin D binding protein genes retain normal serum levels of calcium and PTH, despite having
markedly reduced levels of $25(OH)D$ and $1,25(OH)_2D$ [107]. On the other hand, mice expressing
vitamin D binding protein compared to their knock-out counterparts, are able to prolong the half-
life of $25(OH)D$ in response to a vitamin D deficient diet and are less susceptible to toxicity and
hypercalcaemia from surplus vitamin D intake [102]. It therefore seems likely that the role of
vitamin D binding protein is to prevent rapid fluctuations in vitamin D metabolites [7]. Vitamin
D receptors are also known to undergo regulation. When dietary calcium intake is sufficient,
$1,25(OH)_2D$ up-regulates the vitamin D receptor gene and PTH down-regulates it [94]. On a low
calcium diet though, $1,25(OH)_2D$ stimulation is attenuated and the repressive effect of PTH
dominates [95].

Despite its high activity, the tight regulation of $1,25(OH)_2D$ makes it unsuitable as an indicator
of vitamin D status [8]. Moreover, the levels of this metabolite are low and unstable compared to
the major transport form, $25(OH)D$ [97]. Levels of $25(OH)D$ undergo seasonal fluctuations and
correlate more strongly with the levels of other calcitropic hormones and markers of calcium
status [108]. They are also a good parameter of gut calcium absorption [109]. These factors make
$25(OH)D$ the accepted measure of vitamin D status.
2.2 Vitamin D Deficiency & Insufficiency

2.2.1 Osteomalacia, Rickets & Secondary Hyperparathyroidism

Severe vitamin D deficiency causes osteomalacia, or rickets when it occurs before puberty. The condition results from impaired mineralisation of newly formed bone, causing an excess of extracellular non-mineralised bone matrix, called osteoid tissue [10]. Vitamin D deficiency may be due to inadequate sunlight or dietary supply, chronic liver or renal disease, anticonvulsant therapy, 1α-hydroxylase deficiency, or vitamin D resistance. Hypophosphataemia may also result in similarly impaired mineralisation since sufficient levels of both calcium and phosphate are required at the site of mineralisation.

Osteomalacia presents as nonspecific, isolated or generalised bone pain, muscle aches and weakness [8, 9, 110]. It is diagnosed histologically by quantifying the degree of osteoid tissue and rate of mineralisation [10]. Vitamin D deficiency is also commonly indicated by elevated alkaline phosphatase and low serum calcium, phosphate, and urine calcium excretion with associated bone pain and muscle weakness [8]. In children, long bone growth, known as endochondral growth, occurs via an intermediate cartilaginous stage. Rickets is characterised by an overgrowth of cartilage at the epiphyseal growth plates, causing severe bone pain, bone end deformities and stunted growth [10]. Despite lowered serum 25(OH)D, levels of 1,25(OH)₂D tend to be maintained or increased due to hyperparathyroidism secondary to a lack of absorbed calcium [7, 8]. Other blood chemistry can be within the normal range, although serum calcium and phosphate and urine calcium may be reduced, especially in malabsorptive or solar/dietary deficiency [8]. Urine phosphate and alkaline phosphatase can be increased as consequences of the inhibitory effect of raised PTH on renal phosphate reabsorption and its accelerative effect on bone resorption respectively [8]. In chronic renal disease, while serum calcium is likely to be low, serum phosphate may actually be high due to decreased glomerular filtration [8].

Rickets and osteomalacia are generally thought to be associated with levels of 25(OH)D less than 20 to 30 nmol/L, and consequently these levels often demarcate definitions of vitamin D deficiency [8, 111, 112]. However, severe dietary calcium deficiency is recognised to contribute to rickets in sunny parts of the third world [113], so direct comparison with rachitic levels of 25(OH)D that would occur in a normal Western diet is not possible. In a recent systematic review of studies investigating vitamin D efficacy and safety, Cranney et al., noted a surprising large range of mean 25(OH)D levels associated with rickets in infants and children under 5 years. About half the relevant studies obtained reported levels less than 27.5 nmol/L, as would be
anticipated, but in the other half, means ranged from 36 to 50 nmol/L. The authors attributed the higher levels to low calcium intakes that in the study populations, which were predominantly from Africa and the Middle East [11].

A state in which low levels of vitamin D, though asymptomatic, are thought likely to increase the occurrence of adverse skeletal (or other) outcomes has been termed vitamin D insufficiency. The term vitamin D deficiency is used to refer to a more extreme state in which histological bone changes, such as high turnover or defects in mineralisation, may be evident [8]. In these states, the risk of osteoporotic fractures is thought to rise due to lowered bone mineral density (BMD) and possibly through reduction of muscle strength and increased falls [8]. Commonly, PTH is chronically elevated in vitamin D deficient populations [8] and this secondary hyperparathyroidism is likely to be the primary mechanism of bone loss. In chronic hyperparathyroidism, especially that resulting from renal insufficiency when 1,25(OH)2D levels are diminished, circulating calcium is low, and phosphate elevated, normal regulatory processes are disrupted and a perpetuating positive feedback cycle may arise. Hypocalcaemic stimulation, lack of inhibition by 1,25(OH)2D and hyperphosphataemia each independently increase PTH secretion [60]. In addition, pathologies of parathyroid tissue characterised by collections of cells with no vitamin D receptor, and hence an unresponsiveness to the PTH-lowering effects of 1,25(OH)2D, can occur [2].

2.2.2 Optimal Level of 25-Hydroxyvitamin D

There is substantial uncertainty about the levels of serum 25(OH)D that indicate vitamin D insufficiency or deficiency, and consequently below what level supplementation ought to occur. When osteomalacia, related to vitamin D deficiency, is established histologically 25(OH)D is often less than 15 nmol/L, and can be below 5 nmol/L [114]. Notwithstanding, risk of osteoporosis is probable at levels considerably above this. Estimates of the lower reference limit vary considerably: from as low as 10 nmol/L up to 50 nmol/L [8]. Divergence of opinion as to what are optimal levels is even greater. Recently, Dawson-Hughes et al. [13] reported that estimates by a panel of six prolific authors in the vitamin D field, of minimum serum 25(OH)D levels optimal for fracture prevention, varied from 50 to 80 nmol/L. Some prominent researchers in the field argue strongly in favour of higher minimum reference levels, e.g. Hollis, 2005 for 80 nmol/L [115]; Bischoff-Ferrari, 2007 for 75 nmol/L [116]; and Vieth, 2006 for 75 nmol/L [117]. Another [86] has argued that current knowledge only justifies a minimum reference level of 50 nmol/L and this viewpoint was more recently supported in a round table discussion by the European Society on Clinical and Economic Aspects of Osteoporosis and Osteoarthritis [118].
Delegates at a recent osteoporosis forum in Australia contended that bone health may be improved by attaining levels above 50 nmol/L but failed to reach a consensus regarding what the lower reference limit ought to be [119]. The difference between these recommendations is not a trivial matter in economic terms if broad scale supplementation is contemplated. Whilst 32% of postmenopausal European women have been shown to have 25(OH)D levels below 50 nmol/L, 80% are below 80 nmol/L [120]. Similarly, recent population data from New Zealand adults shows that 52% and 86% of females and 45% and 82% of males fall below 50 nmol/L and 80 nmol/L respectively [121]. The cost of supplementing all New Zealand adults below the lower reference limit with therapeutic vitamin D₃ (cholecalciferol) would be 75% greater if 80 nmol/L was used as the cut-off compared to 50 nmol/L. In practice, the cost of testing 25(OH)D levels is vastly greater than supplementation. Consequently, if evidence was considered sufficient to support improved health outcomes above 80 nmol/L, population-wide supplementation or food fortification would need to be seriously considered.

Formerly, definitions of vitamin D sufficiency were commonly based on levels of 25(OH)D present in healthy populations. Population norms vary widely, however, due to differences in latitude, climate and behavioural factors which affect typical UVB exposure [8, 122]. Besides, it has been argued that modern vitamin D levels are an inappropriate standard as they are unlikely to match those that humans have evolved in response to [39, 115]. Individuals exposed to sunshine or high levels of oral vitamin D typically exhibit 25(OH)D levels in excess of 140 nmol/L with no adverse effect [123]. The degree of physiological redundancy in these levels for maintaining maximal calcium absorption and minimal calcium loss from bone is not known. Furthermore, it is not clear if the endocrine activity of 25(OH)D was comparable during earlier stages of human evolution: racial differences in PTH response to 25(OH)D have been noted [26, 124], and increased fracture rates or other morbidities in the aged population today is undoubtedly a more detrimental trade-off to higher vitamin D status than in prehistoric times when the human lifespan was likely to be markedly shorter [125].

Various outcome measures have been used to establish functional sufficiency and optimisation of vitamin D status. Vitamin D’s primary physiological role is to elevate calcium absorption through the action of its metabolites and osteoporosis is the most important potential consequence of its chronic insufficiency. Accordingly, the most apposite indicators of vitamin D status might be expected to be the levels of 25(OH)D which maximise calcium absorption [16, 115, 126], those that provide no substrate limitation to 1,25(OH)₂D production [86], or those that minimise adverse bone measurements such as bone mineral mass or fracture rates [8, 13, 86,
The concept of a 25(OH)D threshold that denotes the boundaries of these effects has become an implicit indicator of optimal vitamin D status [16, 86, 115]. Since it is generally accepted that sustained elevation of PTH secondary to vitamin D insufficiency is the primary mechanism for an adverse effect on bone [8], the level of 25(OH)D that results in a subsidence in secondary hyperparathyroidism has also been identified as a key indicator of optimal vitamin D status [8, 115]. Indeed, identification of an upper level of 25(OH)D which results in a plateau in the decline of PTH level has been the most frequently employed research paradigm aimed at defining optimal vitamin D status [19, 128-131].

Notwithstanding its key role in bone health, vitamin D supplementation, or a higher vitamin D status, has been linked with other beneficial health effects. These have been well reviewed and include improved muscle function and functional activities [9, 23, 36, 132-134] improved insulin sensitivity and glucose levels [36, 135] reduced hypertension [36] and reduced risk of autoimmune diseases and some cancers including breast and colorectal varieties [9, 39, 66, 136-138]. Improved muscular functioning or glucose handling, associated with vitamin D levels, may also reduce the rate of falling. A number of cross-sectional, prospective and interventional studies have investigated falls risk in relation to 25(OH)D levels, or the effect of vitamin D supplementation on falls. Whilst a meta-analysis and some larger interventional studies tend to support an effect of vitamin D supplementation on falls, particularly in less active cohorts and when it is provided with calcium [23, 139-141], results are equivocal with some interventions reporting no effect [25, 142-144]. Although there exist far fewer randomised controlled trials of calciferol on non-skeletal than bone-related health, Bischoff-Ferrari et al. have reviewed levels of 25(OH)D associated with improvements in multiple health outcomes and suggested an overall minimum target level of 75 nmol/L [42]. A recent meta-analysis of controlled calciferol interventions has also shown a slightly reduced all-cause mortality relative risk of 0.93 [38]. Although the level of 25(OH)D associated with increased mortality has yet to be established, this study is of note because it highlights the possibility that even very small beneficial effects of vitamin D in the prevention of a range of common life-threatening conditions may have important implications for public health.
2.3 Seeking a 25-Hydroxyvitamin D Threshold From Investigations of Vitamin D-Health Outcome Relationships

2.3.1 Measurements of Calcium Absorption & 1,25-Dihydroxyvitamin D

Since they are functionally indicative of its role in calcium homeostasis, data from a number of studies employing 1,25(OH)₂D levels and calcium absorption as outcome variables provide evidence about optimal vitamin D status. Some earlier studies examined vitamin D sufficiency by establishing the threshold for substrate-dependent synthesis of 1,25(OH)₂D [reviewed in 86]. In cross-sectional investigations, positive correlations between 25(OH)D and 1,25(OH)₂D are presumed to indicate a dependence on the 25(OH)D substrate for 1,25(OH)₂D production, and hence suboptimal status. This presumption is not necessarily valid since both metabolites bind to a common protein, and levels of the vitamin D binding protein might also tend to create a positive relationship between them. Competition between 25(OH)D and 1,25(OH)₂D for binding sites on the protein might also create more complex associations since unbound levels of both metabolites may directly or indirectly affect levels of the other. Also, since 1,25(OH)₂D circulates at levels 1000-fold lower than 25(OH)D and is under tight endocrine control, very small alterations in 1,25(OH)₂D levels might have important effects on the observed relationship between the two metabolites. Another fundamental difficulty with using the 1,25(OH)₂D versus 25(OH)D relationship to establish optimal vitamin D status is that 25(OH)D itself almost certainly engenders some biological activity [7]. Hence, there may very well be enhanced physiological benefits at higher levels of 25(OH)D than those required to optimise 1,25(OH)₂D production.

Despite the theoretical difficulties of estimating optimal level of 25(OH)D from its relationship with 1,25(OH)₂D, positive correlations have been demonstrated in populations with very low levels of 25(OH)D (mean ± sd) (19-29 ± 11-21 nmol/L) [145-147] and in another population with moderate levels (68 ± 29 nmol/L) [148]. In contrast, another study which calculated the production rate of 1,25(OH)₂D observed an inverse relationship between this rate and 25(OH)D levels in a 51 healthy women with moderate 25(OH)D levels (62 ± 22 nmol.L) [149]. Since aging increased 1,25(OH)₂D and reduced 25(OH)D levels in this study, age may be a confounding factor which masked a possible substrate dependence for 1,25(OH)₂D. Need et al. [15] observed a relationship between 1,25(OH)₂D and 25(OH)D that was only positive above a 25(OH)D level of 40 nmol up to a peak of 100 nmol/L. Below 40 nmol/L 25(OH)D was inversely related to 1,25(OH)₂D and 1,25(OH)₂D was most strongly correlated with levels of PTH. It appears from this study that when vitamin D insufficiency is more severe, in this case
<40 nmol/L, elevated PTH may serve to raise 1,25(OH)₂D at the expense of 25(OH)D. More recent work by the same authors, analysing the clinic records of 319 osteoporosis patients without renal insufficiency whose 25(OH)D levels were <40 nmol/L, found that decreasing 25(OH)D levels were not associated with reduced 1,25(OH)₂D levels until 25(OH)D fell below 15 nmol/L [150]. In older populations, renal insufficiency is common, and a reduction in 1,25(OH)₂D would very likely be apparent at higher levels of 25(OH)D. Although, these findings may call into question the utility of the 1,25(OH)₂D versus 25(OH)D positive relationship as a indicator of vitamin D status, one interpretation of data from the earlier Need et al. study [15] is that since a positive relationship between 25(OH)D and 1,25(OH)₂D was noted above 40 nmol/L up to a 25(OH)D level of 100 nmol/L, that 25(OH)D limits 1,25(OH)₂D production to at least this level and the optimal 25(OH)D level is actually higher.

A longitudinal approach, which investigates the effect of initial 25(OH)D on 1,25(OH)₂D increase following vitamin D supplementation, provides more conclusive evidence for establishing a causative relation between the two metabolites, although estimation of optimal vitamin D status from these data is still subject to many of the theoretical difficulties associated with the cross-sectional approach. Two vitamin D interventional studies that have investigated 1,25(OH)₂D change following supplementation have noted an increase when baseline 25(OH)D was less than 30 nmol.L⁻¹ [14] and no increase when mean baseline 25(OH)D was greater than 40 nmol.L⁻¹ [151].

Studies of calcium absorption employ measurements of isotopically-labelled calcium, which is not readily available, or alternatively, they may estimate it from the area under the curve for a load-induced change in serum calcium. Although calcium absorption varies markedly throughout a typical range of 25(OH)D levels [109], studies investigating calcium absorption as an indicator of vitamin D status do not typically display clear 25(OH)D thresholds. An earlier example of such a study, of high-dose vitamin D₃ and 25(OH)D supplementation by healthy young men, showed that change in absorption efficiency was significantly related to post-treatment 25(OH)D levels [98]. No threshold in the level of change in calcium absorption with increased change in 25(OH)D was apparent from the data. In fact, the relationship between changes in absorption efficiency and 25(OH)D changes held for very large changes in 25(OH)D of up to 1000 nmol.L⁻¹. When the authors combined their data with an analysis of data from a previous study [152], they showed an increasing linear relationship between treatment doses of 25(OH)D up to about 500 μg.day⁻¹ (1.25 mmol.day⁻¹) and changes in calcium absorption [98]. From these data, it appears that calcium absorption continues to increase even when 25(OH)D levels are likely to be
elevated approximately 10 to 20 fold greater than normal. Vieth, [39], also argues that the relationship between 25(OH)D and calcium absorption does not appear to reach a plateau, citing data from an unpublished abstract (Bischoff-Ferrari et al, 2003 In: Proceedings of the 5th International Symposium on Nutritional Aspects of Osteoporosis). Similarly, Hansen et al. found 12.5% increases in calcium absorption following high dose vitamin D2, in postmenopausal women with initial 25(OH)D levels between 40 and 60 nmol/L, but do not report a threshold [153]. The absence of a useful calcium absorptive threshold might not be surprising given that high doses of vitamin D are toxic due to the resulting hypercalcaemia and hypercalciuria which develop [154]. Therefore, the results of a more recent study of healthy men whose 25(OH)D levels were well above average presents somewhat of a conundrum [155]. In this study, mean 25(OH)D levels dropped from 122 to 74 nmol.L⁻¹ from late summer to late winter. Despite this, there was no significant concurrent reduction in calcium absorption fraction. Thus, Heaney and coauthors have shown that while estimated calcium absorption increases within the 25(OH)D range of 50 to 87 nmol.L⁻¹ [109], within the range of 74 to 122 nmol.L⁻¹ calcium absorption fraction does not [155]. On this basis and incorporating estimated calcium absorption data from lower 25(OH)D levels [140], Heaney has argued for the existence of a 25(OH)D threshold of 80 nmol.L⁻¹ [16]. Whether calcium absorption is indeed able to provide a solid basis for calculation of such a threshold is still equivocal.

In summary, evaluation of data from studies examining the relationships between levels of 25(OH)D and 1,25(OH)₂D or calcium absorption do little to elucidate what are optimal levels of 25(OH)D. Prospective analyses of the 25OH)D relationship with 1,25(OH)₂D suggest optimal 25(OH)D levels of 30 – 40 nmol.L⁻¹ [14, 151] whilst investigations of the 25(OH)D – calcium absorption relationship suggest optimal levels of around 80 nmol/L [109]. Furthermore, theoretical difficulties associated with the assumptions made in establishing optimal levels from these relationships may impact upon conclusions.
2.3.2 Muscle Functioning or Falls (Table 2.1)

A growing body of evidence can be used to estimate optimal vitamin D status with respect to a secondary physiological role associated with improved muscle physiology. A number of larger descriptive studies provide evidence of a positive relationship between 25(OH)D and muscle function [134, 156-160], though not all [161]. Three of these studies report cross-sectional associations between 25(OH)D and functional test results in 237 postmenopausal women [156] and in 4100 older participants in the third National [US] Health and Nutrition Examination Survey (NHANES III) [160], but do not show any plateau in test outcomes below 25(OH)D levels of at least 120 nmol/L. In the NHANES III study [160], a locally-weighted regression plot showed that the greatest rate of improvement in lower extremity function occurred below 25(OH)D levels of 40 nmol.L\(^{-1}\), although there was a continued gradual improvement above this level. Another large cross-sectional study, with 976 older participants [157], reported differences in physical performance and hand-grip strength above and below 25 nmol/L and 50 nmol/L respectively. One prospective study noted a cross-sectional relationship between 25(OH)D levels and various measures of muscle function and an increased 3-year fracture risk when baseline 25(OH)D levels were less than 50 nmol/L although the difference in fracture rate was not significantly different when levels above and below 75 nmol/L were compared [158]. Levels of 25(OH)D are likely to be highly correlated with sun exposure, which could itself be strongly associated with many factors influencing muscle function and falls risk including physical activity, mobility, and other lifestyle behaviours. Although multiple statistical corrections for socio-demographic and lifestyle variables were performed in the NHANES III study, prospective or interventional studies have the potential to better elucidate links between 25(OH)D, muscle function and falls. A 3-year prospective Dutch study demonstrated that low levels of 25(OH)D (<25 nmol/L compared to >50 nmol/L) and elevated PTH levels (≥4.0 pmol/L compared to <3.0 pmol/L) increased the risk of sarcopenia, defined by the degree of reduced grip strength and appendicular skeletal muscle loss. Similarly, a recent prospective study showed that functional recovery during acute post-hip-fracture rehabilitation was independently associated with both 25(OH)D (positively) and PTH levels (inversely) [134].

Currently, the interventional evidence that vitamin D supplementation reduces falls risk is equivocal. In fact, two recent meta-analyses have drawn seemingly contradictory conclusions. Whereas Latham et al. [25] showed no evidence of the effect of vitamin D supplementation on falls risk, a meta-analysis published a year later by Bischoff-Ferrari et al. [23] showed more than a 20% reduction in falls risk following vitamin D supplementation. The different findings can be
explained by the studies that were included or omitted from these analyses and by the fact that Latham et al. quantitatively analysed only studies providing calciferol, but not other vitamin D metabolites. Two studies favouring vitamin D treatment and supplementing with non-calciferol vitamin D metabolites were included in the primary Bischoff-Ferrari et al. meta-analysis but not included in that of Latham et al. [162, 163]. One of these was published subsequently to the Latham et al. meta-analysis [163]. A third study favouring treatment also included in the Bischoff-Ferrari et al. analysis was not included in the Latham et al. analysis because insufficient data were available, since, at that time, it was only published in abstract form [140]. In addition, two studies showing no effect of vitamin D supplementation on falls risk that were included in the Latham et al. meta-analysis were omitted from that of Bischoff-Ferrari et al. [143, 144]. The reasons for exclusion from the Bischoff-Ferrari et al. analysis may have been the lack of an explicit definition of a falls, no regular recording of how falls were ascertained in the case of one of the studies [143], or that study participants recruited as hospital geriatric rehabilitation patients were deemed to be in an unstable health state in the case of the other [144]. The effects of vitamin D supplementation may be contingent upon sufficient dietary calcium, since one of the studies included in both meta-analyses showing a positive effect of supplementation on the number of falls provided additional calcium to both the treatment and control groups [139]. In addition, a more recent study comparing intramuscular ergocalciferol interventions with and without supplemental calcium has shown a non-significant trend for a greater 25(OH)D increment and PTH drop in the group which received calcium, although this was not translated into a benefit to falls or fracture risk [164].

Assuming that vitamin D intervention presents a modest effect on muscle function and hence falls risk, it is of interest to ascertain the level of 25(OH)D at which this occurs. The few studies that have supplemented with either 1α-hydroxyvitamin D₃ (alfacalcidol) or 1,25(OH)₂D are not included in this analysis as their results may not be comparable to those providing calciferol. This is because these treatment strategies bypass the normal calcium homeostatic control mechanisms. In fact, levels of 25(OH)D may remain unchanged [165] or fall [163] with 1α-hydroxyvitamin D₃ and generally fall following 1,25(OH)₂D administration [88, 162], probably due to stimulation of PTH secretion (refer to Chapter 2.2.1 Osteomalacia, Rickets & Secondary Hyperparathyroidism, pg ). This is in contrast with vitamin D₃ supplementation which invariably results in large rises in 25(OH)D (Table 2.1, Figure 2.4b). When the effect of initial and changing levels of 25(OH)D in calciferol intervention studies on falls risk is analysed, however, there does not seem to be a clear relationship (Figure 2.4). Although, two of the three studies in which baseline 25(OH)D was close to or above 40
nmol/L did not show an effect of vitamin D supplementation on falls risk [144, 166] (Figure 2.4a), a recent study of active older people with higher initial levels found a reduced falls risk in women [141]. These spurious results may be due to supplemental calcium, which was provided in the third study [141]. A high calcium intake due to provided calcium supplements may also account for the beneficial effect on falls risk noted in three other studies with initially low 25(OH)D levels [139, 140, 164]. A notable exception is a 2002 French study which provided oral calciferol and 1200 mg/day calcium to institutionalised elderly women with low initial 25(OH)D levels [143]. In this study, the intervention had no observed effect on falls. The lack of effect may arise from errors of participant recollection, since falls were assessed only during 3-monthly visits with no regularly-kept falls diary nor a clear definition of falls specified. In addition to these reasons for a lack of an observed effect, assay methods used to assess 25(OH)D differ between studies, compromising direct comparisons and making the estimation of optimal levels of this metabolite difficult.

Figure 2.4 a&b. The Effect of Baseline Levels of 25-Hydroxyvitamin D (a) and Its Change (b) on Relative Risk of Falls Following Calciferol Intervention. High relative risks of close to or above 1.0 indicate no effect of the intervention on falls risk. Citations for above figures: 1 Grafmans et al., 1996 [142]; 2 Pfeifer et al., 2000 [139]; 3 Chapuy et al., 2002 [143]; 4 Bischoff et al., 2003 [140]; 5 Latham et al., 2003 [144]; 6 Trivedi et al., 2003 [166]; 7 Dhesi et al., 2004 [167]; 8 Harwood et al., 2004 [164]; 9 Flicker et al., 2005 [168] 9 Bischoff-Ferrari et al., 2006 [141].
Table 2.1 Calciferol Intervention Studies Assessing Changes in Muscle Function or Falls

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Treatment</th>
<th>Duration</th>
<th>Initial 25(OH)D (nmol/L) †</th>
<th>Change in 25(OH)D (nmol/L) †</th>
<th>Outcome &amp; Calcium Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D₂ or D₃ VERSUS PLACEBO</strong></td>
<td></td>
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<tr>
<td>Corless et al., 1985 [169]</td>
<td>82 (32)</td>
<td>9000 IU D₂ / day</td>
<td>2-9 months</td>
<td>16.6</td>
<td>≈90 (estimated from figure)</td>
<td>No difference in activities of daily living</td>
</tr>
<tr>
<td>Graafmans et al., 1996 [142]</td>
<td>368 (354*)</td>
<td>400 IU D₃ / day</td>
<td>28 weeks</td>
<td>not stated est.</td>
<td>not stated est.</td>
<td>Ca intake not stated</td>
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<tr>
<td>[sample from Lips et al. 1996]</td>
<td></td>
<td></td>
<td></td>
<td>≈27 from Lips et al., 1996</td>
<td>≈20 from Lips et al., 1996</td>
<td>Ca intake not stated</td>
</tr>
<tr>
<td>Latham et al., 2003 [144]</td>
<td>243 (108)</td>
<td>300 000 IU D₃</td>
<td>6 months</td>
<td>37.4 (median)</td>
<td>22.5 (median) (3 month)</td>
<td>No difference in falls risk (RR=1.0)</td>
</tr>
<tr>
<td>Trivedi et al., 2003 [166]</td>
<td>2686 (1345)</td>
<td>100 000 IU D₃/4mth</td>
<td>4 years</td>
<td>≈53 (est. from placebo final value)</td>
<td>≈21 (difference between treatment &amp; placebo final value)</td>
<td>No difference in falls risk (RR=1.02)</td>
</tr>
<tr>
<td>Dhesi et al., 2004 [167]</td>
<td>139 (62)</td>
<td>600 000 IU D₂ im</td>
<td>6 months</td>
<td>26.7</td>
<td>17.2</td>
<td>Improved functional performance, reaction time and balance</td>
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<td></td>
<td>No difference in strength or falls risk (RR=0.78) Ca intake not stated</td>
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<tr>
<td><strong>D₂ or D₃ AND CALCIUM VERSUS PLACEBO</strong></td>
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<tr>
<td>Honkanen et al., 1990 [170]</td>
<td>139 (55)</td>
<td>1800 IU D₃ / day</td>
<td>11 weeks</td>
<td>42.8 (independent)</td>
<td>37.9 (independent)</td>
<td>No difference in grip strength</td>
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<td></td>
<td>66 (25)(independent)</td>
<td>73 (30) (hospital)</td>
<td></td>
<td>24.5 (hospital)</td>
<td>39.9 (hospital)</td>
<td>Ca supplement 1558mg/day; Intake not stated Total Ca &gt; 1600mg/day</td>
</tr>
<tr>
<td>Chapuy et al., 2002 [143]</td>
<td>583 (279)</td>
<td>800 IU D₃ / day</td>
<td>2 years</td>
<td>21.2±13.2 (Gr1)</td>
<td>≈55 (both groups)</td>
<td>No difference in falls risk (RR=1.03)</td>
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<td></td>
<td>199 (142) (Gr1)</td>
<td></td>
<td></td>
<td>22.5±16.5 (Gr2)</td>
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<td>Ca supplement 1.2g/day; Intake 555mg/day Total Ca 1755mg/day</td>
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<tr>
<td></td>
<td>194 (137) (Gr2)</td>
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<tr>
<td>Harwood et al., 2004 [164]</td>
<td>150 (113 in 3 treatment groups)</td>
<td>300 000 IU D₂; 300 000 IU D₂ + 1g Ca / day; 800 IU D₃ / day + 1g Ca / day</td>
<td>1 year</td>
<td>29</td>
<td>16</td>
<td>Difference in falls risk (RR=0.48)</td>
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<td></td>
<td>Ca supplement 1g/day; Intake not stated Total Ca &gt; 1200mg/day</td>
</tr>
<tr>
<td>Study</td>
<td>n*</td>
<td>Treatment</td>
<td>Duration</td>
<td>Initial 25(OH)D (nmol/L) †</td>
<td>Change in 25(OH)D (nmol/L) †</td>
<td>Outcome &amp; Calcium Intake</td>
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<tr>
<td>Bischoff-Ferrari et al., 2006 [141] [Dawson-Hughes et al., 1997 companion study [129]]</td>
<td>445 (182)</td>
<td>700 IU D₃ / day</td>
<td>3 years</td>
<td>81.9±37.4 (♂)</td>
<td>28.0 (♂)</td>
<td>No difference in falls risk (RR=0.77; ♂)</td>
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<tr>
<td></td>
<td>199 (83) (♂)</td>
<td>199 (83) (♂)</td>
<td></td>
<td>69.9±32.9 (♂)</td>
<td>33.9 (♂)</td>
<td>Difference in falls risk (RR=0.54; ♀)</td>
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<tr>
<td></td>
<td>246 (99) (♀)</td>
<td>246 (99) (♀)</td>
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<td>75.2 (weighted mean)</td>
<td>31.2 (weighted mean)</td>
<td>Large difference in inactive ♀ (RR=0.64 weighted mean)</td>
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<td></td>
<td>Ca supplement 500mg/day; Intake 576mg/day</td>
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<td></td>
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<td></td>
<td></td>
<td>Total Ca 1076mg/day</td>
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<tr>
<td>Dowler et al., 2007 [141]</td>
<td>82 (36)</td>
<td>700 IU D₃ / day</td>
<td>6 months</td>
<td>58.3±41.4 (♂)</td>
<td>≈7.7±5.2</td>
<td>No difference in falls risk (RR=0.77; ♂)</td>
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<td></td>
<td></td>
<td>Difference in falls risk (RR=0.54; ♀)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Large difference in inactive ♀ (RR=0.64 weighted mean)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplement 500mg/day; Intake 576mg/day</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca 1076mg/day</td>
</tr>
<tr>
<td>Gloth et al., 1995 [171]</td>
<td>32</td>
<td>400 IU D₂/day</td>
<td>1 &amp; 6 months</td>
<td>&lt;37.4</td>
<td>≈3.2-3.5±10</td>
<td>Difference in self-assessed function</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplement dose not stated; Intake not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca &gt; 1200mg/day</td>
</tr>
<tr>
<td>Binder et al., 1995 [172]</td>
<td>34 (11)</td>
<td>1 000 000 IU D₃ + 50 000 IU D₃ /week</td>
<td>8 weeks</td>
<td>57.7±20.2</td>
<td>24.0±24.7</td>
<td>No difference in muscle function</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplementation 1g/day; Intake not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca &gt; 1200mg/day</td>
</tr>
<tr>
<td>Pfeifer et al., 2000 [139]</td>
<td>148 (70)</td>
<td>800 IU D₃/day</td>
<td>1 year</td>
<td>25.7±13.6</td>
<td>40.5±27.0</td>
<td>↓ sway &amp; falls risk (RR=0.55)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplement 1.2g/day; Intake not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca &gt; 1400mg/day</td>
</tr>
<tr>
<td>Bischoff et al., 2003 [140]</td>
<td>122 (62)</td>
<td>800 IU D₃/day</td>
<td>3 months</td>
<td>30.7(IQR given)</td>
<td>34.7(IQR given)</td>
<td>↓ falls risk (RR=0.75) (↓49% falls: RR=0.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplement 1.2g/day; Intake not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca &gt; 1400mg/day</td>
</tr>
<tr>
<td>Kenny et al., 2003 [173]</td>
<td>65 (est. 30)</td>
<td>1000 IU D₂/day</td>
<td>6 months</td>
<td>65 ± 17</td>
<td>22 (estimated from figure)</td>
<td>No difference in change between groups for strength or physical performance measures</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca Intake 1454 ± 357</td>
</tr>
<tr>
<td>Flicker et al., 2005 [168]</td>
<td>625 (269)</td>
<td>10 000 IU D₂/week then 1000 IU D₂/day</td>
<td>2 years</td>
<td>42 (estimated from frequency distribution)</td>
<td>not stated</td>
<td>No difference in falls risk (OR=0.82, RR=0.92) but reduced falls (and fallers) in PP analysis (OR=0.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplement 800mg-1.2g/day; Intake not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca &gt; 1g/day</td>
</tr>
<tr>
<td>Glerup et al., 2000 [174]</td>
<td>55‡</td>
<td>700 000 IU D₃ + 400-600 IU D₂/day</td>
<td>3 months</td>
<td>6.7±0.6</td>
<td>27.7</td>
<td>14%↑ maximal voluntary contraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ca supplement 800mg-1.2g/day; Intake not stated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total Ca &gt; 1g/day</td>
</tr>
</tbody>
</table>

*D Sample size of entire group randomised to receive calciferol or placebo in a randomised controlled trial unless otherwise stated. Sample size of the per protocol (completing) calciferol treatment group is included in parentheses where this information is available. # indicates that the number in parentheses is the sample size of the per protocol treatment and control groups.

†Data (means ± standard deviations unless otherwise stated) are for vitamin D treatment group only.

‡No control group in sample severely deficient young Asian women (Group B in study)

D₃ = vitamin D₃ cholecalciferol; D₂ = vitamin D₂ ergocalciferol; RR = relative risk; OR = odds ratio; Ca = calcium; PP = per protocol
2.3.3 Disease Risk Indicators (Table 2.2)

Recently, a number of reviews have highlighted the potential role of vitamin D in a range of other physiological functions which are linked to the prevention of infections, autoimmune and chronic diseases such as heart disease, insulin-dependent diabetes, multiple sclerosis, rheumatoid arthritis, respiratory infections, inflammatory bowel diseases and many types of cancer [33, 36, 39, 66, 137, 175-178]. There are also suggestions that the higher incidence of cardiovascular disease, diabetes, infections and some cancers in African Americans might be, in part, related to a lower vitamin D status [178-180]. The mechanisms for these effects are thought to be mediated via paracrine or autocrine production of 1,25(OH)\(_2\)D which is not subject to the PTH regulatory processes affecting bone metabolism [78] (refer Chapter 2.1.3 Vitamin D Synthesis & Metabolism, pg. 11). Consequently, target levels of 25(OH)D for minimising the risk of these conditions may be different from the optimal levels for calcium absorption and bone health. Estimates of optimal levels of 25(OH)D for disease prevention are possible in studies which report assay results of this metabolite, or perhaps those that estimate it from other indicators of vitamin D status such as reported outdoor time and vitamin D intake.

The purpose of this section of the review is to examine the evidence supporting the benefits of vitamin D on disease-related outcomes and establish whether there is any evidence of an associated target or optimal level of 25(OH)D. This is achieved by comparing the levels of 25(OH)D reported in studies that establish or fail to establish a vitamin D effect (Table 2.2). If improvements in population vitamin D status do indeed have small effects on the incidence of common conditions associated with high morbidity and mortality costs, widespread vitamin D supplementation may be a cost effective public health strategy.

2.3.3.1 Cancer

Of the chronic diseases to which vitamin D status has been linked, cancer, particularly colorectal and breast, has been the most heavily researched of these. Certain tumour cells have been known to bind 1,25(OH)\(_2\)D for some years [181]. Similarly, 1,25(OH)\(_2\)D has been shown to inhibit proliferation of a range of cells [66, 78, 182, 183] and the potential use of vitamin D analogues for cancer therapy has been postulated [184-187]. Early phase I and II trials have shown promise, with reductions in PTH and tumour reduction or stability in some patients [188-190] although concerns have been raised as to the efficacy of treatment in metastatic cancer since an increase in cancer cell proliferation was noted when 1,25(OH)\(_2\)D was applied to cell cultures within living bone tissue [191]. There is also a rapidly growing body of epidemiological evidence linking vitamin D status with cancer risk reduction, which has been more extensively reviewed elsewhere [42, 136, 138, 175, 192-194].
Geographical and seasonal differences in cancer risk provide the first line of evidence. In the US, solar UVB radiation levels associated with latitude and atmospheric pollution have been shown to be inversely correlated with cancer incidence, mortality and survival rates [195-202]. Data showing similar relationships between UV exposure and cancer risk have been also been shown in other parts of the world [203-211]. Similarly, in one Northern European study, an improved prognosis following breast, colon and prostate cancer diagnosis was noted in summer and fall compared to other times of the year [212].

Many studies also report a cross-sectional association between vitamin D status or indices such as reported outdoor activity, sunbathing habits or vitamin D intake and cancer risk [196, 213-220], though not all have identified this association [221-224]. Some longitudinal data confirms a negative association of vitamin D intake (or combined calcium and vitamin D intake) with the subsequent incidence of certain cancers [225-229]. However, in other similar studies, a relationship between vitamin D intake and cancer has not been firmly established [230-234].

Prospective cohort studies or case-control studies nested within larger cohort studies provide a stronger design (Table 2.2). Some of these have reported inverse associations between 25(OH)D and colon or colorectal cancer [235-237], breast cancer [238] and prostate cancer [239, 240]. In contrast, similar studies have not found an effect of 25(OH)D on colorectal cancer precursor adenomas [241, 242], prostate cancer [243, 244] or ovarian cancer risk [245], and one study reported a higher risk of prostate cancer in the highest compared to the lowest quintile of baseline 25(OH)D [246]. A recent meta-analysis identified five nested case-control studies investigating the association between 25(OH)D and colorectal cancer [247]. The analyses showed a 50% reduced risk of colorectal cancer when 25(OH)D levels were ≥82 nmol/L compared with <30 nmol/L and also a significant trend across quintiles of 25(OH)D. Two prospective studies that have adjusted for a wide range of confounders, report an effect of 25(OH)D on colorectal cancer risk. The first of these, a US nested case-control study investigating colorectal cancer incidence matched cases with controls by age and month of blood collection and additionally adjusted for body mass index, physical activity, smoking, menopausal status, hormone replacement therapy, aspirin use, family history of colorectal cancer and intake of a range of nutrients [237]. The authors reported an odds ratio of 0.5 in the highest compared to the lowest quintile of 25(OH)D which failed to attain significance, although a significant reduction in risk across all quintiles of increasing 25(OH)D was noted ($P = 0.02$). A more recent prospective study of data from the Third National Health and Nutrition Examination Survey
(NHANESIII), investigating the risk of total and site-specific cancers, reported no effect of baseline 25(OH)D on 13 – 19 year risk of total cancers, but did find a significant effect on colorectal cancer risk [236]. When adjusted for age, sex, race/ethnicity and smoking history, relative risks compared to 25(OH)D levels <50 nmol/L, were 0.4 from 50 – 80 nmol/L, and 0.3 when levels were ≥80 nmol/L.

Two prominent interventional studies in postmenopausal women with conflicting findings also exist (Table 2.2). One was an analysis of data from 36 282 participants in US Women’s Health Initiative (WHI) trial of calcium (1 g/day) and vitamin D (400 IU/day), which failed to find an effect on the 7-year incidence of colorectal cancer [248]. The other was a smaller 4-year Central US (Nebraska) trial of calcium (1.4 – 1.5 g/day) and vitamin D (1100 IU/day), which reported a protective effect (Relative Risk = 0.4) on the risk of all cancers [249]. The conclusions of both studies have drawn critical comment. In the WHI, both the dose and compliance were low, probably resulting in low end-of-study 25(OH)D levels (59 nmol/L in a subsample of women during the study) which might have been insufficient to observe an effect [250]. Also, participants in the study were allowed to take substantial quantities of “personal” calcium or vitamin D supplementation (≤1000 IU/day vitamin D by the end of the study). Dietary supplement use amongst the control group has been argued to be the cause of a lack of reduction in bone mineral density (BMD) in that group and for the lack of an observed effect on fracture risk [251]. It may also explain the lack of effect on colorectal cancer in the WHI study [248]. Conversely, in the Nebraska study [249], the observation of an uncharacteristically high cancer rate amongst control participants has been argued to have resulted in a false positive identification of an effect of vitamin D supplementation on cancer [252, 253]. However, since this was an interventional rather than large cohort study, the 95% confidence intervals were quite broad and not incompatible with previous cancer incidence data. Two smaller interventions also provide relevant data. The first, found a decrease in adenoma proliferative indices, precursors to colorectal cancer, following daily 400 IU vitamin D supplementation [254]. The other, a calcium intervention investigating risk of colorectal adenoma recurrence, reported a reduction in risk compared to the placebo group, but only in those with 25(OH)D levels >73 nmol/L [255]. One reason for inconsistent results across prospective investigations and randomised trials, and the failure to find an effect of vitamin D supplementation or status on cancer risk, even in large trials, could be due to the timing of vitamin D exposure or lack, relative to the pathological stage of cancer, which can progress over many years [256]. Factors such as the age of study participants and the length of follow-up may be highly pertinent.
There are a number of other possible reasons for inconsistent findings across studies. The most likely is the failure to adequately control for any of the many possible confounding variables. Dietary calcium intake is one possible confounding variable in investigations of the role of vitamin D status in cancer incidence. In the afore mentioned calcium intervention, Grau et al., 2003 found that the risk reduction of 1.2 g/day calcium on colorectal adenoma recurrence was restricted to those above the median baseline 25(OH)D level of 73 nmol/L [255]. Conversely, 25(OH)D was only inversely related to adenoma recurrence in those randomised to receive calcium and not in the placebo group. A second possible reason for inconclusive data is that the effects of vitamin D status on cancer incidence may be non-linear. An early nested case-control study noted a decreasing trend in colon cancer incidence from the lowest to the second to highest quintile, which attained significance compared to the lowest quintile for quintiles 3 and 4. However, the risk increased again in the highest quintile (5), and was not different from lowest [235]. Data from another prospective study suggest that both low (≤19 nmol/L) and high (≥80 nmol/L) levels of 25(OH)D are associated with increased prostate cancer risk [257], relative to intermediate values. Whilst these authors attribute the increased risk associated with high levels to possible vitamin D resistance through enhanced 24-hydroxylase activity, there may also be a confounding influence of outdoor smoking, of calcium or dairy intake, which has been associated with increased prostate cancer risk [258], or of an interaction with vitamin D receptor genotypes [215, 217, 218, 259, 260].

Overall, no estimate of optimal levels of 25(OH)D can be made on the basis of the sparse interventional studies of cancer incidence or risk. A meta-analysis of nested case-control investigations of the effect of 25(OH)D on colorectal cancer suggests that 25(OH)D levels in excess of 82 nmol/L are preferable to levels <30 nmol/L [138]. Results from other studies of differential cancer risk above and below this levels of 25(OH)D may imply target levels. For example, Garland et al. reported a one third risk of colon cancer [235] and Freedman et al., a 0.3 – 0.4 relative risk of colorectal cancer [236] with initial 25(OH)D levels above compared to below 50 nmol/L. Grau et al. reported an effect on colorectal adenoma response to calcium, only with 25(OH)D levels above 73 nmol/L [255]. Nonetheless, linear trends across quintiles in the colorectal cancer meta-analysis [138], and reported dose-response relationships between 25(OH)D levels and cancer risk of the lower digestive tract [41, predicted levels, 237, 248] or breast [238] up to levels of 100 – 150 nmol/L do not lend to support the existence of a threshold level of 25(OH)D at which further increases do not further reduce cancer risk [42, 138, 261].
2.3.3.2 Other Chronic Diseases

Evidence linking vitamin D to the risk of multiple sclerosis, type 1 and 2 diabetes, cardiovascular disease and arthritis are based primarily on trials in animal models indicating potentially deleterious effects on the aetiology of these chronic conditions and cross-sectional epidemiological and case-control data [66, 175]. Notwithstanding, a strengthening basis exists for a vitamin D effect on cardiovascular disease risks [262] and autoimmune conditions, specifically multiple sclerosis, rheumatoid arthritis and type 1 diabetes [263].

Preclinical study results suggest a range of potential mechanisms by which low vitamin D status might influence non-cancer chronic disease risk. Elevated blood pressure may come about via enhanced renin production since vitamin D deficient or receptor knockout mice display increased renin biosynthesis which is reduced with exogenous 1,25(OH)₂D administration [264]. Additionally, 1,25(OH)₂D is thought to enhance extracellular calcification through the action of metalloproteinases [265]. In turn, cardiovascular disease risk may be increased in vitamin D deficient states both by hypertension and by alterations to the structural integrity of the myocardial extracellular matrix [266]. These changes may explain previously observed increases in myocardial contractility in vitamin D deficient rats [267, 268]. Increased risk of autoimmunity is thought to occur as a result of 1,25(OH)₂D regulation of T lymphocyte development and function [66, 78, 269] and 1,25(OH)₂D administration prevents the development of autoimmune diabetes, multiple sclerosis and arthritis in mice [270-273].

Latitude, season and other indices of UVB exposure are cross-sectionally associated with the incidence of hypertension [274], multiple sclerosis [275-280] and diabetes [281, 282]. Similar prospective associations with vitamin D intake and the incidence or progression of these conditions and both rheumatoid and osteoarthritis in addition [283-286]. A few case-control, prospective and interventional studies have assayed 25(OH)D levels and, with improved vitamin D status or following vitamin D supplementation, demonstrate reduced risk of osteoarthritis [285, 287], multiple sclerosis [288, 289], diabetes [290] and vascular disease [40, 291-294]. In a number of these studies, benefits associated with higher 25(OH)D occur at levels of 50-80 nmol/L compared to 20-40 nmol/L, indicating that optimal status may be attained at some point between these two ranges (Table 2.2). Results of the prospective studies show benefits at slightly higher 25(OH)D levels (above 75 nmol/L) compared to lower levels. Similarly, data from two randomised controlled trials show reduced blood pressure when initial levels of around 60 nmol/L are increased to levels exceeding 150 nmol/L [292, 293].
2.3.3.3 Overview

Much of the evidence supporting an effect of vitamin D status on disease outcomes is cross-sectional. All of these associations are confounded by the likelihood of poor prior health status and less time spent outdoors. Correlates with vitamin D status, such as age, physical activity, adiposity and dietary factors [295, 296], are other likely confounders which figure in the aetiology of many diseases with which vitamin D has been linked. Another source of variability, which might obscure 25(OH)D effects if they exist, is the time of the year that measurements are taken. Reported seasonal variation in 25(OH)D between annual peak and trough ranges from 6 nmol/L in sub-tropical Florida (26°N) [297] to almost 40 nmol/L in Northern Europe (51°N) [298] and, depending upon the latitude that a study was undertaken, could therefore inject a large degree of noise into data from studies which failed to control for it in their design.

Even longitudinal assessment of chronic disease risk, to establish an association with 25(OH)D, is subject to the same confounders. Most of the prominent studies have corrected for some of these confounders, either by matching cases and controls or via statistical adjustment, but few studies have adequately adjusted for all important confounders. In many reports, the statistical processes followed to decide which covariates to adjust for are not clearly explained. For example, matters such as whether covariates were decided upon a priori or post hoc, and which potential covariates were included in multiple regression models, including those that did not enter such models are seldom clear. Furthermore, because of the complex inter-relationships between confounders, statistical methods of addressing this problem may result in erroneous conclusions by either exaggerating the effect of an overlooked confounding variable, resulting in a false positive finding, or by removing a real effect of vitamin D status.

Overall, data relating vitamin D status to the risk of cancer and other chronic diseases spans a range of study designs and particular conditions, but is sparse when restricted to those from sound analyses of cohort studies or randomised controlled trials. Optimal levels of 25(OH)D may be different for body systems other than those affected by the regulation of circulating calcium. However, at this stage, the evidence remains insufficient to provide compelling assessments of optimal 25(OH)D levels based on non-musculoskeletal outcomes.
### Table 2.2 Studies Showing Beneficial Associations of Vitamin D Supplementation or Status With Chronic Disease Risk

#### CROSS-SECTIONAL CASE-CONTROL STUDIES

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Comparison</th>
<th>Lower 25(OH)D (nmol/L)</th>
<th>Higher 25(OH)D (nmol/L)</th>
<th>Outcomes or Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zittermann et al., 2003 [291]</td>
<td>88</td>
<td>54 cases versus 34 controls</td>
<td>≈26 est.</td>
<td>≈50 est.</td>
<td>Lower status in congestive heart failure patients</td>
</tr>
<tr>
<td>Poole et al., 2006 [294]</td>
<td>140</td>
<td>44 cases versus 96 controls</td>
<td>≈40 est.</td>
<td>≈70 est.</td>
<td>Lower status in stroke patients</td>
</tr>
<tr>
<td>Soilu-Hanninen et al., 2005 [288]</td>
<td>80</td>
<td>40 cases versus 40 controls</td>
<td>≈60 est.</td>
<td>≈80 est.</td>
<td>Lower status in multiple sclerosis patients in summer</td>
</tr>
</tbody>
</table>

#### PROSPECTIVE OR NESTED CASE-CONTROL STUDIES

<table>
<thead>
<tr>
<th>Study</th>
<th>From cohort of 25 620</th>
<th>n*</th>
<th>Comparison</th>
<th>Lower 25(OH)D (nmol/L)</th>
<th>Higher 25(OH)D (nmol/L)</th>
<th>Outcomes or Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garland et al., 1989 [235]</td>
<td>34 cases versus 67 controls</td>
<td>10-47 Quintile 1</td>
<td>82-102 Quintile 4 (5 not different from 1)</td>
<td>Lower 8 yr risk of colon cancer with 25(OH)D ≥ 50 nmol/L and in quintiles 3 (OR=0.25)and 4 (OR=0.21) compared to quintile 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Braun et al., 1995a [241]</td>
<td>57 cases versus 114 controls</td>
<td>&lt;43 Quintile 1</td>
<td>&gt;75 Quintile 5 (not different from 1)</td>
<td>No effect of 25(OH)D on 7 yr colon cancer risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Braun et al., 1995b [243]</td>
<td>61 cases versus 114 controls</td>
<td>&lt;60 Quintile 1</td>
<td>&gt;124 Quintile 5 (not different from 1)</td>
<td>No effect of 25(OH)D on 12 yr prostate cancer risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McAlindon et al., 1996 [285]</td>
<td>25(OH)D levels: lowest versus highest tertile</td>
<td>12-60 Tertile 1</td>
<td>90-197 Tertile 3</td>
<td>Levels of 25(OH)D inversely associated with ≈9 yr risk for osteoarthritis progression (RR=2.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lane et al., 1999 [287]</td>
<td>25(OH)D levels: lowest two versus highest tertile</td>
<td>20-74 Tertile 1 &amp; 2</td>
<td>75-180 Tertile 3</td>
<td>Levels of 25(OH)D inversely associated with ≈8 yr risk for osteoarthritic joint space narrowing (RR=3.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ahonen et al., 2000 [240]</td>
<td>149 cases versus 566 controls</td>
<td>41 (for cases)</td>
<td>44 (for controls)</td>
<td>Lower 13 yr risk of prostate cancer with 25(OH)D &gt;40 nmol/L compared to &lt;40 nmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grau et al., 2003 [255]</td>
<td>25(OH)D levels: &lt;73 nmol/L versus &gt;73 nmol/L and per 30 nmol/L change</td>
<td>&lt;73</td>
<td>&gt;73</td>
<td>Lower risk of bowel adenoma recurrence risk with 1.2 g/day calcium when 25(OH)D &gt; 73 nmol/L and inverse association of 25(OH)D with risk (RR=0.7 / 30 nmol/L increase in 25(OH)D) for treated group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>n*</td>
<td>Comparison</td>
<td>Lower 25(OH)D (nmol/L)</td>
<td>Higher 25(OH)D (nmol/L)</td>
<td>Outcomes or Conclusions</td>
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</tr>
<tr>
<td>Feskanich et al., 2004 [237]</td>
<td>[same cohort as Bertone-Johnson et al., below]</td>
<td>From cohort of 32 826</td>
<td>193 cases versus 383 controls</td>
<td>40 Quintile 1 median</td>
<td>Decreasing 11 yr risk in colorectal cancer risk across increasing quintiles (P = 0.02)</td>
<td></td>
</tr>
<tr>
<td>Platz et al., 2004 [244]</td>
<td>From cohort of 18 018</td>
<td>460 cases versus 460 controls</td>
<td>61 (for cases) 35 annualised Quartile 1 median</td>
<td>60 (for controls) 71 annualised Quartile 4 median</td>
<td>No effect of 25(OH)D on 12 yr colorectal cancer risk</td>
<td></td>
</tr>
<tr>
<td>Bertone-Johnson et al., 2005 [238]</td>
<td>From cohort of 32 826</td>
<td>701 cases versus 724 controls</td>
<td>&lt;60 Quartile 1</td>
<td>&gt;110 Quartile 5 (not different from 1)</td>
<td>Trend of decreasing risk in breast cancer across increasing quintiles (P = 0.06) Effect significant in women ≥50 years</td>
<td></td>
</tr>
<tr>
<td>Wactawski-Wende, 2006 [248]</td>
<td>From cohort of 36 282</td>
<td>317 cases versus 317 controls</td>
<td>&lt;31 Quartile 1</td>
<td>&gt;58 Quartile 4</td>
<td>No effect of 25(OH)D on 7 yr colorectal cancer risk</td>
<td></td>
</tr>
<tr>
<td>Forman et al., 2007 [40]</td>
<td>1811</td>
<td>25(OH)D levels: &lt;37 nmol/L versus &gt;75 nmol/L</td>
<td>&lt;37</td>
<td>&gt;75</td>
<td>Levels of 25(OH)D inversely associated with 4 yr risk of hypertension (RR=3.18)</td>
<td></td>
</tr>
<tr>
<td>Freedman et al., 2007 [236]</td>
<td>16 818</td>
<td>25(OH)D levels: &lt;50 nmol/L versus 50-80 and &gt;80 nmol/L</td>
<td>&lt;50</td>
<td>50-80 &amp; &gt;80</td>
<td>Lower 9 yr risk of colorectal cancer mortality with 25(OH)D ≥ 50 nmol/L compared to 50-80 nmol/L (RR=0.44) and ≥80 (RR=0.28) No effect of 25(OH)D on total cancer mortality</td>
<td></td>
</tr>
<tr>
<td>Li et al., 2007 #2162 [239]</td>
<td>14 916</td>
<td>25(OH)D Levels: &lt;70 nmol/L versus &gt;70 nmol/L</td>
<td>&lt;70 est. median</td>
<td>&gt;70 est. median</td>
<td>Effect of 25(OH)D levels on prostate cancer risk in one vitamin D receptor polymorphism</td>
<td></td>
</tr>
<tr>
<td>Tworoger et al., 2007 [245]</td>
<td>From 3 large cohorts 278 185</td>
<td>224 cases versus 603 controls 25(OH)D Levels: &lt;60 nmol/L versus &gt;60 nmol/L</td>
<td>≤60</td>
<td>&gt;60</td>
<td>No effect of 25(OH)D on ovarian cancer risk</td>
<td></td>
</tr>
</tbody>
</table>
### Table 2.2 cont.

**INTERVENTIONAL STUDIES**

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Treatment</th>
<th>Duration</th>
<th>Initial 25(OH)D (nmol/L) †</th>
<th>Change in 25(OH)D (nmol/L) †</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krause et al., 1998 [292]</td>
<td>18</td>
<td>UVB 3x/week (control UVA)</td>
<td>6 weeks</td>
<td>58</td>
<td>94</td>
<td>Reduced ambulatory systolic and diastolic blood pressure by 6 mmHg from 155/95 mmHg</td>
</tr>
<tr>
<td>Pfeifer et al., 2001 [293]</td>
<td>148</td>
<td>800 IU/day D₃</td>
<td>8 weeks</td>
<td>64±34</td>
<td>98</td>
<td>Reduced systolic blood pressure by 13 mmHg from 144/85 mmHg 1200 mg supplemental calcium/day to treatment and control groups</td>
</tr>
<tr>
<td>Borissova et al., 2003 [290]</td>
<td>10</td>
<td>1332 IU/day D₃</td>
<td>1 month</td>
<td>35±15</td>
<td>27</td>
<td>Change in 1st phase insulin secretion proportional to change in 25(OH)D</td>
</tr>
<tr>
<td>Mahon et al., 2003 [289]</td>
<td>39</td>
<td>800 IU/day D₃</td>
<td>6 months</td>
<td>42±15</td>
<td>27</td>
<td>Increased cytokine TGFβ1 levels (indicates reduced risk of multiple sclerosis) 800 mg supplemental calcium/day to treatment and control groups</td>
</tr>
<tr>
<td>Wactawski-Wende et al., 2006 [248]</td>
<td>36 282</td>
<td>400 IU/day D₃ + 1 g/day calcium</td>
<td>7 years</td>
<td>≈60 nmol/L est.</td>
<td>not reported</td>
<td>No effect of 25(OH)D on risk of colorectal cancer in response of vitamin D and calcium</td>
</tr>
<tr>
<td>Lappe et al., 2007 [249]</td>
<td>1179</td>
<td>1100 IU/day D₃ + 1.4-1.5 g/day calcium</td>
<td>4 years</td>
<td>72±20</td>
<td>24±18</td>
<td>Reduced risk of any cancer</td>
</tr>
</tbody>
</table>

*Sample size of entire group randomised to receive calciferol or placebo in a randomised controlled trial unless otherwise stated. Sample size of the per protocol (completing) calciferol treatment group is included in parentheses where this information is available. # indicates that the number in parentheses is the sample size of the per protocol treatment and control groups.

†Data (means ± standard deviations unless otherwise stated) are for vitamin D treatment group only in interventions. D₃ = vitamin D₃ cholecalciferol; D₂ = vitamin D₂ ergocalciferol; 25(OH)D = 25 hydroxyvitamin D; UV = ultra-violet; RR = relative risk ratio; est. = estimated (from figure)
2.3.4 Skeletal Outcomes

The vast majority of studies investigating the benefits of vitamin D examine bone outcomes or PTH, thought to be the primary mediator of bone-related effects. Data come mainly from cross-sectional investigations of the association with 25(OH)D levels and a few notable case-control studies, although there are a growing number of prospective cohort analyses and randomised placebo-controlled trials [8, 11, 66, 299]. Evidence for beneficial effects of vitamin D or circulating concentrations of its metabolites is inconclusive. Whilst a majority of cross-sectional and prospective cohort studies show such an effect [127, 158, 300, 301], some do not [302-305]. Similarly, although there is evidence amongst some groups of people for skeletal effects of combined vitamin D and calcium supplementation [11, 306], few, if any, randomised controlled trials demonstrate an effect of baseline 25(OH)D [128, 307-309].

It is possible that the equivocal results from vitamin D supplementation studies with bone mineral or fracture outcomes are influenced by differing baseline levels of 25(OH)D and post-intervention changes in this metabolite. The apparent larger effect on fracture risk in institutionalised individuals receiving daily doses of ≥700-800 IU vitamin D, in whom baseline 25(OH)D levels are likely to be low, supports this possibility. If there were concurrent negative results from studies with higher starting 25(OH)D levels and smaller changes in this metabolite, it is possible that a threshold effect could be identified, above which vitamin D supplementation was no longer beneficial. Similar thresholds for associations between 25(OH)D and skeletal outcomes might also exist for cross-sectional or longitudinal studies. More detailed consideration of studies investigating the relationship between 25(OH)D and bone outcomes has therefore been provided in this review, with the aim of uncovering evidence for an optimal level of 25(OH)D.

2.3.4.1 Cranney et al. Report

During the preparation of this literature review, a systematic review of studies published earlier than July, 2006, commissioned by the United States Agency for Healthcare Research and Quality, into the efficacy and safety of vitamin D in relation to bone health and an accompanying summary were published [11, 12]. In these reports, Cranney et al. identified almost 10 000 potentially relevant citations, a total reduced to 6566 articles following the removal of duplicates and review articles [11, 12]. Using previously defined criteria, they selected 682 studies that specifically addressed five key questions, which were then classified by study design and narrowed further to include only randomised controlled trials for three of the questions for which sufficient data were available. The first question of this systematic review aimed to establish
levels of 25(OH)D associated with specified bone-health outcomes in three age-groups: children and infants, women of reproductive age and elderly men and postmenopausal women [11]. The vast majority of identified studies were in the older age group (41, of which 10 were randomised controlled trials) compared to 5 (no randomised controlled trials) during pregnancy or lactation and 27 in infants and children (6 randomised controlled trials), including 13 (1 randomised controlled trial) which investigated established rickets (72 unique studies in total) [12]. Consequently, in the older age-group, the outcomes included in the selection criteria set by the authors were also narrower than in the other age categories including only bone mineral density, fractures and falls. The authors did not therefore include studies in which the outcomes were PTH or biochemical markers of bone turnover, for which a sizeable body of literature exists, and did not include studies reporting these data when not part of the specified research questions.

For the purposes of the current literature review, important studies providing data on bone mineral content or density (BMC or BMD), assays of biochemical markers of bone turnover and fracture rates in relation to 25(OH)D levels are reviewed. These key bone outcomes are addressed in turn, followed by studies assessing PTH in a separate section.

2.3.4.2 Bone Mineral Content or Density (Tables 2.3 – 2.6)

Many studies, particularly those of older women, have reported positive cross-sectional relationships between 25(OH)D and BMD at various sites (Table 2.3) [130, 147, 156, 310-312]. Although some studies have reported such associations in adults of both sexes [313, 314] and in young men [315], other studies have shown no relationship [316-324].

In addition to age, gender group and differences in the 25(OH)D assays used in these cross-sectional studies, it is very likely that the range of 25(OH)D levels encompassed within the study will determine whether or not a significant association is identified. Prevailing levels of 25(OH)D may also play a part. If a threshold for 25(OH)D existed, above which there was no association with BMD levels, this might suggest that the threshold level was an indication of attainment of optimal 25(OH)D. Associations between 25(OH)D and BMD outcomes have been found in cross-sectional studies with mean (or median) levels ranging from ~30 – 110 nmol/L (Table 2.3). Most of the studies summarised in Table 2.3 identifying these associations have established this relationship in populations with mean levels <30 nmol/L, or with higher levels but a large range (standard deviations > 30 nmol/L) [for example 130, 147, 310, 314]. In contrast, those studies not finding significant associations between these variables have invariably reported mean 25(OH)D levels >35 nmol/L [for example 311, 320]. One interpretation of this pattern is that vitamin D status does not influence bone mineral density...
when its levels are >35 nmol/L. However, since these studies also tend to encompass a smaller range of values and are therefore less likely to find an association, the interpretation is not clear. In addition, since some studies have noted a relationship in people with mean levels well above this threshold [for example, 130, 156, 312, 315], suggesting that heterogeneity of the sample in relation to vitamin D status is the overriding factor in determining whether or not this relationship has been found. In contrast to the overall view of cross-sectional data, an analysis of data from a single large United States cohort (13 432 people) specifically addressing optimal 25(OH)D levels observed a plateau in BMD in older men and women at approximately 90 to 100 nmol/L [313]. The reason for the high threshold is unclear, though it may be due to the low dietary calcium intake observed in the older group (less than 750 mg/day in white Americans). It may be pertinent to note that amongst younger white Americans in the same study, whose calcium intake was higher at 880 mg/day, a reduction in the increase in BMD was noted above 25(OH)D levels of 60 nmol/L [313].

Other support for a role of vitamin D status in determining bone mineral indices comes from seasonal data, collected both cross-sectionally by recruiting throughout the year, and longitudinally (Table 2.4). Seasonal variation in lumbar BMC, 1.5% higher in summer compared to winter months, was established in the early 1980s although these authors attributed this change to seasonal differences in mechanical loading [325]. Two longitudinal studies in postmenopausal women in the early 1990s also established small seasonal changes in vertebral BMD associated with vitamin D intake, when mean 25(OH)D levels were between 70 and 75 nmol/L [305, 326]. More recent studies have found mixed results, some observing seasonal changes in BMD [108, 327] but others not [318, 328].

Most prospective studies (Table 2.5) which have examined the effect of baseline 25(OH)D levels on subsequent BMD changes have not shown a relationship [147, 308, 324], although one study found a positive correlation between 25(OH)D and change in hip BMD when dietary calcium intake was less than 716 mg/day [329].

The recent systematic review by Cranney et al. [11, 12] also investigated the level of circulating 25(OH)D associated with a benefit to BMC or BMD in different age-groups. A total of 30 studies investigating this relationship were identified, 10 in people up to the age of adolescence, 1 in pregnant women and 19 in older adults. The authors reported inconsistent findings of a relationship between 25(OH)D and bone mineral in young people. For infants, they found no evidence that vitamin D intervention affects BMC, with minimal effects even when there were
large differences in 25(OH)D between cases and controls. In older children and adolescents, most cross-sectional and cohort studies observed relationships between 25(OH)D and some indices of regional bone mineral. The results from the two interventional studies identified were inconsistent in terms of any benefit to bone: a Lebanese study found small changes in hip BMC with weekly high-dose (14 000 IU) vitamin D supplementation and slightly greater changes in premenarcheal girls [330] whilst a Finnish study found no effect on distal radius BMC [331]. However, the former study with mean 25(OH)D levels of 35 nmol/L noted an inverse relationship between baseline 25(OH)D and percent change in lumbar spine BMD with mean 25(OH)D levels of 35 nmol/L [330]. There were a lack of data in premenopausal women, and no evidence of a relationship in the single study of pregnant women [332]. For this relationship in older adults Cranney et al. identified 6 randomised controlled trials, 7 prospective cohort studies and 6 case-control studies, and concluded that there was fair evidence of an association between 25(OH)D and hip BMC [11, 12]. This conclusion was based primarily on prospective cohort studies (four out of seven studies finding this relationship) [127, 305 are in Tables 2.4 & 2.5] and cross-sectional studies (also see Table 2.3). No randomised controlled trials identified by these authors noted an association between baseline 25(OH)D and changes in BMD [128, 307, 308, 309 are in Table 2.6], however one noted an effect of supplementation on BMD after adjusting for baseline 25(OH)D [128].

Comparison of 25(OH)D levels between randomised controlled studies in relation to whether or not an effect on BMD changes was reported might provide an alternative means of estimating optimal 25(OH)D levels (Table 2.6). Randomised controlled vitamin D interventions investigating effects on BMD appear to have generally found effects when baseline levels of 25(OH)D are ≤40 nmol/L [128, 143, 164, 333], although effects have been noted in studies with much higher baseline 25(OH)D levels of 77 – 78 nmol/L [162, 334]. A previous meta-analysis of vitamin D interventions on bone mineral density (BMD) has, however, failed to identify a clear benefit. Pooled analyses noted only small early (less than 1 year) reductions in bone loss at the lumbar spine and later similar reductions at the femoral neck [335]. Although this meta-analysis did consider differences in trial designs as possible explanatory variables for the differences noted, the co-administration of calcium in particular, it did not examine differences in baseline 25(OH)D between the studies.

In their meta-analysis, Cranney et al., did address the question of 25(OH)D levels associated with beneficial changes in BMD [12]. The authors identified 13 trials with BMD endpoints that reported baseline 25(OH)D, 15 trials which reported follow-up levels or change in BMD and 12
that reported both. Five of these studies were in people with vitamin D deficiency [mean 25(OH)D ~ 30 nmol/L], and all of these studies reported significant effects [128, 143, 164, 336, 337]. However, because of differences and ambiguity about 25(OH)D assays used, the authors were unable to establish, amongst all studies, whether there was an interaction between baseline or change in 25(OH)D or determine any upper limit for an effect. The authors also identified 4 trials which assessed the effect of baseline 25(OH)D on BMD response to vitamin D, less than the 6 identified above due to stricter study selection criteria for this analysis. As previously stated, none of these four studies reported such as association [128, 307-309].

Here, a review of studies investigating BMC or BMD changes in relation to baseline levels or changes in 25(OH)D has failed to identify any clear relationships between these variables. Similarly, in intervention studies, although most studies of people with baseline levels <40 nmol/L appear to show an effect, there does not appear to be a threshold 25(OH)D level above which effects are never shown. The Cranney et al. report was published after this literature review was undertaken [12]. It is notable that this report indicates that a number of the authors also attempted to establish 25(OH)D thresholds, but were unable to do so. From the prospective evidence, Cranney et al. noted a large range in baseline 25(OH)D levels (from 30 to 80 nmol/L) below which bone loss at the hip increased. They also noted that most of these studies failed to adequately adjust for important confounding variables.
<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Population*</th>
<th>Season</th>
<th>Correlation</th>
<th>25(OH)D (nmol/L)</th>
<th>Other Outcomes or Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan et al., 1991 [316]</td>
<td>164</td>
<td>Children 9±4 yr (Range: 2-16 yr)</td>
<td>Not stated</td>
<td>No relationship between 25(OH)D and BMC estimated from SPA scan of radius</td>
<td>≈70 (Range: 8-138)</td>
<td></td>
</tr>
<tr>
<td>Meier et al., 1991 [317]</td>
<td>137</td>
<td>Premenopausal women 37±7 yr</td>
<td>Throughout year</td>
<td>No relationship between 25(OH)D nor PTH and radial or vertebral BD</td>
<td>49±26</td>
<td></td>
</tr>
<tr>
<td>Khaw et al., 1992 [310]</td>
<td>138</td>
<td>Perimenopausal women 57±5 yr (Range: 45-65 yr)</td>
<td>Throughout year</td>
<td>25(OH)D positively correlated with lumbar and femoral BMD (r=0.2) PTH also negatively correlated with lumbar and femoral BMD (r=-0.2)</td>
<td>29±12 (Range: 4-58)</td>
<td></td>
</tr>
<tr>
<td>Ooms et al., 1995 [147]</td>
<td>330</td>
<td>Elderly women 80±6 yr (70+ yr)</td>
<td>Throughout year</td>
<td>25(OH)D positively related to femoral but not radial BMD PTH also negatively related to femoral and radial BMD</td>
<td>28±13</td>
<td>25(OH)D threshold of 30 nmol/L: estimated from best fit to curve (log femoral BMD versus 25(OH)D) No gender difference in 25(OH)D</td>
</tr>
<tr>
<td>Scharla et al., 1996 (EVOS Study) [311]</td>
<td>415</td>
<td>Older adults (Range: 50-80 yr)</td>
<td>Throughout year</td>
<td>25(OH)D positively correlated with femoral BMD in women (r=0.3) but not in men – greater correlation in summer than winter</td>
<td>Summer 67±25 Winter 42±22</td>
<td>No gender difference in 25(OH)D</td>
</tr>
<tr>
<td>Tsai et al., 1997 [318]</td>
<td>262</td>
<td>Older women (Range: 40-72 yr)</td>
<td>Throughout year</td>
<td>No relationship between 25(OH)D and BMD at any site</td>
<td>77±20</td>
<td></td>
</tr>
<tr>
<td>Kristinsson et al., 1998 [319]</td>
<td>259</td>
<td>Young women (Range: 16-20 yr)</td>
<td>Late winter</td>
<td>No relationship between 25(OH)D and BMD at any site for whole group 25(OH)D positively correlated with forearm BMC &amp; BMD in youngest group, whose 25(OH)D was slightly lower (r=0.3)</td>
<td>44±21</td>
<td>16yr 41±20</td>
</tr>
<tr>
<td>Sigurdsson et al., 2000 [320]</td>
<td>248</td>
<td>Elderly women (70+ yr)</td>
<td>Autumn to spring</td>
<td>No relationship between 25(OH)D and whole body or lumbar BMD PTH negatively related to whole body (r²=2.2%) but not lumbar BMD</td>
<td>53±20</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3 cont.

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Population*</th>
<th>Season</th>
<th>Correlation</th>
<th>25(OH)D (nmol/L)</th>
<th>Other Outcomes or Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamberg-Allardt et al., 2001 [321]</td>
<td>328</td>
<td>Adults 38±3 yr (Range: 31-43 yr)♂ 126 37±4 ♀ 202 38±3</td>
<td>Late winter</td>
<td>No correlation of 25(OH)D or PTH with forearm BMD</td>
<td>46±34</td>
<td>25(OH)D threshold based on PTH plateau♂ 40 nmol/L (PTH 2.4 pmol/L)♀ 80 nmol/L (PTH 2.7 pmol/L)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lips et al., 2001 [130]</td>
<td>7564</td>
<td>Postmenopausal women 67±7 yr (Range: 31-80 yr)</td>
<td>Throughout year</td>
<td>25(OH)D&lt;25 nmol/L associated with lower trochanter BMD but not femoral neck or lumbar BMD</td>
<td>71±31</td>
<td>25(OH)D threshold of 50 nmol/L based on 6 month PTH change in placebo group</td>
</tr>
<tr>
<td>Outila et al., 2001 [322]</td>
<td>178</td>
<td>Adolescent females 15±1 yr (Range: 14-16 yr)</td>
<td>Late winter</td>
<td>No relationship between 25(OH)D nor PTH and radial or ulnar BMD</td>
<td>39±14</td>
<td></td>
</tr>
<tr>
<td>Pfeifer et al., 2001 [156]</td>
<td>237</td>
<td>Postmenopausal women 63±7 yr</td>
<td>Throughout year</td>
<td>25(OH)D correlated with femoral BMD (details not stated)</td>
<td>70±31</td>
<td></td>
</tr>
<tr>
<td>Segal et al., 2003 [338]</td>
<td>78</td>
<td>Lactose intolerance patients (Range: 20-78 yr)</td>
<td>Not stated (6 month period)</td>
<td>Age-corrected 25(OH)D correlated with femoral and hip BMD z-scores (r≈0.3) and lumbar BMD z-score in postmenopausal women (r=0.5)</td>
<td>Not stated</td>
<td>(21% &lt; 37 nmol/L)</td>
</tr>
<tr>
<td>Bischoff-Ferrari et al., 2004 [313]</td>
<td>13432</td>
<td>Adults (20+ yr)</td>
<td>Throughout year</td>
<td>White Quintiles of 25(OH)D associated with hip BMD Hispanic Quintiles of 25(OH)D associated with hip BMD Black Quintiles of 25(OH)D associated with hip BMD for ≥50 yrs</td>
<td>White 77±28 Hispanic 61±23 Black 49±21</td>
<td></td>
</tr>
<tr>
<td>Arya et al., 2004 [314]</td>
<td>92</td>
<td>Asian Indians adults 34±7 yr (Range: 24-53 yr)</td>
<td>Not stated</td>
<td>25(OH)D positively correlated with hip and femoral BMD (r≈0.5)</td>
<td>31±27</td>
<td></td>
</tr>
<tr>
<td>Välimäki et al., 2004 [339]</td>
<td>220</td>
<td>Young men 20 yr median (Range: 18-21 yr)</td>
<td>Baseline in summer</td>
<td>25(OH)D positive determinant of femoral, hip and lumbar BMD with age, height, weight and lifestyle factors included in model (uncorrected r=0.04-0.07)</td>
<td>110 median (Range: 37-267)</td>
<td>No 25(OH)D threshold for rise in PTH observed</td>
</tr>
<tr>
<td>Study</td>
<td>n*</td>
<td>Population*</td>
<td>Season</td>
<td>Correlation</td>
<td>25(OH)D (nmol/L)</td>
<td>Other Outcomes or Conclusions</td>
</tr>
<tr>
<td>------------------------------</td>
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<td>-----------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Nakamura et al., 2005 [323]</td>
<td>108</td>
<td>Young women 21±1 yr (Range: 19-25 yr)</td>
<td>Autumn &amp; early winter</td>
<td>No relationship between 25(OH)D and lumbar or femoral BMC &amp; BMD PTH negatively related to femoral and lumbar BMC &amp; BMD (r=-0.2-0.3)</td>
<td>36±11</td>
<td></td>
</tr>
<tr>
<td>von Muhlen et al., 2005 [312]</td>
<td>615</td>
<td>Postmenopausal women 75±10 yr (Range: 50-97 yr)</td>
<td>Throughout year</td>
<td>25(OH)D positively and PTH negatively associated with hip BMD in a model including age, body mass index, drug and supplement use</td>
<td>102±35</td>
<td></td>
</tr>
<tr>
<td>Garnero et al., 2007 (OFELY study) [324]</td>
<td>671</td>
<td>Postmenopausal women 62±9</td>
<td>Throughout year</td>
<td>No relationship between 25(OH)D or PTH and hip, femur or radial BMD</td>
<td>(35% ≤ 50) (n=569)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means or means ± standard deviations unless otherwise stated
*Sample size of and age for entire cohort unless otherwise stated
D3 = vitamin D3 cholecalciferol; D2 = vitamin D2 ergocalciferol; 25(OH)D = 25 hydroxyvitamin D; BMD = bone mineral density; BMC = bone mineral content; SPA = single photon absorptiometry
<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Population*</th>
<th>Study Design</th>
<th>Nature of Seasonal Changes</th>
<th>25(OH)D (nmol/L)</th>
<th>Outcomes or Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawson-Hughes et al., 1991 [326]</td>
<td>125</td>
<td>Postmenopausal women 62±0.5 yr (placebo)</td>
<td>longitudinal</td>
<td>25(OH)D 25% lower in winter/spring than summer/autumn with similar 1-1.5% fall in lumbar but not whole body BMD between these months</td>
<td>≈71 (mean between seasons)</td>
<td>seasonal difference less in those given vitamin D supplements</td>
</tr>
<tr>
<td>Rosen et al., 1994 [305]</td>
<td>15</td>
<td>Elderly women 77 yr</td>
<td>longitudinal</td>
<td>25(OH)D 13% lower in late winter (Feb) than in late summer (Aug) (PTH 27% higher) with similar 4.2% and 2.4% fall in lumbar and femoral BMD respectively</td>
<td>≈75 (mean between seasons)</td>
<td>dietary vitamin D correlated with femoral BMD changes and degree of winter drop in 25(OH)D may be key determinant of lumbar BMD seasonal differences may not affect BMD in healthy women</td>
</tr>
<tr>
<td>Tsai et al., 1997 [318]</td>
<td>262</td>
<td>Adult women 55±1 yr (Range: 40-72 yr)</td>
<td>cross-sectional</td>
<td>25(OH)D 13% lower in winter than in summer but no difference in BMD</td>
<td>77±20</td>
<td></td>
</tr>
<tr>
<td>Yonei et al., 1999 [328]</td>
<td>31</td>
<td>Adult men 35±7 yr (Range: 24-51 yr)</td>
<td>longitudinal</td>
<td>25(OH)D 16-22% lower in spring than in summer but no seasonal change in calcaneous BMD</td>
<td>57±12</td>
<td>no change in BMD during Antarctic winter</td>
</tr>
<tr>
<td>Rapuri et al., 2002 (STOP IT study) [108]</td>
<td>251</td>
<td>Elderly women (Range 65-77 yr)</td>
<td>cross-sectional</td>
<td>25(OH)D 20% lower in late winter than in late summer (PTH not significant) with similar 6-8% fall in lumbar, total body and radial, but not hip BMD in same period</td>
<td>74±1</td>
<td>small seasonal changes in bone outcomes</td>
</tr>
<tr>
<td>Viljakainen et al., 2006 [327]</td>
<td>196</td>
<td>Adolescent girls 11±0.4 yr</td>
<td>cross-sectional</td>
<td>25(OH)D 37% lower in late winter than in autumn (PTH not significant) with similar 7-10% fall in lumbar and femoral BMD</td>
<td>60±13</td>
<td>small seasonal changes in bone outcomes in adolescent girls</td>
</tr>
</tbody>
</table>

Data are means or means ± standard deviations unless otherwise stated

*Sample size of and age for entire cohort unless otherwise stated

D<sub>3</sub> = vitamin D<sub>3</sub> cholecalciferol; D<sub>2</sub> = vitamin D<sub>2</sub> ergocalciferol; 25(OH)D = 25 hydroxyvitamin D; BMD = bone mineral density
Table 2.5 Studies Investigating Other Longitudinal Associations Between Vitamin D Status and Change in Bone Mineral Content or Density

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Population*</th>
<th>Duration</th>
<th>Correlation</th>
<th>25(OH)D (nmol/L)</th>
<th>Outcomes or Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ooms et al., 1995 [128]</td>
<td>348</td>
<td>Elderly people (70+ yr) 80±6 yr</td>
<td>1st year in 2 year trial</td>
<td>Baseline 25(OH)D did not modify the effect of vitamin D supplementation on 1 year bone loss</td>
<td>27 (median)</td>
<td></td>
</tr>
<tr>
<td>Dawson-Hughes et al., 1995 [308]</td>
<td>247</td>
<td>Postmenopausal women 64±5 yr</td>
<td>2 yr</td>
<td>Levels of 25(OH)D in any season not correlated with whole body, lumbar or hip BMD change</td>
<td></td>
<td>Baseline levels not stated 700 IU/day but not 100 IU/day attenuated seasonal 25(OH)D change</td>
</tr>
<tr>
<td>Peacock et al., 2000 [329]</td>
<td>438</td>
<td>Elderly people (&gt;60 yr) 74±8 yr</td>
<td>6mth &amp; 1yr in 4 yr trial</td>
<td>Positive relationship (r=0.13) between 25(OH)D and change in total hip BMD when calcium intake &lt; median (716 mg) (n=393)</td>
<td>62±28</td>
<td>No seasonal PTH change</td>
</tr>
<tr>
<td>Bischoff-Ferrari et al., 2005 [127]</td>
<td>327</td>
<td>Elderly people 74±11 yr</td>
<td>1 – 2 yr</td>
<td>Positive association between 25(OH)D and BMD corrected for confounders; Higher group have higher BMD</td>
<td>70±31</td>
<td></td>
</tr>
<tr>
<td>Garnero et al., 2007 (OFELY study) [324]</td>
<td>671</td>
<td>Postmenopausal women 62±9 yr</td>
<td>11.2 yr median</td>
<td>No relationship between 25(OH)D and radial bone loss</td>
<td></td>
<td>Not stated (35% ≤ 50) (n=569)</td>
</tr>
</tbody>
</table>

Data are means or means ± standard deviations unless otherwise stated
*Sample size of and age for entire cohort unless otherwise stated
D$_{3}$ = vitamin D$_{3}$ cholecalciferol; D$_{2}$ = vitamin D$_{2}$ ergocalciferol; 25(OH)D = 25 hydroxyvitamin D; BMD = bone mineral density
Table 2.6 Vitamin D Interventions Reporting 25-Hydroxyvitamin D & Bone Mineral Density or Content Outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Population</th>
<th>Treatment</th>
<th>Duration</th>
<th>Initial 25(OH)D (nmol/L) †</th>
<th>Change in 25(OH)D (nmol/L) †</th>
<th>Outcome Supplemental Calcium or Intake Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapuy et al., 1992</td>
<td>3270</td>
<td>Elderly women 84±6 yr (Range: 69-106yr)</td>
<td>800 IU/day D₃ + 1.2 g/day calcium</td>
<td>18 month</td>
<td>40±27</td>
<td>105±22</td>
<td>Treatment increased femoral BMD (proximal 7% difference) Calcium supplement to treatment group</td>
</tr>
<tr>
<td>Dawson-Hughes et al., 1995 [308]</td>
<td>247</td>
<td>Postmenopausal women 64±5 yr</td>
<td>100 IU/day D 700 IU/day D</td>
<td>2 yr</td>
<td>not stated</td>
<td>66±26</td>
<td>700 IU/day reduced femoral BMD loss (1% difference) but no difference in lumbar or total body BMD Calcium supplement to both groups 0.5 g/day</td>
</tr>
<tr>
<td>Ooms et al., 1995</td>
<td>348</td>
<td>Elderly people 80±6 yr (70+ yr)</td>
<td>400 IU/ day D₃</td>
<td>2 yr</td>
<td>27 (median)</td>
<td>35 (median change at 1 yr)</td>
<td>Treatment increased femoral (2% difference) but not radial BMD Median calcium intake 870 mg/day</td>
</tr>
<tr>
<td>Dawson-Hughes et al., 1997 [334]</td>
<td>389</td>
<td>Elderly people (65+ yr)</td>
<td>700 IU D₃ / day + 500 mg calcium / day</td>
<td>3 yr</td>
<td>77±37</td>
<td>35±33</td>
<td>Treatment increased femoral (1%), lumbar (3%), and total body (1% differences) BMD Calcium supplement to treatment group and mean intake 730 mg/day</td>
</tr>
<tr>
<td>Gallagher et al., 2001 [162]</td>
<td>246</td>
<td>Elderly women 72±4 yr (Range: 65-77 yr)</td>
<td>calcitriol</td>
<td>3 yr</td>
<td>78±22</td>
<td>-17±20</td>
<td>Treatment improved lumbar (2%), total body (1%), and radial (1%) but not femoral/hip BMD PTH declined in treatment group Calcium intake 763±303 mg/day</td>
</tr>
<tr>
<td>Patel et al., 2001 [340]</td>
<td>70</td>
<td>Adult women 47±14 yr (Range: 24-70 yr)</td>
<td>800 IU/day D₃</td>
<td>2 yr cross-over</td>
<td>72±20 (Range: 30-119)</td>
<td>≈10-15</td>
<td>No treatment effect on any BMD measure Treatment prevented winter dip in 25(OH)D with maximum difference between groups ≈30 nmol/L</td>
</tr>
<tr>
<td>Chapuy et al., 2002</td>
<td>610</td>
<td>Elderly people 85±7 yr (Range: 64-99 yr)</td>
<td>2 treatment groups both: 800 IU/day D₃ + 1.2 g/day calcium</td>
<td>2 yr</td>
<td>22±15</td>
<td>≈22-25</td>
<td>Treatment increased femoral (3%/yr difference) but not radial BMD Calcium supplements to treatment groups and mean intake 560 mg/day</td>
</tr>
<tr>
<td>Study</td>
<td>n*</td>
<td>Population</td>
<td>Treatment</td>
<td>Duration</td>
<td>Initial 25(OH)D (nmol/L) †</td>
<td>Change in 25(OH)D (nmol/L) †</td>
<td>Outcome</td>
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<tr>
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<tr>
<td>Harwood et al., 2004 (NONOF Study) [164]</td>
<td>150</td>
<td>Elderly women 81 yr</td>
<td>3 treatment groups: 300 000 IU stat D2; stat + 1 g/day calcium; 800 IU/day D3 + 1 g/day calcium</td>
<td>1 yr</td>
<td>29 (Range: 6-85)</td>
<td>≈45 (final value not change)</td>
<td>Treatment increases femoral/hip (3-5% difference) but not lumbar BMD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Range: 67-92 yr)</td>
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<tr>
<td>Cooper et al., 2003 [309]</td>
<td>187</td>
<td>Postmenopausal women 56±4 yr</td>
<td>10 000 IU/wk D2 + 1g Ca/day</td>
<td>2 yr</td>
<td>82±26</td>
<td>not reported</td>
<td>No benefits beyond those with 1g calcium alone</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>No correlation between baseline 25(OH)D or BMD and ΔBMD</td>
</tr>
<tr>
<td>Aloia et al., 2005 [307]</td>
<td>208</td>
<td>Postmenopausal women 61±6 yr</td>
<td>88 IU D3/day 2y then 2000 IU D3/day</td>
<td>3 yr</td>
<td>45 ± 19</td>
<td>not reported</td>
<td>No effect on bone loss or bone turnover markers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>No association between 25(OH)D and ΔBMD</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No influence of 25(OH)D above and below 40 nmol/L or high PTH levels on ΔBMD</td>
</tr>
<tr>
<td>Flicker et al., 2005 [168]</td>
<td>625</td>
<td>Elderly people 83 ± 8 yr</td>
<td>10 000 IU/wk D2 then 1 000 IU/day</td>
<td>2 yr</td>
<td>(Range: 25-90)</td>
<td>43</td>
<td>Reduced number of people who fell but no change in fracture rates</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serhan et al., 2005 [341]</td>
<td>143</td>
<td>Adults 49 ± 12 yr</td>
<td>400 IU/ day D + 1-1.25 g/day calcium</td>
<td>1 yr</td>
<td>11 ± 6</td>
<td>35 ± 16</td>
<td>No effect on BMD at any site</td>
</tr>
</tbody>
</table>

Data are means or means ± standard deviations unless otherwise stated

*Sample size of and age for entire cohort unless otherwise stated (intention to treat in interventional studies)

† For vitamin D treatment group only

D3 = vitamin D3 cholecalciferol; D2 = vitamin D2 ergocalciferol; 25(OH)D = 25 hydroxyvitamin D; (Δ)BMD = (change in) bone mineral density; BMC = bone mineral content; HRT = hormone replacement therapy
2.3.4.3 Bone Turnover Markers

A number of studies provide cross-sectional data from investigations of associations between 25(OH)D and biochemical markers of bone turnover. Some of these have found either negative correlations with, or inverse seasonal fluctuations compared to resorption markers, for example deoxypyridinoline [29, 342], or formation markers, for example osteocalcin [147] or bone specific alkaline phosphatase [342]. Other studies report similar positive correlations between PTH and bone turnover markers, including deoxypyridinoline, osteocalcin and bone specific alkaline phosphatase [338, 343]. However, the majority of these studies have not found any association between circulating 25(OH)D and bone turnover markers [148, 318, 324, 339] or any seasonal fluctuation [298, 318], and one study reported a weak positive association between osteocalcin and 25(OH)D [29].

One calcium intervention in elderly women reported winter increases in N-telopeptide (a resorption marker) and osteocalcin [344]. The findings of other longitudinal studies have been inconsistent. One German study noted distinct seasonal fluctuations, with a winter peak in deoxypyridinoline, N-telopeptide, osteocalcin and bone specific alkaline phosphatase [345]. A second US study reported winter to spring increases in bone formation markers (alkaline phosphatase and 24-hour whole body retention of 99mTc-diphosphonate) but no change in resorption markers [346]. In contrast, a study of a Japanese Antarctic wintering team of men reported increases in osteocalcin and bone specific alkaline phosphatase during summer and reductions in pyridinoline and deoxypyridinoline at the end of winter [328]. These seemingly paradoxical data may have reflected true seasonal lags between increasing UV exposure, resorption and subsequent formation, or may have been an acute effect of the substantial change in circadian behaviour, diet and lifestyle resulting from living in this extreme environment. In addition to these two longitudinal studies showing seasonal alterations in bone turnover markers, other such studies have found none [305, 328, 340, 347].

No attempt can be made to establish optimal levels of 25(OH)D from between study comparisons of relationships between 25(OH)D and bone turnover markers due to substantial inconsistency in these data. Nonetheless, two cross-sectional studies have sought thresholds for 25(OH)D in these relationships and have reported that levels below, compared to above, 30 nmol/L are inversely associated with circulating osteocalcin [147], and that the greatest differences in bone turnover marker levels occur above and below cut-points for 25(OH)D of 60 nmol/L [348].
2.3.4.4 Fracture Rates

Cranney et al. identified 15 studies, all in older adults, investigating an association between 25(OH)D and fractures [11, 12]. Of these, three were prospective cohort investigations and the remainder were case-control studies. Ten out of the 12 case-control studies found significantly lower 25(OH)D in those with hip fracture than controls. Only one prospective cohort study found an association between 25(OH)D and fracture rate [158]. In this study, 21% of participants with 25(OH)D levels less than 50 nmol/L sustained a fracture during the study compared to 12% with levels of 50 nmol/L or above. The other two prospective studies did not find an association between 25(OH)D and the risk of hip or vertebral fracture [349] or any fracture [303] after adjusting for important confounding variables. Two recent prospective analyses in cohort studies add to these data. A prospective nested case-control assessment of data from the Women’s Health Initiative reported no difference in baseline 25(OH)D levels between those who did or did not sustain hip fractures, whether or not the combined calcium/vitamin D intervention was adjusted for [350]. Similarly, a more recent prospective study [324], found no association between 25(OH)D levels and fracture risk, changes in bone mineral density or bone turnover markers after adjusting for age.

Therefore, there is little evidence for a relationship between 25(OH)D and fracture risk in prospective of randomised controlled trials. For conclusions based upon interpretations of cross-sectional data, much the same problems exist as for data from studies of the association of vitamin D status with cancer and other chronic diseases. Those who fracture are highly likely to be frail pre-fracture and may therefore spend less time outdoors and have lower 25(OH)D levels. Additionally, in many of the studies, 25(OH)D was assayed following hip fractures and intervening periods of immobility and hospitalisation may be extended and are variable.

Meta-analyses of vitamin D interventions on fracture risk suggest an effect in some situations, but have also been equivocal. One meta-analysis showed a positive effect of 700 – 800 IU vitamin D on non-vertebral fracture risk [127]. Another has found an effect of vitamin D given in conjunction with calcium on hip and non-vertebral fractures, but noted that this effect was restricted to studies of people in institutional care [306]. A third, restricting its analysis only to hip fracture, concluded that the effect on vitamin D is dependant upon adjunct supplemental calcium [351]. Closer scrutiny of data from these meta-analyses suggests that the results are likely to be strongly influenced by a sizeable effect from a small handful of studies conducted in institutionalised elderly. In particular, a French study, and its follow-up, reported sizeable effects of supplemental calcium (1.2 mg/day) and vitamin D (800 IU/day), relative to double placebo, in
frail but ambulatory elderly women living in residential care, in whom a large number of hip fractures were sustained [333, 352]. In contrast, data from three large, recent randomised controlled trials, in which no effect of vitamin D on fracture risk was shown, call into question the efficacy of vitamin D treatment (with and without calcium) to prevent osteoporotic fractures [350, 353, 354]. Nonetheless, compliance to treatment was low in all of these with 55% taking any study pills at 2 years of a 5-year trial (Randomised Evaluation of Calcium Or Vitamin D: RECORD) [353], 59% taking at least 80% of their study pills at the end of the trial (Women’s Health Initiative: WHI; refer also to other criticisms of this study in Chapter 2.3.3.1 Cancer, pg. 32) [350] and 69% taking study pills at 2 years [354]. The last of these studies was also unique in that it investigated secondary prevention of fractures in those already assessed to be at high risk [353].

It may be possible to estimate optimal levels of 25(OH)D by comparing the results of randomised controlled trials of vitamin D in relation to 25(OH)D levels. In all, approximately 15 randomised control trials evaluate the effect of oral vitamin D (mainly D₃) supplementation on fractures in postmenopausal women and older men [12] and two more evaluate annually administered parenteral vitamin D₂ [299]. These studies have been frequently reviewed and are tabulated in a number of other publications [12, 127, 306, 351], including tabulation of baseline 25(OH)D levels in the treatment group of all vitamin D randomised controlled trials identified by Avenell et al. in their meta-analysis [306]. Cranney et al. also attempted to separate studies reporting and not reporting effects by baseline and changes in 25(OH)D [12]. In both reports [12, 306] the authors highlighted the likely influence on the analyses of the previously reviewed French study of elderly women living in residential care [352], in whom baseline 25(OH)D levels were 40 nmol/L [352]. This finding suggests that optimal concentrations of 25(OH)D are at least above this level. Changes in 25(OH)D following vitamin D interventions or end-of-study levels may be a stronger predictor of an effect, although not all studies report these. To this end, Cranney et al. [12] noted a significant reduction in fracture risk (odds ratio = 0.73) when the 4 identified trials with end-of-study 25(OH)D levels of ≥74 nmol/L were combined [143, 166, 326, 352]. Three of these studies reported a reduction in total fracture risk [all except 143]. In contrast, combining trials with end-of-study 25(OH)D levels <74 nmol/L resulted in no effect on fracture risk. Both Avenell et al. and Cranney et al. advise caution in the interpretation of any of these results due to differences in assays between studies [11, 12, 306]
2.3.5 Summary

In summary, the present examination cross-sectional and longitudinal data from the literature has been unsuccessful in determining an optimal level of 25(OH)D. Analyses of well designed epidemiological studies of 25(OH)D and lower bowel cancer, suggest that levels of >75 – 80 nmol/L may be preferable to levels below this, but few other data are convincing. There is no evidence of threshold behaviour in the cross-sectional relationships noted between 25(OH)D and bone mineral or fracture outcomes, the main conclusion being that low levels of 25(OH)D (21 – 40 nmol/L) combined with moderately high doses of intervening vitamin D (≥700 IU/day) tend to result in positive findings, with respect to fracture risk. Similarly, in longitudinal studies investigating skeletal outcomes that report 25(OH)D, no level of 25(OH)D can be identified, below which effects are invariably demonstrated, and above which they disappear. This conclusion corresponds to that of Cranney et al. [11, 12]. In those reports the authors concluded that there was insufficient data to establish a specific concentration of 25(OH)D associated with optimal bone health outcomes, mainly due to the difficulties associated with inter-laboratory assay comparisons, a conclusion reiterated in a recent review by Greer on the subject [355].

2.3.6 Parathyroid Hormone & Optimal Vitamin D Status

The greatest number of investigations specifically investigating optimal 25(OH)D status have used PTH as an outcome variable and these have been well summarised in a systematic review by Aloia et al. [17]. Estimates a based on an upper threshold level of 25(OH)D associated with PTH plateau and vary from 25 nmol/L to 122 nmol/L. The median threshold level reported in studies reviewed by Aloia et al. was just above 50 nmol/L and these authors noted two clusters of estimates both between 40 to 50 nmol/L and between 70 to 80 nmol/L [17]. A great many other studies report a cross-sectional inverse correlations ranging from r = -0.14 to -0.55 between circulating levels of the two hormones (often from baseline or pooled measurements in an interventional or prospective study), but do not specifically seek a threshold in this relationship [for example 14, 69, 149, 343, 356] or do not find one [18, 324].

The concept of a threshold 25(OH)D based on a plateau of PTH has been criticised by some for a number of reasons. One reason is that although the majority of cross-sectional studies investigating relationships between 25(OH)D and fractures or BMD show an effect, the results from prospective studies are less clear (refer Chapter 2.2.2 Optimal Levels of 25-Hydroxyvitamin D, pg. 19). Hence, the clinical utility of using PTH as an indicator of optimal vitamin D status has been questioned [17, 324]. Others argue that PTH does not reach a threshold but continues to decrease as 25(OH)D rises, even in cases of vitamin D toxicity or high
doses of calciferol [357-359]. In a positioned review, Vieth & El-Hajj Fuleihan, 2005 [359] cite data from a graded dosing study which indicates dose-related suppression of PTH with 8-week dosing ranging from 1000 – 50 000 IU/day [88]. In addition, a recent meta-analysis of 52 clinical trials failed to identify a clear threshold in the pooled relationship between changes in 25(OH)D and changes in PTH [360]. However, whether PTH is likely to be suppressed by vitamin D supplementation depends on both the baseline levels and subsequent change in 25(OH)D. Although baseline PTH instead of baseline 25(OH)D entered into a multiple regression model predicting PTH change in this meta-analysis, a clearer demonstration of a lack of a threshold would have made some correction for the initial state. Another reason is that a great deal of variability in PTH at a particular level of 25(OH)D is observed, and models which force a cut-point onto these data are subjective [359]. For example, a Danish study of 2016 healthy perimenopausal women found only 16% of women with very low 25(OH)D (25 nmol/L or less) had levels of PTH elevated above the reference range [18]. Although few women had elevated PTH when 25(OH)D levels were above 75 nmol/L, it was not until levels exceeded 100 nmol/L that all values of PTH were within the reference range [18]. The variability in PTH associated with a particular level of 25(OH)D may arise from methodological issues, including: assay variability, the model used to establish a threshold, lag in the seasonal changes in PTH occurring as a result of changing 25(OH)D, or individual factors, including: age, sex and race, renal function, calcium intake, adiposity or reproductive hormonal levels.

2.3.7 Factors Affecting the Relationship Between PTH & 25(OH)D

2.3.7.1 Assays and Techniques Used to Estimate a Threshold for PTH

The assay for 25(OH)D, in particular, is subject to substantial variability. In addition to both inter- and intra-assay variability in 25(OH)D measurement within a laboratory, there may be considerable variability between laboratories, even those employing the same assay. When laboratories employed their routine 25(OH)D assay methods one study noted a coefficient of variation of 35% for levels in eight plasma samples measured at 19 laboratories [361] and two other studies noted maximum mean differences of approximately 47 nmol/L between the same samples measured at different laboratories [362, 363]. Consequently, as a result of this variability, differences in threshold levels of the metabolite at which PTH plateaus may fluctuate within a study, and certainly between studies.

Developing valid assays for 25(OH)D presents a technical challenge due to the hydrophobic nature of this metabolite and the fact that it may exist in its two, D2 and D3, forms [364, 365]. Consequently, the first competitive protein binding assay for 25(OH)D assessment, which used a
tritium label, was not developed until the 1970s [366]. More recently, other assays have become popular. These include radioimmunoassays, such as that developed by DiaSorin Corporation, and assays allowing direct determination from plasma or serum using competitive chemiluminescence, for example the Nichols Advantage® (now withdrawn) and DiaSorin Liaison® Systems. Physical detection of 25(OH)D is also possible using high-performance liquid chromatography or liquid chromatography and mass spectrometry. Although these techniques are considered more valid with experienced users, they are generally more cumbersome and, in the case of mass spectrometry, require expensive equipment [365]. Competitive binding assays and chemiluminescent assays were shown to overestimate levels compared to liquid chromatography [362, 363].

Further assay-related difficulties occur when oral vitamin D₂ supplementation contributes to a population’s vitamin D status. Although, in particular, the DiaSorin radioimmunoassay equally detects 25(OH)D₂ and 25(OH)D₃, other radioimmunoassays and chemiluminescent methods do not [365, 367] and differences in the availability and use of D₂ and D₃ preparations amongst the study population might also be a source of error in 25(OH)D threshold estimates.

Additionally, a range of quantitative and qualitative techniques have been used to determine a threshold level of 25(OH)D for a PTH plateau. Different mathematical models, which are all likely to produce disparate results, have been utilised to provide estimates of 25(OH)D threshold. Commonly used approaches employ the locally weighted scatterplot smoothing (LOWESS or LOESS) model, which makes no prior assumptions about the shape of the relationship; a model of exponential decay, from which an inflection point may be calculated; and a spline function and associated cut-point, which produces maximal difference in the slopes of the lines above and below it. In the final model, the additional stipulation that the slope of the line associated with higher levels of 25(OH)D is not different from zero is often set. Their pros and cons have been recently reviewed [17].

2.3.7.2 Seasonal Variation
Both 25(OH)D and PTH have been shown to vary seasonally with opposing peaks and nadirs. These seasonal variations have been established in a range of ages from both from cross-sectional data [129, 312, 368-370] and longitudinal data [305, 345, 371-374], although PTH does not always exhibit seasonal fluctuation when mean annual 25(OH)D levels are greater than 50 to 60 nmol/L [108, 327, 340, 342, 375]. However, the timing of the peaks and nadirs does not coincide exactly. Pasco et al., in cross-sectional data from a population-based study, have noted that PTH periodicity has a 1 month phase-shift delay relative to 25(OH)D [370]. It is therefore
possible that there may be transient alterations in the relationship between 25(OH)D and PTH when no adjustment is made for this lag, and this could be another source of error in estimates of 25(OH)D threshold based on this relationship.

2.3.7.3 Age, Sex & Race
The relationship between 25(OH)D and PTH could also be influenced by age, sex and race. Age differences in the slope of the cross-sectional relationship between log PTH on log 25(OH)D have been reported [357], those over 70 years tending to have a less steep negative slope with PTH levels declining less with increases in 25(OH)D in this age-group. Age-related hyperparathyroidism could have a number of causes including endocrine changes with age such as depleted levels of reproductive hormones or relative resistance of gut calcium absorption to 1,25(OH)₂D, reduced calcium intake, or loss of kidney function with impaired 1,25(OH)₂D synthesis [8, 357]. It is not clear, however, how the change in slope might translate to differences in threshold estimations for different age groups, although in this study, Vieth et al. have argued that no threshold is evident from their data since a non-biased LOWESS regression plot, which makes no assumptions about the nature of the relationship, of the logarithms of both hormones returned a shape not outside the 95% confidence limits for linear regression [357]. In the review by Aloia et al. of studies investigating a threshold effect in the relationship between PTH and 25(OH)D, there was a minimal difference in the mean threshold in studies of people less than compared to greater than 50 years of age, 59 nmol/L compared to 63 nmol/L [17].

It is also possible that data from elderly populations might artefactually emphasise a threshold effect, since the most frail have both poor vitamin D status and secondary hyperparathyroidism compounded by renal insufficiency, inadequate dietary calcium and impaired absorption of this nutrient. Although less data is available in younger populations, an analysis of previous studies shows that the existence of a PTH versus 25(OH)D relationship from which a plateau can be investigated is less clear in younger age-groups, even when the levels of 25(OH)D are below those at which hyperparathyroidism is normally observed in older cohorts. On the one hand, studies of men and women >50 years of age have invariably observed cross-sectional correlations between 25(OH)D and PTH and often reported a biphasic relationship which exhibits a 25(OH)D threshold. On the other hand, similar investigations in younger adults and children have produced more variable results. A sizeable proportion (approximately one third of such studies identified here) do not report a 25(OH)D threshold [30, 315, 323, 373, 376, 377], and a further proportion (also around one third of identified studies) report no association at all between 25(OH)D and PTH [317, 371, 378-381]. Of the remaining studies in younger age-groups that have estimated an optimal level of 25(OH)D level, this estimate has seldom been
based on a demonstrated PTH plateau and more commonly on the level of 25(OH)D associated with a lack of elevated PTH levels, defined as either above the reference range or above the study’s median PTH level [31, 322, 382].

In addition to age, there may be gender differences in 25(OH)D threshold. Very few studies have reported estimations of threshold separately for men [17]. In one study of 202 premenopausal Finnish women and 126 men of a similar age, the estimate of threshold, based on non-linear regression, for men compared to women was approximately 40 nmol/L higher at 80 nmol/L. However, no gender difference in the relationship between 25(OH)D and PTH was reported in another study of elderly men and women [129].

Racial differences in calcium homeostasis may be another source of variation in estimates of 25(OH)D threshold based on a plateau in PTH. Higher levels of circulating PTH for a given 25(OH)D concentration compared to Caucasians have been reported in Chinese [302], Indian migrants to the United States (Awumey et al, 1998) and African Americans [383, 384], though not in all studies for African Americans [317]. Observations that African Americans, compared to Caucasian controls with similar dietary calcium intakes, excrete less urinary calcium, have led some to postulate that calcium might be more efficiently absorbed by this group [317, 384-386]. However, there are many determinants of urinary calcium which might also be responsible for this observation [57] (Refer to Chapter 2.1.2 Parathyroid Hormone Structure, Regulation & Action, pg. 7). In the study comparing Chinese and British elderly, PTH also exhibited a lesser reduction with increasing 25(OH)D [302] and it therefore seems very likely that ethnic differences in the relationship between PTH and 25(OH)D would also result in differing threshold estimates.

2.3.7.4 Renal Function
Renal insufficiency results in impaired production of 1,25(OH)₂D, reducing calcium absorption and thus resulting in an elevation of PTH that is independent of 25(OH)D. Consequently, gradual impairment of renal function which occurs with aging [8] is likely to alter the relationship between 25(OH)D and PTH and any resulting estimate of a threshold within it. One recently reported retrospective analysis of hospital biochemistry records has substantiated this [387]. The authors found that estimated glomerular filtration rate was highly inversely correlated with PTH but not 25(OH)D and that in the lowest tertile of estimated glomerular filtration rate (less than 60 mL/min), 25(OH)D levels less than 50 nmol/L resulted in additional elevation of PTH compared to estimated glomerular filtration rates above this level. The higher levels of PTH, particularly at lower concentrations of 25(OH)D, might be anticipated to raise indicated 25(OH)D threshold levels in situations of impaired kidney function.
2.3.7.5 Calcium Intake

Another factor that can impact upon the relationship between PTH and 25(OH)D is calcium intake. PTH secretion and suppression are ultimately controlled by serum calcium levels. For active vitamin D metabolites to usefully increase calcium absorption, sufficient dietary calcium must be available. Very low calcium intake causes rickets in children [113] and high dietary calcium results in PTH suppression due to increased intestinal calcium absorption via non-vitamin D dependent paracellular routes [388, 389]. Accordingly, low calcium intake is associated with elevated PTH independently of 25(OH)D in the elderly [329, 390], in younger adults [323, 338, 391], and in adolescent girls [381]. Calcium intake has also been shown to modify the relationship between 25(OH)D and PTH [322, 391, 392] and would be likely therefore to influence 25(OH)D thresholds determined from it.

2.3.7.6 Body Mass & Composition

Percent body fat has been shown to cross-sectionally affect PTH levels independently of 25(OH)D [393]. This effect may be mediated by leptin. A recent cross-sectional investigation reported that secondary hyperparathyroidism with low levels of 25(OH)D, and hence a strong inverse association, was primarily found in women with elevated circulating leptin [394]. The independent association between PTH and adiposity, might also explain a recently identified link between PTH and mortality, which was independent of vitamin D status and renal function [395]. Although, no studies to date have specifically investigated the effect of indices of body mass or composition on the relationship between 25(OH)D and PTH, it is quite likely that calculations of a 25(OH)D threshold might be altered by changes in the relationship that occur due to differences in mass or adiposity.

2.3.7.7 Oestrogen Levels

Whilst it is clear that maintenance of oestrogen levels prevents both bone mineral loss [396] and fractures [349], the exact mechanism for this is uncertain. The effect may be direct on bone cells or may alter the calcium-regulatory endocrine system. Evidence for the latter is conflicting. Harris et al. found that oral contraceptive use was associated with a 40% elevation in 25(OH)D levels and discontinuation of use lead to mean reductions in the metabolite of over 25 nmol/L. However, Finkelstein and Schoenfield, 1999 found drug-induced ovarian suppression for endometriosis treatment, with confirmed oestrogen suppression, did not change the response of 1,25(OH)₂D, blood calcium or skeletal sensitivity to PTH challenge. If levels of reproductive hormones do alter the balance of circulating PTH and 25(OH)D, then they also have the potential to alter the determination of thresholds in the relationship between these metabolites.
2.3.7.8 Overview

It is apparent that a number of factors may affect either PTH and 25(OH)D independently of each other, therefore altering the relationship between these two variables, and hence any estimated threshold in the relationship. Cross-sectional data is particularly susceptible to errors associated with individual factors. These could be vastly reduced by using change in PTH in a longitudinal design as an outcome variable in the determination of a 25(OH)D threshold.

2.4 Vitamin D Supplementation & Safety

2.4.1 Pharmacodynamics of Vitamin D Supplementation

Assuming that vitamin D supplementation is likely to be beneficial for a population, there is a question surrounding the best supplementation regimen. Three recent studies, two in healthy adult men from Heaney et al.’s group, have documented 25(OH)D response to daily vitamin D3 doses [88, 93, 397]. The earliest of these studies assessed short-term (up to 8 week) rate of increase in 25(OH)D following different doses of vitamin D3, 25(OH)D3 and 1,25(OH)2D3 [88]. Linear regression of 25(OH)D response to three vitamin D3 doses between 1000 IU/day and 50 000 IU/day established a dose response of 0.014 nmol/L per IU vitamin D3/day (or 0.54 nmol/L per μg/day) (Table 2.7). When only the lowest dose (1000 IU/day) was included in the regression model, the dose response slope was somewhat higher (0.02 nmol/L), and the authors suggested that change in 25(OH)D was a curvilinear function of dose, with a steeper rate of change at lower doses and a flattening off of the 25(OH)D response at higher doses. In addition, these authors found that pretreatment 25(OH)D was inversely correlated with the rise in 25(OH)D [88]. Both the lessening 25(OH)D response to increasingly high doses, and the smaller dose response when pre-existing levels of 25(OH)D are already high, suggests that there may be some hepatic saturation or regulatory feedback of 25(OH)D production from vitamin D. Further confirmation of this hypothesis comes from two further studies, one using high doses of vitamin D and the other lower doses [88, 397]. The first, a later study by Heaney and coauthors, again followed 25(OH)D response to three doses of vitamin D3 (between 1000 IU/day and 10 000 IU/day) and a control dose, this time for a longer (4 to 5 month) duration when plateaux were observed. When all doses were included in a linear regression model the calculated dose response of 0.015 nmol/L per IU vitamin D3/day (0.6 nmol/L per μg/day) was similar to the earlier study [93] (Table 2.7). The other, a Finnish study, which administered lower vitamin D3 doses (between 200 and 800 IU/day) to elderly women for 8 weeks, reported higher 25(OH)D responses of 0.03 to 0.06 nmol/L per IU vitamin D3/day than the earlier studies [397] (Table 2.7). Relative to a control group, in which 25(OH)D levels fell, the 25(OH)D response in this study was even higher (between 0.04 to 0.1 nmol/L per IU vitamin D3/day).
Hydroxylated forms of the vitamin D metabolites can also be given. The dose response of 25(OH)D to administered 25(OH)D₃ has been shown to be 7.4 times higher than to the same daily drug mass of vitamin D₃ [88].

Table 2.7 Response of 25(OH)D to Oral Doses of Vitamin D₃

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants, n (Age, yr: mean±sd)</th>
<th>Baseline 25(OH)D: mean±sd (nmol/L)</th>
<th>Dose(s) (IU Vitamin D₃ / day)</th>
<th>Duration (weeks)</th>
<th>Response (nmol/L per IU/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barger-Lux et al., 1998</td>
<td>116 men (28±4)</td>
<td>67 ± 25</td>
<td>1 000 1 000 – 50 000</td>
<td>8</td>
<td>0.020 0.014</td>
</tr>
<tr>
<td>Heaney et al., 2003 [93]</td>
<td>67 men (39±11)</td>
<td>70 ± 20</td>
<td>1 000 – 10 000</td>
<td>≈20</td>
<td>0.015</td>
</tr>
<tr>
<td>Viljakainen et al., 2006</td>
<td>49 women (71±4)</td>
<td>46 ± 14 47 ± 10 44 ± 14</td>
<td>200 400 800</td>
<td>6</td>
<td>0.055 0.036 0.030</td>
</tr>
<tr>
<td>Chel et al., 2008 [398]</td>
<td>338 elderly: 76 men/262 women (84±6)</td>
<td>24 ± 9 27 ± 13 24 ± 9</td>
<td>600 (as 600/day) 600 (as 4200/wk) 600 (as 18000/mth)</td>
<td>17</td>
<td>0.075 0.070 0.047</td>
</tr>
<tr>
<td>Ish-Shalom et al., 2008</td>
<td>48 elderly women (81±8)</td>
<td>38 ± 17 39 ± 25 40 ± 25</td>
<td>1500 (as 1500/day) 1500 (as 10500/wk) 1500 (as 45000/mth)</td>
<td>8</td>
<td>0.030 0.022 0.035</td>
</tr>
</tbody>
</table>

Data are means or means ± standard deviations

More recently, there has been interest in the pharmacodynamics of high doses, which can be delivered less frequently. These regimens might be more convenient than daily dosing in some situations, for example in the elderly, and might result in greater overall compliance to the treatment. The time-course of 25(OH)D following an oral dose of vitamin D₃ is not equivalent to the pharmacokinetics of administered 25(OH)D (refer Chapter 2.1.3 Vitamin D Synthesis & Metabolism, pg. 12). It depends on the absorption of vitamin D₃, the hepatic conversion of vitamin D₃ to 25(OH)D and, in addition, to the disappearance half-life of serum 25(OH)D itself. Based on the earlier physiological studies of Mawer and coworkers [400], Vieth has estimated a half-life of combined vitamin D metabolites of around 2 months [401]. This estimate has been confirmed in a recent study which followed healthy American adults after a 100 000 IU dose of vitamin D₃ and found that peak circulating 25(OH)D was obtained at 7 days with an ensuing linear decline that displayed a half-life of approximately 50 days [402]. An earlier New Zealand study, in which 300 000 IU vitamin D₃ was administered to 49 elderly men and women recruited
from hospital wards, observed a slightly longer 25(OH)D half-life of 90 days following a peak occurring between 13 and 21 days [92]. The longer half-life in the New Zealand study may have been due to a reduced rate of vitamin D₃ hydroxylation with higher vitamin D₃ concentrations following this high-dose therapy [403], or could simply have resulted from greater levels of sun exposure in the study group during follow-up.

### 2.4.2 Efficacy of High-Dose Vitamin D Supplementation

In general, higher vitamin D₃ doses administered at approximately the frequency of the half-life would be expected to have similar effects to same cumulative dose administered daily [404]. Thus, large intermittent doses of vitamin D, ranging from 10 000 IU weekly to 300 000 IU annually, are effective and have been shown to increase bone mineral density [164], and to prevent falls [164, 168] and fractures [166, 405]. A recent Dutch study, however, reported that the frequency of dosing did affect the size of the dose response. After 4 months treatment with the equivalent of 600 IU/day vitamin D₃, provided as 600 IU/day, 4200 IU/week or 18 000 IU/month to frail elderly people in residential care, the authors reported a smaller increase in 25(OH)D following monthly dosing compared to daily dosing [398]. Even with monthly dosing, the dose response was high, 0.05 nmol/L per equivalent IU/day (Table 2.7), and this may have been due to the very low initial vitamin D status of the participants (around 25 nmol/L). In contrast, another recent study did not show any significant difference in 25(OH)D response of elderly women to daily, weekly or monthly dosing equivalent to 1 500 IU/day [399] (Table 2.7). A criticism of all these studies is that the treatment period is relatively short, and normally a duration of 4.5 times the half-life (at least 8 to 9 months) would be considered adequate for attaining a new steady state [404].

Additionally, there is some concern about the increased potential for toxicity compared to smaller, more regular doses (refer Chapter 2.4.4 Safe Dose of Vitamin D, pg. 64). An Australian study which treated 50 men and women who had low vitamin D status [25(OH)D < 50 nmol/L] with intramuscular 600 000 IU vitamin D₃, found that at 12 months, 20% had hypercalciuria (urine calcium/creatinine excretion index of 0.6 or greater), and 4% had mild hypercalcaemia (mean level 2.67 mmol/L compared to upper reference range of 2.65 mmol/L) [406]. Nonetheless, it is not clear whether these observations resulted from the vitamin D intervention. Because it would have been unethical to fail to provide vitamin D to individuals with pre-established insufficiency, there was no control group in this study. Furthermore, three individuals who developed hypercalciuria (6% total) also had elevated values at baseline, and primary hyperparathyroidism was uncovered in an additional person. Furthermore, since the time to peak

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25(OH)D after oral administration of a similarly high dose [92] is in the region of 2–3 weeks, it is unclear why calcium levels at 4 months were not also higher than baseline [406]. The possibility of increased fracture rates following high dose vitamin D is suggested by the results of a recent large (n=9440) intervention in which annual 300 000 IU intramuscular vitamin D2 was provided for 3 years[407]. In this study, increased rate of hip and femur fractures (hazard ratio = 1.49) was found for those randomised to vitamin D, though this effect was evident only in women.

2.4.3 Comparison of Vitamins D2 & D3 Supplementation Regimens

Based on the treatment of rickets from the 1930s, vitamins D2 and D3 have generally been considered to be equally effective in humans [408] and nutritional guidelines do not differentiate between the two [39]. However, more recent work has suggested that the form of vitamin D administered may be another determinant of its effect. A number of studies have shown that vitamin D3 compared to D2 results in greater increases in 25(OH)D levels of around two fold [409, 410] or up to 9.5 times greater when the area under the curve is considered [408]. Confirming these findings, a South American study reported 3-month 25(OH)D responses to vitamin D2 supplementation (5 000 to 10 000 IU/day) relative to a control group, of around a third of that expected for similar doses of vitamin D3 (between 0.005 to 0.009 nmol/L per IU/day) [411]. On the other hand, recent data reported by Holick and coworkers introduce some uncertainty about the relative advantage of vitamin D2 or D3, as these authors failed to find any difference in 25(OH)D response between daily 1000 IU doses of either form [412]. More uncertainty arises from assay-related difficulties, when comparing 25(OH)D response to vitamin D2 and D3 therapies, since some assays unevenly measure 25(OH)D2 and 25(OH)D3 [364, 367] (refer Chapter 2.3.6.1 Assays and Techniques Used to Estimate a Threshold for PTH, pg 56).

Despite the observations of increased 25(OH)D responses to vitamin D3 compared to vitamin D2 treatment, not all studies confirm their clinical importance. The advantage of vitamin D3 over D2 was not translated into 1-year changes in bone mass, for which vitamin D2 was shown to be superior in one study [413]. Similarly, in another study, no difference in winter levels of 25(OH)D was found between women who reported taking vitamin D2 compared to D3 [414]. Between study comparisons of the effectiveness of the two forms or vitamin D on falls or bone outcomes are difficult because randomised controlled trials supplementing with daily doses have invariably provided vitamin D3, whilst those using higher, less frequent doses invariably used vitamin D2 [12]. Vitamin D3 preparations delivering more than 1000 IU per dose are not readily available in Australia, Europe or North America and this explains why their efficacy has seldom
been investigated. High-dose studies have used oral vitamin D2 doses of 10 000 IU weekly [168, 309], 100 000 IU quarterly [415], 50 000 IU, 3 times weekly [416] or as large intramuscular injections (doses ranging from 150 000 to 600 000 IU) [164, 167, 405]. An exception is one high-dose British study, which provided oral D3 [166]. Of the five randomised controlled trials investigating skeletal outcomes or falls following high-dose vitamin D2 intervention, none found a treatment benefit on fracture rates and only two noted any significant skeletal effects, on bone mineral density and falls risk [164] or on falls risk alone [415]. In contrast, the high-dose study that provided vitamin D3, did report a lower fracture rate [166].

2.4.4 Safe Dose of Vitamin D

High doses of vitamin D are toxic and may result in hypercalcaemia, renal stones and the calcification of soft tissue and blood vessels [12]. Although it was earlier assumed that rises in 1,25(OH)2D were responsible for the toxicity-associated pathology, the expected substantial elevations in this metabolite have not been consistently demonstrated in cases of toxicity [417]. It is now believed that pharmacological concentrations 25(OH)D and/or other vitamin D metabolites exceed the vitamin D binding protein capacity, causing free 25(OH)D or displaced 1,25(OH)2D to enter cells and stimulate gene transcription [417]. Vieth has suggested that homeostatic control mechanisms, potentially hepatic, prevent the large increases in 25(OH)D necessary for toxicity with regular vitamin D intakes <10 000 IU/day [123].

Toxicity, in the form of hypercalcaemia, has been recorded following long-term (greater than 1 year) doses exceeding 10 000 IU/day or shorter-term doses of 20 000 to 50 000 IU/day or more [123]. The lowest levels of 25(OH)D associated with toxicity are 221 nmol/L, although they are normally higher than 500 nmol/L [123, 417]. By comparison, the highest recorded levels associated with natural sun exposure are 225 nmol/L, and 274 nmol/L following deliberate ultra-violet exposure [123, 292]. Currently established recommendations for safe upper intake levels, which range from 1 000 IU/day in the UK to 2 000 IU/day in Europe and the US, have been heavily criticised by Vieth for being based largely on a single study in which no measurements of 25(OH)D were reported, in the face of a body of evidence showing no adverse effects with much higher doses [418, 419].

More recently, Cranney et al., in their published systematic review and associated report (refer to Chapter 2.3.4 Skeletal Outcomes, pg. 39), identified 22 trials which reported data on adverse events with doses of vitamin D3 ranging from 400 to 4000 IU/day and vitamin D2 ranging from 5000 to 10 000 IU/day [11, 12]. Most data come from small studies with low event rates and
inadequate statistical power to detect adverse events. Meta-analyses performed in this review focused on hypercalcaemia, hypercalciuria and kidney stones in postmenopausal women and elderly men. In all, the authors identified 31 cases of hypercalcaemia in treatment groups versus 19 cases in placebo groups, although this difference was not significant in a total of 10 535 individuals. Of the eight randomised controlled trials that reported hypercalcaemic events, four, each reported a single case [14, 169, 333, 420] and one, 3 cases [143], which were all explained by previously undiagnosed primary hyperparathyroidism, a malignant tumour [143], or a reaction to a thiazide diuretic [14]. Another of the randomised controlled trials reported 18 cases of hypercalcaemia, but only seven in the treatment group [421]. This study also provided 1 g/day supplemental calcium in addition to 800 IU/day vitamin D₃ to participants, so it is not possible to distinguish between the effects of the two nutrients. Cranney et al., obtained unpublished data from a large British vitamin D intervention [RECORD, reported in 353] showing that 21 cases of hypercalcaemia were identified, but that there was no difference in numbers between the control group and three treatment groups [12]. The final study reported nine cases of mild and transient hypercalcaemia, and though six were in the treatment group, this difference was not denoted as statistically significant [307].

No evidence of harm has been identified from a meta-analysis of hypercalciuric adverse events. Cranney et al., identified nine randomised controlled trials providing these data [12] and one dose comparison study [308]. Only four of the controlled trials identified any hypercalcaemic events: two assessed vitamin D₃ and calcium versus a placebo [143, 334] and only two determined the additional effect of vitamin D₃ by providing calcium to both treatment and control groups [307, 411]. Neither the meta-analysis nor any of the individual studies reported any significant difference in hypercalciuric events between groups. One randomised controlled trial [421] and one dose comparison study [418] have observed calcium to creatinine ratios elevated above different and relatively arbitrary thresholds in the treated or higher dose compared to the control or lower dose groups. A very recent study assessed the 6-month efficacy and safety of high doses of vitamin D₃, starting at 2000 to 4000 IU/day and adjusted at 2-month intervals according to assayed 25(OH)D₃ to reach a median daily dose of 3800 IU [422]. The authors identified four cases of elevated calcium/creatinine ratio, but there was no difference in the number of cases between treatment and placebo groups, and in three cases the elevation was temporary and within the normal range 1 week later. No cases of hypercalcaemia were noted in this study [422].
Seven trials identified by Cranney et al. reported renal stone incidence, though only two of these observed any events [12]. The larger of these population-based studies, the Women’s Health Initiative trial, noted an increased 7-year occurrence of renal stones amongst the treatment group (2.7%) compared to the controls (2.3%) from a total of 36 282 postmenopausal women participants [350]. However, this study compared dual vitamin D₃ (400 IU/day) and calcium (1 g/day) therapy with a double placebo control and it is therefore not possible to ascertain the effect of vitamin D. Cranney et al. report that the RECORD study observed four cases of renal stones, two each in the daily 800 IU vitamin D₃ plus 1 g calcium group and in the placebo group after a 5-year follow-up, although these data are not reported in the original paper [12, 353].

Overall, there is a lack of data documenting changes in serum or urinary calcium along with 25(OH)D following sustained doses of vitamin D above 800 IU/day. This is despite there being no strong evidence of any adverse events associated with vitamin D supplementation at these levels, good evidence that these doses may not be sufficient to correct the low vitamin D status that exists in many populations (based on dose responses of 0.02 to 0.03 nmol 25(OH)D per IU Vitamin D / day, Table 2.7), and no evidence of frank hypercalcaemic toxicity in the few reports of sustained doses more than ten times this level.

### 2.4.5 Other Factors Determining the Efficacy of Vitamin D Supplementation

Efficacy of vitamin D supplementation also depends on season of delivery, probably as a consequence of the effect of pre-treatment vitamin D status. Supplementation in winter has been shown to reduce the proportion of those with low vitamin D status (<37.5 nmol/L) [414] and reduce bone loss in postmenopausal women [326]. The failure of a large daily dose of vitamin D₃ (2000 IU/day) to adequately raise 25(OH)D in a large proportion of postmenopausal African American women, suggested the possibility that race might also affect supplementation efficacy [307]. However, because a later study by the same authors found no significant difference in 25(OH)D response to oral vitamin D₃ between healthy white and African American adult women, it is likely that lower pre-existing vitamin D status also explains these ethnic differences [422].

Body composition may be another determinant of vitamin D supplementation efficacy, although mechanisms surrounding this effect are uncertain. In the previously reviewed graded dosing study by Heaney and coworkers, body mass index was inversely correlated with post-supplementation rise in 25(OH)D [88], although in another study no association was found between percent body fat and 25(OH)D response to weekly 7000 IU doses of vitamin D₃ [423].
2.4.6 Summary

Assuming that vitamin D supplementation has an effect on either skeletal or non-skeletal outcomes, a number of factors will effect its efficacy. These include dose regimen, the form of vitamin D taken, pretreatment 25(OH)D levels (and hence season of delivery), and potentially body composition parameters. Higher doses, vitamin D\textsubscript{3} compared to D\textsubscript{2}, and higher pre-existing 25(OH)D levels are all associated with a lesser relative increase in 25(OH)D following supplementation. Notwithstanding, the disadvantage of high doses may be offset by improved treatment compliance associated with less frequent delivery.

2.5 Determinants of Vitamin D Status

Regardless of the exact levels of 25(OH)D regarded as optimal, it is evident that vitamin D status is low in many sectors of the population. This is surprising, since human skin has a vast capacity for vitamin D production and for most humans, 90% or more of its requirement is met in response to sunlight [9, 424]. An estimated 10 000 – 25 000 IU vitamin D\textsubscript{3} is produced when most of the body’s surface is exposed to 1 minimal erythemal dose (MED) [9]. This dose is the amount required to cause minimal redness, equivalent to approximately 20 – 30 minutes of sun exposure for a fair skinned person in midday sun in temperate regions [119]. A modest single-exposure dose (0.5 MED) to the arms and legs is therefore equivalent to around 3000 IU [33]. By comparison, vitamin D obtained from natural food sources is very low. About ten 100g cans of sardines, 100 eggs or 300 L standard New Zealand full-cream milk would be required to deliver this dose [33, 425]. Even for the richest natural source of vitamin D, cod liver oil, 15 teaspoons would be required. Vitamin D fortification of food is also rare in Southern Europe, the United Kingdom, Australia and New Zealand. Some margarines and a few types of drinking milks are fortified in New Zealand with a maximum of 20 IU vitamin D per 100 mL of milk. In North America, milk typically contains 100 IU / 8 ounces (44 IU / 100mL) [33], thus providing 110 IU per standard glass. Consequently, because minimal vitamin D is obtained from any normal diet, it is clear that individuals who do not obtain exposure to outdoor sun are very likely to have low vitamin D status.

Elderly people commonly have low 25(OH)D levels [426] and these findings are consistent in both cooler and warmer climates [122, 427-429]. This trend is compounded by inactivity [430 Mason, & Fraser, 2007, 431] and is likely to result primarily from reduced outdoor time and increased clothing due to impaired mobility [432]. Deliberate avoidance of direct sun exposure, probably to reduce the risk or exacerbation of skin cancer [433] or for cosmetic protection, may
also contribute. In addition, aging reduces the ability of the skin to produce vitamin D, by more than half in 80 year olds compared to adolescents, through lowered epidermal 7-dehydrocholesterol concentrations [434, 435] and increased skin thickness [436].

Low vitamin D status is not particular to older people. Mounting data indicate that other population groups are also at risk. Notable amongst these are African American adults and children [437 Gunter, & Sahyoun, 2002, 438 & Holick, 1995, 439, 440 Norris, & Martins, 2005], Māori and Pacific Islanders in New Zealand [121, 441, 442] and many groups of non Western peoples, particularly women of childbearing age and their children living in Eastern Europe, Northern Africa, the Middle East, Southern or Eastern Asia or migrated to the West [26-29, 31, 32, 130, 379, 441, 443-447]. Furthermore, low 25(OH)D levels have been reported in substantial proportions even amongst healthy independently-living people from temperate and sunny regions of the United States, Europe, Asia, South America and Australia, particularly in the winter months [19, 30, 314, 426, 448-452]. In Australasia, Europe and the United States, women have a poorer vitamin D status than men of a similar age [121, 129, 453, 454]. Recently published population-based data from the United States suggest that 25(OH)D levels may have declined in the last decade, especially in men [455].

The causes of low levels of vitamin D are multifactorial. Women who cover their face, hands and other skin as part of religious and cultural practice are at obvious risk of low vitamin D status. When 25(OH)D has been assessed in these groups, levels are extremely low [456, 457]. Low 25(OH)D levels in urban or factory workers, notably in Asia, are most likely due to indoor lifestyles with apartment living and long working hours [379, 392, 444, 445, 447]. Air pollution, outdoor avoidance and excessive use of sunscreen or skin shade fuelled by a desire for fair skin may also contribute [458, 459]. Deeply pigmented skin colour is another important risk factor for low vitamin D status. Fair skinned individuals exhibit a greater response to fixed UV doses than their darker skinned counterparts [460, 461]; vitamin D status of Black Americans is lower than White Americans with Hispanic Americans commonly recording intermediate values [375, 383 Mudgal, & Dawson-Hughes, 2000, 437, 440, 448, 462]. It is notable that despite exhibiting higher levels of 1,25(OH)2D and PTH for the same level of 25(OH)D, bone mass and fracture risk are polygenic and African Americans, compared to non-Hispanic White Americans, have higher femoral bone mineral density and lower rates of fracture [463-466]. Although higher body mass, biomechanical and bone structural advantages may also contribute to the lower fracture rates [385, 467], bone-protective compensatory homeostatic mechanisms, such as enhanced renal tubular resorption or skeletal resistance to PTH actions, have been suggested [124, 179, 384].
2.5.1 Sun Exposure & Skin Protection

While the total duration of sun exposure on skin is likely to be the greatest determinant of vitamin D status in healthy individuals who are not taking dietary supplements, this variable is very difficult to quantify accurately. It depends on a number of factors which have themselves been associated with levels of 25(OH)D. These include environmental factors such as the weather or air pollution levels, and personal factors such as mobility, level of outdoor physical activity, clothing, use of sunscreen and outdoor behaviour, particularly typical regimens of sun exposure and avoidance of direct or reflected sunlight [468-470]. Consequently, when they are reported, univariate correlation coefficients between indices of outdoor exposure and 25(OH)D levels tend to be modest, ranging from $r = 0.2 – 0.5$ [343, 444, 471].

In addition to duration of skin sun exposure, the angle of the sun relative to the local vertical position, known as the solar zenith angle, is also crucial in determining the amount of vitamin D production [470]. The Earth’s atmosphere blocks much of the UV radiation emanating from the sun. Therefore, a far greater amount of UV reaches the Earth’s surface when the sun, high in the sky, creates a sharp solar zenith angle and passes through the atmosphere for a relatively short distance. Thus, vitamin D production is highest during sun exposure around the middle of the day, in the summer time and at lower latitudes (closer to the equator) [66, 472]. In fact, at 42° North latitude, sun-exposed skin will only produce vitamin D for eight months of the year and at 52° N, this is reduced to six months of the year [472]. Similarly, at 40° N during midsummer, the duration required for a standard UV dose is 2 – 3 times longer 3 hours either side of midday and 5 – 7 times longer 4 hours either side of midday than at midday itself [473]. For a lightly pigmented person living at latitudes greater than around 30°, obtaining an erythemal dose would not be possible before approximately 10 a.m. or after 4 p.m. [473].

Because the activity spectrum for isomerisation of vitamin D$_3$ to non-calcaemic biproducts is thought to be broader than that for previtamin D$_3$ production from 7-dehydrocholesterol, there is the theoretical possibility that prolonged exposure to UV at wavelengths longer than 315 nm, for example during winter at higher latitudes, might actually reduce 25(OH)D levels [67, 472]. One piece of clinical evidence in support of this hypothesis is reported by Dawson-Hughes and coworkers who observed an interaction between vitamin D intake in winter time and reported sunlight exposure [474]. For those reporting high dietary intake, levels of 25(OH)D were lower in those also reporting high sunlight exposure compared to those reporting low exposure.
As outlined previously in relation to elderly and skin colour (Refer to Chapter 2.3.6.3 Age, Sex & Race, pg. 57), characteristics of an individual’s skin will also determine vitamin D production. Fundamentally, electromagnetic radiation within the appropriate UVB range must energise a molecule of 7-dehydrocholesterol, more commonly located within the deeper epidermal layers. Since an over-lying melanin molecule may prevent this from occurring, the type, location and concentration of these molecules [103, 475] and the thickness of more superficial skin layers [476] are all important (refer Chapter 2.1.5 Regulation of Vitamin D Activity, pg. 14). Melanins are now known to encompass a multitude of compounds of differing colours and photochemical properties, although it is only the deeply pigmented “eumelanin” subtypes which are usually considered in relation to photoprotection [103]. Additional protection from UV damage to skin cells may come from antioxidant properties of various melanin molecules [103]. Probably, the overall degree of skin reflectance (lightness of colour) remains as the most valid index of skin capacity for vitamin D production, since the degree of skin pigmentation underlies its radiation-blocking ability. Armas et al. [477] have shown that the 25(OH)D response to standardised doses of UVB is dependent on skin lightness.

Despite the importance of the skin reflectance, almost all of the evidence relating to vitamin D status and skin type relies on inter-ethnic comparisons [317, 439, 440, 460 & Holick, 1982, 461, 462, 478 & Martin, 2007, 479] or sun-reactive skin-type [480, 481]. Drawing conclusions about skin colour from data classified along racial lines may be flawed since there is undoubtedly a large variability within any ethnic group in both constitutive (natural) and facultative (sun-exposed or tanned) skin colour, and there may be confounding racial differences in vitamin D metabolism or action [26, 179, 384]. One recent study observed no relationship between constitutive skin colour and 25(OH)D levels in a New Zealand population of which one quarter were specifically targeted people of Pacific origin and the rest were predominantly European [482]. It may have been that the range of skin colours was not great enough to observe an effect.

The inherent ability of skin to darken (tan) in response to UV exposure could be a determinant of vitamin D status independently of constitutive skin colour. The increased pigmentation of exposed areas would reduce vitamin D production compared to skin which is resistant to tanning but receives the same dose of UVB. Sun reactivity can be measured objectively using multiples of minimal erythemal dose, defined as the minimum UVB radiation dose required to produce detectable erythema (redness). Other simpler indices have been developed, the most well know of these being that of Fitzpatrick [483]. This index categorises white skin into four types based on self-reported tendency to either burn or tan, with two additional categories for brown or black
skin. Although skin-typing is a useful indicator of skin cancer risk, each skin type category represents a considerable overlapping range of minimal erythemal doses [484, 485]. Two descriptive studies which have investigated the relationship between skin type or facultative skin colour and 25(OH)D have found that they are not related when the effects of overall sun exposure are taken into account [480, 482]. Malvy et al. [480] observed individuals with fairer pigmentation actually had lower 25(OH)D levels than their darker counterparts and that this was linked to lower self-assessed global sun exposure. Similarly, Rockell et al. [482] found that a lower reflectance score (more tanning) of sun-exposed outer forearm skin was negatively associated with 25(OH)D levels.

2.5.2 Seasonal Variation

Given the importance of outdoor sun exposure on the skin in determining vitamin D status and the fact that sunlight is an ineffective stimulus for vitamin D production during winter months in many parts of the world, it is not surprising that the time of year when 25(OH)D is assessed is a very important consideration when evaluating vitamin D status. Many studies have confirmed seasonal variation in 25(OH)D levels, even in areas with all-year round sunshine, in elderly, healthy adults and children from cross-sectional data [18, 108, 121, 129, 146, 295, 296, 298, 312, 327, 356, 368-370, 436, 439, 442, 486-489] and longitudinally [297, 305, 340, 345, 371-375, 429, 454, 468, 474, 490]. In frail elderly or institutionalised populations, seasonal variation in 25(OH)D may be less than in independent populations [69, 491], absent [471], or even opposite in hot climates, with lower levels observed in the summer months, indicating an avoidance of the outdoors during summer [492].

The degree of seasonal change in 25(OH)D can be substantial. Differences between summer peak and winter nadir of between 25 and 28 nmol/L have been reported in a longitudinal study of young white women in Boston (42°N) [375], and older women in Auckland, New Zealand (37°S) [296] and southeastern Australia (38-39°S) [370]. Observed differences were substantially greater (almost 40nmol/L higher in summer than winter) in a longitudinal study of young German women recruited from around Bonn (51°N) [298]. Similarly, in Japanese adults recruited throughout the year from a latitude around 35°N, mean 25(OH)D was 41 nmol/L higher in September compared to March [490]. However, the degree of seasonality in 25(OH)D levels in tropical regions is unclear. One study in southern Florida (just outside the tropics at around 26°N) found summer to winter differences in 25(OH)D levels in adult women to be much lower at 6 nmol/L [297].
As a consequence of the striking seasonal variation in 25(OH)D levels, using a target level to
determine vitamin D sufficiency must take into account the time of the year at which samples are
drawn [487]. If, for example, a year-round target level of 50 nmol/L is desired, blood
measurements taken in the New Zealand autumn (March) would need to be 87 nmol/L in men
and 71 nmol/L in women, based on average gender-specific seasonal fluctuations [487].

2.5.3 Vitamin D Intake

Because of the low levels of vitamin D contained in foods, vitamin D intake from foodstuffs
makes only a small contribution to vitamin D status of a population. Intake from food is
normally insufficient to prevent poor vitamin D status, particularly in winter [69, 469, 493-495].
Nonetheless, vitamin D intake has been shown to be an independent determinant of 25(OH)D
levels in winter or in elderly populations when vitamin D status is low [356, 474, 481, 486, 493,
496, 497], though not in all studies [492]. Furthermore, small amounts of vitamin D from
foodstuffs may be sufficient to prevent severe deficiency or winter decline in 25(OH)D levels
[356, 488, 498, 499]. For example, benefits to vitamin D status have been noted with daily
intakes above 220 IU [356], and following modest levels of fortification to milk of 40 – 48 IU
per 100mL [498, 499].

International studies have paradoxically reported better vitamin D status at higher northern
latitudes, in Northern compared to Southern Europe and in North America compared to Latin
America [122, 130, 469, 500, 501]. More common food fortification and use of cod liver oil and
vitamin D-containing supplements are thought to explain the higher levels in North America and
Scandinavia [426, 501, 502]. Consequently, some workers in the field recommend mass
supplementation, additional food fortification (both higher levels in foods already fortified and a
greater range of food vehicles for fortification), or changes in diet to encompass more fish in
order to improve vitamin D status within populations [495, 503, 504]. Others suggest that public
health campaigns should focus solely on diet and supplementation rather than increasing UV
exposure due to the increased risk of skin cancer [125, 505].

An important difficulty of relying on dietary sources of vitamin D is that useful quantities are
naturally present in very few foods and the content is highly variable. Recent analyses of fish
showed variability in vitamin D content ranging from around 60 to 100 IU / 100 g in white fish
to almost 1000 IU / 100 g in wild salmon. Samples from farmed salmon, trout, tuna and species
of oily blue fish contained only 242 to 407 IU / 100 g vitamin D on average, inter-sample
variability was high for all species and frying reduced the vitamin D content by half [506]. In
addition some types of mushrooms contain up to 100 IU / 100 g, egg yolk around 20 IU / 100 g and liver or cured pork around 50 IU / 100 g [33, 425, 504]. Even fortification content may be highly variable. Analyses of fortified milk in the United States in the early 1990s showed that only 29% of samples from 13 different brands contained between 80% and 120% of the stated quantity of vitamin D, although there may have been subsequent improvements in the quality control processes [507]. Moreover, animal products may contain small quantities of 25(OH)D: <0.1 µg / 100 g in milk and fish, 0.2 to 0.4 µg / 100 g in other meat and offal and up to 1 µg / 100 g in egg yolk [508]. This metabolite is more bioavailable and depending on the testing system used has an activity of 60 to 200 IU / µg potentially raising meat, offal and egg content into the low hundreds (IU / 100 g).

Mass oral supplementation is another potential way to raise vitamin D status. Vitamin and mineral supplements are already commonly used in the United States and Northern Europe [497, 509]. Self-reported use of vitamin D supplements has also been shown to be a determinant of winter-time 25(OH)D levels [510] and may prevent severe deficiency in elderly [63]. However, because there is an additional cost to individuals, there is the probability that as a public health strategy vitamin D supplementation would not reach some sectors of the population known to be at risk of deficiency [504, 511]. With oral supplementation, there is also the potential for toxicity, although at levels typically contained in over-the-counter-supplements (100 – 200 IU / dose), accidental overdosing (at least in excess of 2000 – 10 000 IU / day) would be unlikely (Refer to Chapter 2.4.4 Safe Dose of Vitamin D, pg. 64).

2.5.4 Body Composition

Body mass or composition may be another determinant of vitamin D status and of its response to dosing. Lower 25(OH)D levels are observed in obese compared to non-obese people [89, 488, 512], although in non-white cohorts the negative association between adiposity indices and 25(OH)D may be weakened or absent [479, 513]. A parallel inverse association between 25(OH)D and leptin has also been reported [394].

Wortsman et al. noted that the increase in serum vitamin D3 following a standardised dose of UVB was under half that in obese, compared to non-obese participants, and there was a similar trend in the response of serum vitamin D2 to its oral supplementation [89]. Moreover, in another study Arunabh et al., found percent body fat to be a stronger independent correlate of 25(OH)D levels than body mass index after the effects of race, season, age and vitamin D intake were adjusted for [90]. These observations have led to assertions that adipose may act as a reservoir
for inactive vitamin D metabolites, reducing the amount reaching the liver and subsequently 25(OH)D production. However, it is also possible that lower 25(OH)D in obesity may result from feedback inhibition from higher circulating 1,25(OH)₂D [514], although higher levels of 1,25(OH)₂D in obesity have not always been established [515], dietary deficiency [516] or simply from less outdoor sun exposure in obese individuals [517].

Amongst leaner populations, the association of adipose tissue and 25(OH)D appears to be different. In contrast to Arunubh *et al.* [90] who studied American women with a mean body fat of 36% of total body mass, data from a Japanese study, in which participants recorded a mean percentage body fat of 22%, showed that 25(OH)D was positively correlated with body mass and body mass index and not correlated with percent body fat. It could be that in leaner people, non-adipose tissue plays a more important role in the storage or metabolism of vitamin D metabolites.

### 2.5.5 Assay Methods & Other Determinants

Between-study comparisons of 25(OH)D levels and subsequent evaluation of vitamin D status are strongly affected by variability arising from the difficulties associated with measuring the metabolite and differences between laboratories (refer Assays and Techniques Used to Estimate a Threshold for PTH, pg. 56). Comparisons of vitamin D status across continents have also been affected by the differing availability and use of oral vitamin D₂ versus vitamin D₃ preparations since some assays do not evenly detect the two resulting 25-hydroxy metabolites [364, 365, 367].

Levels of 25(OH)D may be affected by a number of other variables. Vitamin D-binding protein phenotype has been shown to affect both the concentration of 25(OH)D and the amount of 25(OH)D per binding protein molecule [518]. Although the affinity of vitamin D metabolites to their binding protein is very high, vitamin D metabolites also cross-bind at lower affinity with more highly concentrated albumin [82, 519]. Thus, positive associations between 25(OH)D and albumin [295, 453, 493, 520-522] and between 1,25(OH)₂D and albumin [523] have been consistently reported amongst older people in the literature. These positive associations in this age-group could also be contributed to by low dietary vitamin D intake combined with protein-energy malnutrition [520].
CHAPTER 3: EFFECTS OF VITAMIN D SUPPLEMENTATION ON PARATHYROID HORMONE LEVELS IN PREMENOPAUSAL CHINESE WOMEN

3.1 Abstract

The level of 25-hydroxyvitamin D [25(OH)D] which constitutes a long-term bone health risk by causing elevated levels of parathyroid hormone (PTH) is not clear. Whilst many studies have investigated optimal 25(OH)D levels using cross-sectional data, few have determined levels required to minimise PTH using a prospective approach. The present study analyses data from 221 Chinese women in Hong Kong, aged 28.0 ± 4.4 years (mean ± sd), taking part in the treatment or control groups of a trial of a dairy product containing 200 IU vitamin D₃ / day. To determine optimal 25(OH)D levels, 3-month changes (Δ) in PTH and 25(OH)D were analysed. Baseline 25(OH)D was 34 ± 11 nmol/L and was inversely related to baseline PTH (r = -0.18, \( P = 0.007 \)), with a plateau in PTH levels when 25(OH)D was greater than 40 nmol/L. After 3 months in the trial, PTH fell 11% and neither \( \Delta 25(OH)D \) nor \( \Delta PTH \) differed between treatment and control groups. PTH change was inversely related to \( \Delta 25(OH)D \) \( (P < 0.001) \) but not to baseline 25(OH)D. Similarly, \( \Delta PTH \) differed between quartiles of \( \Delta 25(OH)D \) \( (P < 0.001) \), but not between quartiles of baseline 25(OH)D. Even in the highest quartile of baseline 25(OH)D (greater than 40 nmol/L), PTH fell 0.4 ± 0.1 pmol/L (mean ± SEM) \( (P = 0.008) \). No interaction was observed between quartiles of baseline 25(OH)D and \( \Delta 25(OH)D \) in determining \( \Delta PTH \). We conclude that vitamin D deficiency is common in young women in Hong Kong, and the fall in PTH following improved vitamin D status suggests that they are likely to benefit from vitamin D supplementation, although 200 IU/day vitamin D₃ was inadequate to raise 25(OH)D compared to the control group in this cohort. The cross-sectional analysis indicates that optimal 25(OH)D is >40 nmol/L, and the longitudinal data is consistent with an optimal level above 40 to 50 nmol/L.

3.2 Introduction

Vitamin D insufficiency, indicated by lowered levels of 25-hydroxyvitamin D [25(OH)D] \[7, 66\], results in elevated parathyroid hormone (PTH) levels \[8, 369\]. Elevated levels of PTH are likely to result in lowered bone mineral density and increased fracture risk \[8\]. Vitamin D supplementation of elderly people with low vitamin D status lowers PTH, reduces bone loss and may prevent hip fractures \[128, 333\]. Despite this, the optimal levels of 25(OH)D are disputed.
[13] and estimates have ranged from 25 nmol/L to 122 nmol/L [14, 17, 19, 29, 69, 129, 147, 323, 356, 357, 391, 493, 524, 525].

A frequently employed methodology for determining optimal vitamin D status uses the relationship between 25(OH)D and PTH in cross-sectional databases to identify a threshold level of 25(OH)D above which a plateau in PTH occurs [13, 19, 86, 129, 357]. An alternative approach for determining optimal 25(OH)D levels is to use a prospective study design to look for a level of baseline 25(OH)D that is not associated with reduction in PTH following vitamin D supplementation. Utilising longitudinal data in this way may be superior to a cross-sectional paradigm since it eliminates bias from confounding effects, such as age, health status or calcium intake, that may be associated with 25(OH)D levels. To date, only three studies have investigated a 25(OH)D threshold longitudinally [17, 130, 131], and have reported 25(OH)D thresholds at the lower end of the range determined from cross-sectional studies (between 40 and 50 nmol/L). Longitudinal data from a further study providing supplemental vitamin D found an association between PTH reductions and initial levels of 25(OH)D [329] but did not report a threshold.

Older adults have higher PTH levels at the same level of 25(OH)D compared to their younger counterparts [357, 390]. This is likely to result from age-related reductions in intestinal calcium absorption [526-528] and renal function [529]. The vast majority of published cross-sectional studies are of adults older than 50 years, although there are some studies in children [31, 322, 371, 373, 377, 381, 382, 530], and younger adults [29, 30, 315, 317, 321, 323, 376, 378-380, 392]. Additionally, most studies of younger people have either not reported a threshold-based optimal 25(OH)D level or have not observed an association between 25(OH)D and PTH at all [30, 31, 315, 317, 322, 323, 371, 373, 376-382]. Furthermore, since all longitudinal investigations have been undertaken in older groups (mean ages between 47 and 67 years) the effect of age in modulating PTH response to changes in 25(OH)D is unknown.

In addition to age, calcium intake is likely to affect the relationship between PTH and 25(OH)D. Low calcium intake is independently associated with elevated PTH in the elderly [329, 390], in younger adults [338, 391], and in adolescent girls [381]. A 25(OH)D threshold has not previously been investigated either cross-sectionally or longitudinally in a Chinese or other East Asian population. Their lower calcium intake [316, 531] may change any relationships between vitamin D status and PTH levels.
The purpose of this study was to investigate whether an optimal level of 25(OH)D could be determined in a population of young, healthy Chinese women through the identification of a threshold level of 25(OH)D above which there is no change in PTH levels following vitamin D supplementation.

3.3 Methods

3.3.1 Participant Recruitment

A randomised controlled trial of the effect of a calciferol-containing dairy product on bone health, carried out in Hong Kong, presented an opportunity for investigating the relationship between 25(OH)D and PTH changes in younger women. Healthy Chinese volunteers aged 20 – 35 years were recruited between February and June 2002 through poster advertisements in public places and mass emailing as previously described [532]. An initial interview excluded women who had a medical history of metabolic bone, liver, endocrine, connective tissue, and respiratory diseases, cancer or previous operations or who were taking calcium or vitamin D supplements or medications (other than oral contraceptives) likely to affect bone metabolism. Women who were amenorrhoeic, lactating or intending to become pregnant during the next two years were also excluded from the study. A total of 327 women were screened, of whom 65 were ineligible, and a further 41 did not attend the first visit. The remaining 221 were randomised to receive either two daily sachets of fortified milk powder (Fonterra Brands Ltd.) containing a total of 1000 mg calcium and 200 IU (5 µg) cholecalciferol (Milk group), or no dairy product (Control group). Randomisation was balanced between two age groups, 20 – 27 years and 28 – 35 years: a total of 111 and 110 women, from each age group respectively, took part in the study. Compliance was checked by counting returned empty sachets. Approval to conduct the study was provided by the Chinese University of Hong Kong Ethics Committee.

3.3.2 Baseline Assessment

All participants completed a questionnaire containing demographic and health information. They also completed baseline assessments of physical activity and dietary intake. Physical activity was measured using a modified version of the Physical Activity Scale for the Elderly [533], with examples of types of activity in the different categories of exercise intensity altered to be more appropriate for the population group. Dietary intake was assessed using a five-day diet record completed prior to their visit. Records were checked during the visit by a nutritionist, who clarified uncertain food item portions using actual size food models and food pictures. Nutritional analysis was undertaken using Food Processor, version 8.0 software (ESHA
Research, OR, USA) with nutrient composition of local Chinese foods added to the database using data from manufacturers and from the Institute of Nutrition and Food Safety of China, China Food Composition [534]. Height and body mass were measured in light indoor clothing without shoes using a Harpenden stadiometer (Holtain Ltd., Crymych, UK) for height and a Healthometer scale (Healthometer Inc., Illinois, USA) for weight. Percent body fat was determined from total body dual energy x-ray absorptiometry measurements made using a Hologic Delphi A scanner (Hologic Inc, Bedford, MA, USA).

### 3.3.3 Blood Collection & Analysis

Fasting blood samples were collected at baseline and 3 months for measurement of serum calcium, albumin, phosphate, 25(OH)D and PTH. Calcium, albumin and phosphate were measured using Roche DP Modular colorimetry. DiaSorin radioimmunoassay (DiaSorin Inc., Stillwater, MN, USA) was used to quantify 25(OH)D (sensitivity 3.7 nmol/L; coefficient of variation (CV) 9.1% at 82 nmol/L) and Immulite 1000 chemiluminescence immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) was used to quantify PTH (sensitivity 0.1 pmol/L; CV 8.6% at 6.2 pmol/L). All assays were carried out at The Chinese University of Hong Kong Pathology Laboratory which is accredited by the Australian National Association of Testing Authorities and the Royal College of Pathologists of Australasia.

### 3.3.4 Statistical Analyses

Statistical analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Linear relationships between 25(OH)D, PTH and their changes were reported as Pearson’s correlation coefficients. One-phase exponential decay curves were fitted to identify possible thresholds in the data. Stepwise, multiple linear regression models (\( p_{in}=0.05, p_{out}=0.10 \)) were used to identify determinants of baseline 25(OH)D and PTH and the change in PTH. We included demographic, nutritional intake, physical activity, adiposity and clinical biochemical variables in the models. We applied a 2-way general linear analysis of variance (ANOVA) to determine whether change (\( \Delta \)) in PTH differed between quartiles of baseline 25(OH)D or with quartile of 3-month change in 25(OH)D [\( \Delta 25(OH)D \)]. Data are presented as mean ± standard error of the mean (SEM) unless otherwise stated.
3.4 Results

3.4.1 Participant Characteristics

Prior to the intervention, 25(OH)D levels ranged from 11 – 70 nmol/L. They were <25 nmol/L in 18%, less <30 nmol/L in 36% and <50 nmol/L in 93% of the women. PTH was elevated above the upper reference level (7.3 pmol/L) in only 5 of the 221 individuals. Baseline characteristics are shown in Table 3.1

Table 3.1 Baseline Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.0 ± 4.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.3 ± 2.6</td>
</tr>
<tr>
<td>Body fat (percent)</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>Physical activity index (PASE score)</td>
<td>131 ± 75</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>450 ± 160</td>
</tr>
<tr>
<td>Vitamin D intake (IU/day)</td>
<td>140 ± 120</td>
</tr>
<tr>
<td>Total protein intake (g/day)</td>
<td>77 ± 18</td>
</tr>
<tr>
<td>Total fat intake (g/day)</td>
<td>70 ± 15</td>
</tr>
<tr>
<td>Total energy intake (kcal/day)</td>
<td>1710 ± 310</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.33 ± 0.08</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>44 ± 2</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.24 ± 0.14</td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D (nmol/L)</td>
<td>34 ± 11</td>
</tr>
<tr>
<td>Plasma parathyroid hormone (pmol/L)</td>
<td>3.5 ± 1.4</td>
</tr>
</tbody>
</table>

Data are mean ± SD
n = 221

3.4.2 Baseline Analyses

Baseline PTH and 25(OH)D were negatively correlated, with PTH showing a gradual curvilinear decline with increasing 25(OH)D (Figure 3.1). There was no further reduction in PTH above a 25(OH)D of 40 nmol/L.

Physical activity index was positively associated with baseline 25(OH)D, whilst baseline PTH was positively associated with age, and negatively associated with serum albumin and calcium and with dietary intakes of protein, fat, and energy (Table 3.2). Multiple regression models retained physical activity index (positive) and baseline PTH (negative) as significant predictors of 25(OH)D, explaining 7.4% of its variance ($P < 0.001$). Determinants of PTH were serum calcium, fat intake and 25(OH)D (all negative), which collectively explained 13% of its variance ($P < 0.001$).
Figure 3.1 The Relationship Between Baseline Serum Parathyroid Hormone (PTH) and Serum 25-Hydroxyvitamin D (25(OH)D) Grouped in Intervals of 5 nmol/L. Correlation for the linear relationship ($r$) = -0.18, $P = 0.007$. The curve represents the best fit to the data of one-phase exponential decay to a PTH plateau of 2.9 pmol/L ($r^2 = 0.04$). Numbers in brackets indicate the sample size for each data point.

Table 3.2 Correlates of Baseline 25-Hydroxyvitamin D and Parathyroid Hormone

<table>
<thead>
<tr>
<th></th>
<th>25(OH)D</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.06</td>
<td>0.16*</td>
</tr>
<tr>
<td>Physical activity index (PASE score)</td>
<td>0.19**</td>
<td>0.01</td>
</tr>
<tr>
<td>Vitamin D intake</td>
<td>0.12#</td>
<td>-0.06</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Total protein intake</td>
<td>0.03</td>
<td>-0.15*</td>
</tr>
<tr>
<td>Total fat intake</td>
<td>0.02</td>
<td>-0.20**</td>
</tr>
<tr>
<td>Total energy intake</td>
<td>0.03</td>
<td>-0.18**</td>
</tr>
<tr>
<td>Body mass index</td>
<td>-0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>-0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum calcium</td>
<td>0.06</td>
<td>-0.28***</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.01</td>
<td>-0.18**</td>
</tr>
<tr>
<td>Serum phosphate</td>
<td>-0.11</td>
<td>-0.13#</td>
</tr>
</tbody>
</table>

Data are Pearson correlation coefficients

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *$P < 0.08$

Abbreviations as follows: 25-hydroxyvitamin D (25(OH)D); parathyroid hormone (PTH)
3.4.3 Changes in 25(OH)D & PTH

Mean levels of 25(OH)D rose and those of PTH fell at 3 months ($P < 0.001$ for both, Table 3.3). There was no significant difference in either $\Delta 25(OH)D$ or $\Delta PTH$ between the milk and control groups, although there was a trend for $\Delta 25(OH)D$ to be higher in the milk group ($P = 0.06$, compared to the control). Data from both groups were combined for longitudinal analyses.

Change in 25(OH)D was $14 \pm 1$ nmol/L in those with baseline values below the median of 33 nmol/L compared with $5 \pm 1$ nmol/L in those above the median ($P < 0.001$).

Change in 25(OH)D was negatively correlated with and explained 5% of the variance in $\Delta PTH$ (Figure 3.2) in a multiple regression model which included both baseline and $\Delta 25(OH)D$ ($P < 0.001$). Baseline 25(OH)D was not related to $\Delta PTH$ (Figure 3.3). Neither baseline 25(OH)D nor treatment group, when it was included, entered the $\Delta PTH$ regression model. Of the 13 women who had baseline 25(OH)D levels of 50 nmol/L or greater, only three experienced increases in this metabolite $\geq 10$ nmol/L.

To further clarify the relationships between baseline 25(OH)D, and the changes in 25(OH)D and PTH, the 25(OH)D variables were expressed as quartiles (Figure 3.4). A two-way fixed factorial ANOVA with $\Delta PTH$ as the dependent variable demonstrated an effect of quartiles of $\Delta 25(OH)D$ ($P = 0.006$), confirming the data shown in Figure 3.2. Bonferroni post-hoc testing showed that the lowest quartile of $\Delta 25(OH)D$ was significantly different from the two highest quartiles, with respect to $\Delta PTH$ values ($P < 0.05$). $\Delta PTH$ was not different between quartiles of baseline 25(OH)D, confirming the data shown in Figure 3.3, and there was no significant interaction between baseline and $\Delta 25(OH)D$. Even in the highest quartile of baseline 25(OH)D (greater than 40 nmol/L), PTH fell $0.4 \pm 0.1$ pmol/L ($P = 0.008$, for a one-sample t-test of $\Delta PTH$ difference from 0). Thus all quartile groups with an increase in 25(OH)D of 10 nmol/L or more showed a mean fall in PTH, and there was no threshold for this effect, with respect to baseline 25(OH)D.

Table 3.3 Three-Month Changes in 25-Hydroxyvitamin D and Parathyroid Hormone

<table>
<thead>
<tr>
<th></th>
<th>Milk (n=110)</th>
<th>Control (n=109)</th>
<th>Combined (n=219)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta 25(OH)D$ (nmol/L)</td>
<td>11 ± 1</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>$\Delta PTH$ (pmol/L)</td>
<td>-0.4 ± 0.1</td>
<td>-0.3 ± 0.2</td>
<td>-0.3 ± 0.1</td>
</tr>
</tbody>
</table>
Figure 3.2 The Relationship Between Changes in 25-Hydroxyvitamin D [Δ25(OH)D] and Parathyroid Hormone (ΔPTH)
Correlation coefficient for the relationship $r = -0.22$, $P < 0.001$)

Figure 3.3 The Relationship Between Baseline 25-Hydroxyvitamin D [25(OH)D] and Change in Parathyroid Hormone (ΔPTH)
Correlation coefficient for the relationship $r = 0.03$, not significant)
Figure 3.4 Change in Parathyroid Hormone (PTH) as a Function of Both Change and Baseline 25-Hydroxyvitamin D [25(OH)D]

Across the entire lowest quartile of Δ25(OH)D, ΔPTH was 0.3 ± 0.2 pmol/L, which was significantly different from ΔPTH in the highest two quartiles (-0.7 ± 0.2 pmol/L and -0.7 ± 0.2 pmol/L for quartiles 3 and 4 respectively). Note that for the entire highest quartile of baseline 25(OH)D (>40 nmol/L), ΔPTH was -0.4 ± 0.1 pmol/L, so a reduction in PTH was still evident.

Sample Sizes

<table>
<thead>
<tr>
<th>Quartiles of Change in 25(OH)D</th>
<th>Quarters of Baseline 25(OH)D</th>
<th>&lt;2 nmol/L</th>
<th>2-9 nmol/L</th>
<th>9-17 nmol/L</th>
<th>&gt;17 nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartiles of Baseline 25(OH)D</td>
<td>&lt;27 nmol/L</td>
<td>25</td>
<td>17</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>27-33 nmol/L</td>
<td>17</td>
<td>13</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>33-40 nmol/L</td>
<td>8</td>
<td>12</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>&gt;40 nmol/L</td>
<td>4</td>
<td>13</td>
<td>17</td>
<td>22</td>
</tr>
</tbody>
</table>
3.5 Discussion

These data demonstrate low vitamin D status in healthy premenopausal Chinese women living in Hong Kong (latitude 22°N). The mean serum 25(OH)D level of 34 nmol/L found here during spring is considerably lower than winter levels in young women in the USA (63 nmol/L) [437], Australia (64 nmol/L) [481] or year-round levels of young women in the UK or New Zealand (49 nmol/L) [121, 535]. Surprisingly, our results indicate lower 25(OH)D levels in the present cohort than in young women of a similar age living in urban Shenyang (North East China: 42°N) [380], in whom 19% had levels less than 30 nmol/L compared with 36% in the present study. They are also lower than young women in tropical Kuala Lumpur and Jakarta [392], in whom around 50% had levels less than 50 nmol/L compared with 93% in the present study and lower than in middle-aged and older Chinese women living in Hong Kong [450], Taipei [318] and Malaysia [536], who have mean levels of 66 – 78 nmol/L.

Although the reasons for such low levels of 25(OH)D were not fully examined, it is possible that they may be related to the long working hours, indoor lifestyle, and commuting patterns of this cohort. Low ultraviolet radiation levels due to pollution may also be a factor explaining the reduced 25(OH)D. Sun avoidance for cosmetic reasons is also likely to play a part. Frequent sunscreen and parasol use and a dislike of going in the sun have been reported amongst older Chinese women [459]. Anecdotal reports suggest that these practices may be even more common in younger women and perhaps adolescent girls. A random sample from a large study of adolescent girls living in Beijing (40°N) found particularly low 25(OH)D levels in both winter and summer (mean ± SD: 13 ± 8 and 27 ± 11 nmol/L, respectively) [28]. In the present study, baseline measurements took place in spring months when sunshine is plentiful in Hong Kong and prior to the rainy typhoon season. Although there is a lack of data to describe seasonal changes in vitamin D status in tropical areas, the 3-month increases in 25(OH)D we observed are likely to be partly contributed to by seasonal changes since they were only slightly greater in the milk group, compared with control.

Our results showed physical activity to be positively associated with 25(OH)D in this cohort, probably because of its association with increased time outdoors [296]. There was borderline association with vitamin D intake: although few foods contain useful quantities of vitamin D, vitamin D intake has been shown to contribute to 25(OH)D levels when sun exposure is minimal [356, 497], and is likely to be important in diets high in fish or fortified foods [39, 537, 538]. In
contrast to a number of previous studies [89, 90, 295, 296], inverse relationships between 25(OH)D and indices of body fat were not observed here.

Our cross-sectional data suggest that a 25(OH)D threshold for decreasing PTH may be reached around 40 nmol/L. Many previous studies have included estimation of optimal 25(OH)D using a range of methods based on cross-sectional data [for example, 19, 31, 321, 391, 525, 530] and produced wide-ranging estimates. Difficulties associated with the identification of optimal 25(OH)D levels through the use of statistical estimates of turning points, plateaus or intersections of 2-slope spline models have been recently addressed [17]. Nonetheless, our data show a distinct increase in PTH levels when baseline 25(OH)D is below 40 nmol/L, and no further decrease above this level (Figure 3.1). Both age [357] and calcium intake [391] affect the relationship between PTH and 25(OH)D. It is possible that our estimate of optimal 25(OH)D levels based on its cross-sectional relationship with PTH may be lower than that observed in many reports because the present study is of young women with low dietary calcium intakes.

The longitudinal data from the present study do not show a 25(OH)D threshold, above which there is no fall in PTH, in contrast to our cross-sectional analysis. As evident from Figure 3.4, young women with baseline 25(OH)D levels in the upper quartile (greater than 40 nmol/L) displayed a potentially beneficial 3-month fall in PTH of 0.4 pmol/L. If 25(OH)D was approaching a threshold, then the reduction in PTH would be expected to be less at higher baseline levels of 25(OH)D, yet we observed no relationship between baseline 25(OH)D and the change in PTH. Of the three studies that have investigated a PTH plateau using a prospective approach, all have reported markedly reduced changes in PTH at baseline 25(OH)D levels of 40-50 nmol/L [17, 130, 131]. Since only three subjects in the present study who had increases in 25(OH)D of 10 nmol/L or greater also had a baseline 25(OH)D of 50 nmol/L or greater, we had insufficient numbers to detect a threshold of this level. Therefore, results from our prospective analysis are not inconsistent with past findings.

In conclusion, the present study demonstrates that serum 25(OH)D concentrations are substantially lower in healthy young women in Hong Kong than in comparable Western populations, and also lower than those reported in postmenopausal women in the same region. Cross-sectionally, 25(OH)D values of less than 40 nmol/L were associated with elevations in PTH. During follow-up, increases in 25(OH)D were related to declines in PTH, but this did not appear to be related to the baseline 25(OH)D concentration. We observed potentially beneficial falls in PTH even for individuals with baseline 25(OH)D levels in the 40 – 50 nmol/L range.
Thus, if there is a threshold above which further increases in 25(OH)D are not accompanied by suppression of PTH, it is above this range. The fall in PTH following improved vitamin D status in these healthy young Chinese women, even those in the highest quartile of baseline 25(OH)D, suggests that this cohort is likely to benefit from vitamin D supplementation.

These data have now been submitted to Osteoporosis International for publication [539].
CHAPTER 4: RELATIONSHIPS BETWEEN 25-HYDROXYVITAMIN D & PARATHYROID HORMONE, AND THEIR DETERMINANTS IN TWO COHORTS OF PREMENOPAUSAL CHINESE WOMEN

4.1 Abstract

Cross-sectional data of the relationship between parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) in young women are sparse. Data from two cohorts of young Chinese women in Beijing and Hong Kong were pooled to investigate determinants of 25(OH)D and PTH and to examine the existence of a threshold level of 25(OH)D above which PTH plateaus at a minimum level. The 441 young Chinese women were aged 27.8 ± 4.4 years (mean±sd). Baseline 25(OH)D was 31.5 ± 11.2 nmol/L and was inversely correlated with baseline PTH (r = -0.25, P < 0.001), with a plateau in PTH levels when 25(OH)D approached 40 to 50 nmol/L. When data from the two cohorts were pooled, 25(OH)D level had an effect on PTH (P < 0.001), though neither tertiles of dietary calcium intake, body mass, body mass index, percent body fat nor their interaction with 25(OH)D groups were associated with PTH. Age, dietary vitamin D intake and physical activity index (positively) and PTH and body mass (negatively) predicted 25(OH)D, whilst calcium intake (positively) and 25(OH)D (negatively) were determinants of PTH. The models predicting 25(OH)D and PTH explained 16% and 7% of the variance respectively (P < 0.001 for both). In a cohort of lean Chinese women of reproductive age, we were unable to clarify an optimal level of 25(OH)D based on cross-sectional relationships with PTH, and have found no evidence that dietary calcium or indices of adiposity affect the relationship between 25(OH)D and PTH.

4.2 Introduction

Optimal levels of 25(OH)D for bone health can be estimated by determining a threshold level of 25(OH)D above which PTH is suppressed at a minimal level. Many studies have established this level using cross-sectional data, and these have been well reviewed [13, 16, 17]. There are few estimates of 25(OH)D threshold in young women reported in the literature. Three studies that have analysed data from women in their 20s to 40s have reported widely varying estimates of 25 nmol/L [29], 52 nmol/L [392] and 80 nmol/L [321].
The availability of cross-sectional data from another cohort of 220 young Beijing women provided an additional opportunity to explore determinants of 25(OH)D and PTH and the relation between them in a larger pooled data set. The aims of this supplemental analysis were firstly to provide further estimates of an upper 25(OH)D threshold at which PTH levels plateau. Secondly, we aimed to examine the role of calcium intake and indices of adiposity in modifying the relationship between 25(OH)D and PTH and identify other determinants of these endocrine metabolites.

4.3 Methods

Female volunteers living in Beijing aged 20 – 35 years were recruited between February and June, 2002 using the same methods and exclusion criteria as the Hong Kong cohort described in Chapter 4. A total of 297 women were screened, of whom 59 were ineligible, and a further 18 did not attend the first visit. A total of 220 Beijing participants are therefore included in the analysis. Approval to conduct the study was obtained from the Peking Union Medical College Hospital Ethics Committee, Beijing.

Physical activity, dietary intake, height and body mass were quantified using identical methods to the Hong Kong cohort. Percent body fat was also determined from total body dual energy x-ray absorptiometry, but used a GE-Lunar Prodigy (Lunar Inc., Madison, WI, USA). Waist and Hip circumference were measured at the narrowest point around the navel and at the widest point around the hips respectively. Grip strength was measured in duplicate on the dominant side using a JAMAR® hand grip dynamometer (Sammons Preston Inc. IL, USA).

Serum calcium, albumin and phosphate were measured using azo arsenic III, bromcresol green, and phosphomolybdic acid colorimetric and spectrophotometric techniques respectively (Olympus, Tokyo, Japan). DiaSorin radioimmunoassay (DiaSorin Inc., Stillwater, MN, USA) was used to quantify 25(OH)D (sensitivity 3.7 nmol/L; coefficient of variation (CV) 9.1% at 82 nmol/L) and Immulite 1000 chemiluminescence immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA) was used to quantify PTH (sensitivity 0.1 pmol/L; CV 8.6% at 6.2 pmol/L). Assays were carried out at the Peking Union Medical College Hospital, Beijing, apart from PTH which was performed at the Chinese University of Hong Kong Pathology Laboratory and 25(OH)D which was performed at the Canterbury Health Laboratories, Christchurch, New Zealand.
Statistical analyses were performed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Univariate relationships between variables were reported as Pearson’s correlation coefficients. One-phase exponential decay curves were fitted to identify possible thresholds in the relationship between PTH and 25(OH)D. The effects of tertiles of calcium intake and adiposity indices on the relationship between PTH and 25(OH)D were analysed using two-way analysis of variance (ANOVA) models with PTH as the dependent variable. Stepwise, multiple linear regression models (p_{in}=0.05, p_{out}=0.10) were used to identify determinants of baseline 25(OH)D and PTH. Age, clinical biochemical, dietary nutrient intakes, anthropometric and physical activity variables were entered into the models in hierarchical blocks based on the degree of biological plausibility. When two variables from the same category (clinical biochemical: serum calcium, serum albumin, serum phosphate; dietary intake: vitamin D, calcium, protein, fat, energy; or anthropometric: height, body mass, body mass index, percent fat, waist:hip ratio) were significantly correlated (P < 0.05), only the variable with the higher univariate correlation with the dependent was entered into a model. A backward entry procedure was used and each model was identified by running the regression procedure an additional time with statistically redundant determinants removed [540]. Data are presented as mean ± standard error of the mean (SEM) unless otherwise stated.
4.4 Results

4.4.1 Participant Characteristics
In the Beijing cohort, 25(OH)D levels ranged from 9 – 74 nmol/L. They were <25 nmol/L in 45%, <30 nmol/L in 65%, and <50 nmol/L in 95% of the women. PTH was elevated above the upper reference level (7.3 pmol/L) in 11 of the 220 individuals. Characteristics of the Beijing and pooled Beijing and Hong Kong cohorts are shown in Table 4.1.

Table 4.1 Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Beijing</th>
<th>Beijing &amp; Hong Kong Cohorts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.6 ± 4.5</td>
<td>27.8 ± 4.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>21.3 ± 2.8</td>
<td>20.8 ± 2.8</td>
</tr>
<tr>
<td>Waist:hip circumference</td>
<td>0.75 ± 0.05</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Body fat (percent)</td>
<td>31 ± 7</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>Physical activity index (PASE score)</td>
<td>145 ± 82</td>
<td>138 ± 79</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>30 ± 4</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Vitamin D intake (IU/day)</td>
<td>38 ± 22</td>
<td>86 ± 101</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>506 ± 189</td>
<td>478 ± 179</td>
</tr>
<tr>
<td>Protein intake (g/day)</td>
<td>65 ± 16</td>
<td>71 ± 18</td>
</tr>
<tr>
<td>Fat intake (g/day)</td>
<td>66 ± 16</td>
<td>68 ± 16</td>
</tr>
<tr>
<td>Energy intake (kJ/day)</td>
<td>7340 ± 1320</td>
<td>7260 ± 1310</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.46 ± 0.09</td>
<td>2.40 ± 0.11</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>47 ± 2</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.23 ± 0.14</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D (nmol/L)</td>
<td>29 ± 11</td>
<td>31 ± 11</td>
</tr>
<tr>
<td>Plasma parathyroid hormone (pmol/L)</td>
<td>4.2 ± 1.8</td>
<td>3.8 ± 1.6</td>
</tr>
</tbody>
</table>

Data are mean ± SD
Beijing: n = 220, Beijing & Hong Kong Cohorts: n = 441

4.4.2 Relationship Between 25(OH)D & PTH

PTH and 25(OH)D were negatively correlated in the Beijing cohort (r = -0.22, P = 0.003) and when data from Beijing and Hong Kong were pooled (r = -0.25, P < 0.001). Both relationships showed a gradual curvilinear decline with increasing 25(OH)D (Figure 4.1 a & b). For the Beijing cohort, the fitted curve showed a plateau in PTH occurring at a 25(OH)D level of 25 nmol/L, however only 12 individuals recorded 25(OH)D levels above 45 nmol/L and PTH in those individuals was over 1 pmol/L lower than those whose 25(OH)D was below this level (P = 0.02 for t-test of this comparison, Figure 4.1a). When Beijing and Hong Kong data were pooled, there was little further reduction in PTH above 25(OH)D levels of 50 nmol/L.
Figure 4.1 a & b. Relationships Between Serum Parathyroid Hormone (PTH) and Serum 25-Hydroxyvitamin D [25(OH)D]
a) in the Beijing cohort ($r = -0.22$, $P = 0.003$ and b) in the pooled Beijing and Hong Kong groups ($r = -0.25$, $P < 0.001$). Levels of 25(OH)D were grouped in intervals of 5 nmol/L. The curves represent the best fit to the data of one-phase exponential decay to a PTH plateau (3.9 pmol/L, $r^2 = 0.07$ in the Beijing cohort; 3.3, $r^2 = 0.08$ in the pooled cohort). Numbers in brackets indicate the sample size for each data point.
4.4.3 Effect of Calcium Intake & Adiposity

In the ANOVA models, grouped 25(OH)D levels were significantly associated with PTH \((P < 0.001, \text{Figure 4.2})\) but tertiles of calcium intake, body mass, body mass index or percent body fat were not (data not shown). There were no significant interactions between 25(OH)D and any of these variables implying that they did not affect the relationship between 25(OH)D and PTH.

![Figure 4.2 Relationship Between Serum Parathyroid Hormone (PTH) and Serum 25-Hydroxyvitamin D [25(OH)D] According to Tertile of Calcium Intake in the Pooled Cohorts of Young Women (n = 441). PTH varied with 25(OH)D category \((P < 0.001)\) but not with calcium intake tertile. There was no significant interaction between 25(OH)D and calcium intake.](image)

4.4.4 Determinants of 25(OH)D & PTH

In an analysis of pooled data from both sites, age, physical activity index, vitamin D intake and protein intake (positively) and albumin, body mass and percent body fat (negatively) were correlated with 25(OH)D (Table 4.2). Univariate correlates of PTH were calcium intake (positively) and vitamin D intake, protein intake and fat intake (negatively) (Table 4.2).

Independent variables initially entered into the regression model to predict 25(OH)D were PTH, age, vitamin D intake, calcium intake, physical activity index, grip strength and body mass. Age, vitamin D intake and physical activity index (positively) and PTH and body mass (negatively)
were retained in the model and collectively explained 16% of the variation in 25(OH)D ($P < 0.001$). The same variables explained 7.9% of the variation in 25(OH)D when PTH was omitted from the model ($P < 0.001$). The model to predict PTH as the dependent variable included 25(OH)D and the same additional independent variables as 25(OH)D. Calcium intake (positively) and 25(OH)D (negatively) were retained in the model and collectively explained 7.3% of the variation in PTH ($P < 0.001$). When 25(OH)D was excluded from the model predicting PTH, vitamin D intake (negatively) was retained and with calcium intake explained 3.1% of the variation in PTH ($P = 0.002$).

Table 4.2 Correlates of Baseline 25-Hydroxyvitamin D and Parathyroid Hormone in the Beijing and in the pooled Beijing and Hong Kong cohorts.

<table>
<thead>
<tr>
<th></th>
<th>Beijing</th>
<th>PTH</th>
<th>Beijing &amp; Hong Kong</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.18**</td>
<td>-0.04</td>
<td>0.19**</td>
<td>0.04</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.03</td>
<td>-0.08</td>
<td>-0.08*</td>
<td>0.01</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>-0.04</td>
<td>-0.04</td>
<td>-0.11*</td>
<td>0.05</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-0.06</td>
<td>&gt;-0.01</td>
<td>-0.08*</td>
<td>0.05</td>
</tr>
<tr>
<td>Waist:hip circumference</td>
<td>-0.03</td>
<td>-0.05</td>
<td>0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body fat (percent)</td>
<td>&lt;0.01</td>
<td>-0.06</td>
<td>-0.10*</td>
<td>0.05</td>
</tr>
<tr>
<td>Physical activity index (PASE score)</td>
<td>0.07</td>
<td>0.03</td>
<td>0.10*</td>
<td>0.04</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.09*</td>
<td>0.06</td>
</tr>
<tr>
<td>Vitamin D intake (IU/day)</td>
<td>0.14*</td>
<td>-0.12</td>
<td>0.21**</td>
<td>-0.14**</td>
</tr>
<tr>
<td>Calcium intake (mg/day)</td>
<td>0.04</td>
<td>0.09</td>
<td>0.01</td>
<td>0.11*</td>
</tr>
<tr>
<td>Protein intake (g/day)</td>
<td>0.02</td>
<td>-0.01</td>
<td>0.11*</td>
<td>-0.14**</td>
</tr>
<tr>
<td>Fat intake (g/day)</td>
<td>0.09</td>
<td>-0.03</td>
<td>0.09*</td>
<td>-0.12*</td>
</tr>
<tr>
<td>Energy intake (kJ/day)</td>
<td>-0.01</td>
<td>-0.03</td>
<td>-0.01</td>
<td>-0.08*</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>0.18**</td>
<td>-0.11</td>
<td>-0.07</td>
<td>-0.02</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>0.07</td>
<td>-0.02</td>
<td>-0.13**</td>
<td>0.05</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>0.03</td>
<td>0.07</td>
<td>-0.02</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

Data are Pearson correlation coefficients
Beijing: n = 220, Beijing & Hong Kong Cohorts: n = 441
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; # $P < 0.1$

Abbreviations as follows: 25-hydroxyvitamin D (25(OH)D); parathyroid hormone (PTH)

### 4.5 Discussion

Analysis of a cohort of young women from Beijing has highlighted very poor vitamin D status amongst women of childbearing age in the North of China. Almost half (45%) had 25(OH)D levels less than 25 nmol/L, which is a proposed delineation of frank deficiency [111]. Very few women (5%) had levels above 50 nmol/L. Levels of 25(OH)D were, on average, 5 nmol/L lower than in the Hong Kong cohort of women of approximately the same age. Because of the urban setting for recruitment, many of the factors contributing to low vitamin D status, including sun
avoidance and indoor work environment and lifestyle in the present group, are likely to be similar to the Hong Kong cohort. However, 25(OH)D levels in Beijing, situated at a latitude of 40°N, are likely to be at the lowest annual level at the end of winter and during the spring months when recruitment took place [296, 298, 370, 375, 490]. Hong Kong, in contrast, lies within the tropics (at 22°N) where the solar zenith angle remains small (the sun is high in the sky) all year round, and recruitment between February and June would fall at a time of year, prior to the rainy season, when sunshine is plentiful. The magnitude of winter to summer seasonal change in 25(OH)D can be substantial, varying from 6 nmol/L in adult women in southern Florida, located just outside the tropics at around 26°N, [297] to almost 40 nmol/L in young German women recruited from around Bonn (51°N) [298]. Thus, seasonal variation may account for the observed difference in vitamin D status between the Hong Kong and Beijing cohorts.

Greater numbers in the pooled analysis allowed a more statistically powerful analysis of the determinants of 25(OH)D than using either cohort independently. Vitamin D intake was positively associated with 25(OH)D as the strongest independent correlate. Whilst food sources contain only small amounts of vitamin D compared to modest doses of UV skin exposure [33], in winter or in populations with low vitamin D status, vitamin D intake has previously been shown to be an independent determinant of 25(OH)D levels [356, 481, 486, 497].

Physical activity was also a significant positive predictor of 25(OH)D levels in agreement with previous studies [158, 295, 296, 479], although in the present study the correlation was not strong (r = 0.1). The association between these variables could be indirect, resulting from an association between physical activity levels and outdoor sun exposure. Most physical activity tools, including the modified Physical Activity for the Elderly tool used in the present study, are limited in their ability to measure total outdoor skin exposure in that they do not distinguish outdoor and indoor forms of exercise and do not account for time outdoors that may be spent sitting down or in inactive modes. A more specific index of outdoor skin exposure might be a much stronger predictor of vitamin D status.

In the present study, body mass was more highly negatively correlated with 25(OH)D than either body mass index or percent body fat and entered the model predicting it. Although percent body fat [90] and body mass index BMI [89, 541] have previously been associated with 25(OH)D levels, a recent study of a Japanese adult population also found body mass to be a stronger predictor than body mass index or percent body fat [490]. In this case, though, the association
was positive and percent body fat was not correlated with 25(OH)D. Adiposity levels were much higher in the American cohort of healthy women in whom the relationship with 25(OH)D was shown (percent fat: 36%; body mass index 24 kg/m²) [90] compared to the Japanese cohort (21%; 21 kg/m²) or in the Chinese pooled cohort of the present study (29%; 21 kg/m²). It may be that amongst lean populations non-adipose tissue, in particular muscular-skeletal sites, plays a greater role in the storage or metabolism of vitamin D metabolites.

Age was also positively associated with vitamin D status. It may be of some concern that young women in their early to mid 20s have poorer vitamin D status that those in their late 20s and early 30s especially if this observation represents a cohort effect in which the lifestyle behaviours precipitating these levels are continued by the younger group as they age.

Analysis of data from the Beijing cohort alone and of pooled data from Hong Kong and Beijing young women showed cross-sectional estimations of optimal 25(OH)D that are likely to be above 40 nmol/L. Although, in all analyses, there were insufficient numbers of young women with 25(OH)D levels greater than 45 nmol/L to clearly identify a PTH plateau, visual inspection of the relationships suggest that a plateau might have occurred above 25(OH)D levels of around 40 to 50 nmol/L.

Estimation of optimal levels of 25(OH)D based on the cross-sectional relationship between 25(OH)D and PTH are likely to be influenced by a number of variables. Low calcium dietary intake is associated with elevated PTH independently of 25(OH)D [323, 381, 390, 391] and intake has also been shown to influence the relationship between the two variables [322, 391, 392]. However, in the pooled Hong Kong and Beijing cohorts, we found no evidence of an effect of calcium intake tertiles upon this relationship. Although higher intakes of calcium are likely to suppress PTH relative to 25(OH)D levels, intakes of around 550 to 800 mg/day, present in the upper tertile of this study group, were particularly low and could have been insufficient to limit PTH.

Since adiposity indices are demonstrated determinants of 25(OH)D [90, 295, 296] and percent body fat has been associated with PTH independently of 25(OH)D levels [521], we hypothesised that these indices might also influence the relationship between 25(OH)D and PTH and hence estimation of a 25(OH)D threshold for PTH suppression. Nonetheless, in the present data there was no evidence of an interaction between tertiles of body mass index or percent body fat and 25(OH)D with PTH as the dependent variable. Bolland et al. investigated these relationships in
116 postmenopausal women with a mean percent body fat of 42% body fat. It is possible that the far lower level of the young women in the current study was too low to be associated with PTH elevation.

In conclusion, cross-sectional data from a pooled cohort of young Chinese women suggest that a plateau in PTH might occur as 25(OH)D approaches 40 to 50 nmol/L, although there are a lack of values in the higher range to clarify optimal 25(OH)D. Although body mass and percent body fat are inversely associated with 25(OH)D, in this young, lean population we have found no evidence that either calcium intake or indices of adiposity modulate the relationship between 25(OH)D and PTH.
CHAPTER 5: PHARMACODYNAMICS OF HIGH-DOSE REGIMENS OF ORAL VITAMIN D₃ IN FRAIL ELDERLY

5.1 Abstract

Most people requiring vitamin D supplementation take this in the form of a daily tablet, but there is concern that the doses used are frequently inadequate, and that compliance with daily medication is likely to be suboptimal. This randomised double-blind trial compares responses to three high-dose vitamin D₃ regimens and estimates optimal 25-hydroxyvitamin D [25(OH)D] levels from changes in parathyroid hormone (PTH) and procollagen type I amino-terminal propeptide (P1NP) in relation to baseline 25(OH)D. Sixty-three elderly hospital inpatients (aged 82 ± 7 years) were randomised on discharge to three regimens of vitamin D supplementation: a 500 000 IU loading dose (Stat group); the loading dose plus 50 000 IU/month (Stat+Monthly group); or 50 000 IU/month (Monthly group). The Stat and Stat+Monthly groups showed increases in 25(OH)D of 58 ± 28 nmol/L to one month. Thereafter, levels gradually declined to plateaux of 69 ± 5 nmol/L and 91 ± 4 nmol/l, respectively. In the Monthly group, 25(OH)D reached a plateau of ~80 ± 20 nmol/L at 3 – 5 months. There were no changes in serum calcium concentrations. PTH and P1NP were only suppressed by vitamin D treatment in those with baseline 25(OH)D levels <50 and <30 nmol/L, respectively. Large loading doses of vitamin D₃ rapidly normalise 25(OH)D levels in the frail elderly, with no adverse effect on serum calcium. Monthly dosing is similarly effective and safe with respect to hypercalcaemia, but takes 3 – 5 months for plateau 25(OH)D levels to be reached.

5.2 Introduction

Adequate circulating concentrations of 25-hydroxyvitamin D [25(OH)D] are important for optimal bone health. Vitamin D status is related to bone density [156, 310, 312, 313, 542], bone turnover markers [311, 348], falls risk, functional stability, strength [156, 160], and the occurrence of hip fractures [145, 324, 543]. Intervention studies with vitamin D suggest beneficial effects on fractures, particularly in institutionalised individuals and when calcium is co-administered [127, 306, 351]. The elderly are particularly susceptible to vitamin D insufficiency [69, 129, 542] as a result of reductions in mobility, time spent outdoors, sun exposure (contributed to by increased skin coverage), intrinsic skin response to UV radiation, and dietary vitamin D intake [432, 544]. The consequences of vitamin D deficiency are
potentially severe in the elderly since it is likely to contribute to their high fracture risk and to the mortality that is associated with fractures in this age group [545, 546].

Vitamin D deficiency is thought to contribute to bone loss by stimulating parathyroid hormone (PTH) secretion, resulting in increased bone resorption [8, 9]. Because of this, determinations of the optimal level of 25(OH)D have been based on estimates of the level of 25(OH)D required to minimise PTH concentrations, most frequently from cross-sectional studies. The values found in such analyses have varied between 25 and 122 nmol/L [17]. This variability may result from other factors that impact on PTH levels (such as dietary calcium intake and renal function) and from assumptions made in fitting curves through such widely scattered data. An approach that circumvents some of these problems is to study the impact of vitamin D supplementation on PTH concentrations longitudinally, in order to identify a level of 25(OH)D above which supplementation has no effect on PTH. There are only three such longitudinal investigations, and these have produced more consistent estimates of optimal 25(OH)D, ranging from 40 to 50 nmol/L [17, 130, 131]. Further corroboration of these results is needed.

Most people requiring vitamin D supplementation take this in the form of a daily tablet, but there is concern that the doses used are frequently inadequate, and that compliance with daily medication is likely to be suboptimal. The long half-life of 25(OH)D following oral calciferol supplementation (estimated at 90 days) [92] means that larger, less frequent doses are a practical alternative to daily supplementation. Despite this, systematic evaluation of regimens using high doses of vitamin D is lacking. The present study seeks to address this need by comparing 25(OH)D responses to three different high-dose cholecalciferol regimens. In addition, we have also used this dataset to determine the optimal level of 25(OH)D in these subjects, by assessing the relationships between baseline 25(OH)D levels and responses of PTH and the bone formation marker P1NP (procollagen type I amino-terminal propeptide) to vitamin D supplementation.

5.3 Methods

5.3.1 Participants
Patients aged ≥65 years in the general medical wards of a metropolitan hospital were recruited between October 2005 and May 2006. Exclusion criteria were a creatinine clearance less than 20 mL/min, current glucocorticoid use for more than 6 months, recent calciferol treatment at doses of more than 600 IU/day, primary hyperparathyroidism or other disorders or drugs that might influence vitamin D or PTH metabolism, and a life expectancy of less than 6 months. Those
taking non-prescribed dietary supplements were asked to continue taking the same product(s) throughout the study. Sixty-three of 183 eligible participants agreed to participate. The main reason given for potential subjects declining was the deteriorating health of themselves or their spouse. All participants provided written informed consent and the study procedures were approved by the local ethics committee.

5.3.2 Treatment
Participants were randomised into one of three treatment groups using a minimisation algorithm to ensure balanced numbers of men and women between groups. One group (Stat) took 10 x 50 000 IU vitamin D₃ tablets (a 12.5 mg loading dose) at the beginning of the study followed by monthly placebo for eight months. A second group (Stat+Monthly) took the 10 x 50 000 IU vitamin D₃ loading dose at study entry, followed by a single 50 000 IU (1.25 mg) tablet monthly. The third group (Monthly) took a 50 000 IU tablet with 9 additional placebo tablets at study entry followed by a 50 000 IU vitamin D₃ tablet monthly. The baseline doses were administered by study personnel, resulting in 100% compliance. Compliance with subsequent doses was assessed by tablet counts. Participants and investigators were blinded to the treatment assignments throughout. Trial medication was supplied by API Consumer Brands, Auckland.

5.3.3 Assays
Plasma and serum were stored at -80°C until the completion of the study, when assays for the measurement of plasma intact PTH and serum 25(OH)D and P1NP were undertaken. PTH and P1NP were measured using automated Roche electrochemiluminescence immunoassays, and 25(OH)D was measured using the DiaSorin radioimmunoassay. Each participant’s samples were measured in the same batch. Inter-assay coefficients of variation were 6.1% for PTH, 7.1% for P1NP and 5.7% for 25(OH)D. Creatinine clearance was estimated using the Cockcroft-Gault formula.

5.3.4 Clinical Assessment
Details of medical and pharmacological history were taken from hospital medical notes or during an interview conducted at baseline. Other information was collected at baseline and at the end of the study. Sun exposure was quantified by summation of reported active and non-active weekly duration of time spent outdoors using the mean of mid-summer and mid-winter values. Calcium and vitamin D intakes were assessed using a food frequency questionnaire [547]. Additional vitamin D-containing foods were added to this questionnaire, and intake was quantified using food composition data from the Foodfiles 2004 © (Revision 18) database (New Zealand Institute
for Crop and Food Research Ltd., Christchurch, New Zealand) accessed through FoodWorks Professional Edition 4 (Xyris Software (Australia) Pty. Ltd., Brisbane, Australia). Calcium and vitamin D intakes included that derived from supplements. Grip strength was measured in triplicate in the dominant hand at baseline and at 9 months.

5.3.5 Statistical Analysis

Data were analysed using SPSS version 14.0 (SPSS Inc., Chicago, IL, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Stepwise, multiple linear regression models (p_{in}=0.05, p_{out}=0.10) were used to identify determinants of baseline 25(OH)D and PTH. A backward entry procedure was used and each model was identified by running the regression procedure an additional time with statistically redundant determinants removed [540]. Two-way (month x dose) mixed model analyses of variance (ANOVAs) were applied to examine changes in 25(OH)D. Differences in the areas under the curves for these variables were analysed using one-way ANOVA. The effects of tertiles of calcium intake, creatinine clearance and BMI on the relationship between ΔPTH and 25(OH)D were analysed using two-way ANOVA models with ΔPTH as the dependent variable. Grip strength change was also analysed via a two-way mixed model ANOVA. Post-hoc multiple comparisons were Bonferroni-adjusted. Linear relationships between 25(OH)D, PTH, P1NP and their changes are reported as Pearson’s correlation coefficients. Data are reported as mean ± SEM unless otherwise stated. All tests were two-tailed and P < 0.05 was considered significant.

5.4 Results

5.4.1 Participants

The study subjects were in hospital at the time of recruitment for the following reasons: vascular disease (26), collapse or falls (15), rehabilitation following orthopaedic surgery (10), infections (6), respiratory disease (3), and other problems (3). Prior to hospital admission, 53 lived at home, 7 in a retirement village, and 3 in residential care. At the time of the 1 month assessment, 81% were independent with respect to mobility and self-cares, and at 9 months this was the case for 88%. At baseline, more than one third of participants took prescribed or non prescribed tablets that contained either vitamin D (21) or calcium (22). Six participants had been on stable doses of bisphosphonates for more than 1 year (5 were taking alendronate and 1 etidronate). Treatment groups were comparable in terms of baseline characteristics and completion rate (Table 5.1). Despite their frailty, age and reported minimal use of vitamin D supplementation, mean baseline
levels of 25(OH)D were 58 nmol/L, though the range was wide and some participants were markedly deficient.

Six participants withdrew within the first study month because of ill health. Four withdrew subsequently for health reasons, one moved away from the area, and five died during the study. Of the 47 subjects who completed the study, one completed a 4 week course of oral steroids for a bronchial infection and another was started on an oral bisphosphonate during the study. Their data are included in the analyses, except where noted otherwise. P1NP data was removed from analysis for another individual with unexplained extremely high values (874 µg/L at baseline). Compliance, defined as the number of monthly tablets taken as a percentage of the number of monthly tablets that should have been taken, was less than 80% in only two participants, and the median was 100% in all groups.
Table 5.1 Baseline Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Stat (n=19)</th>
<th>Stat+Monthly (n=22)</th>
<th>Monthly (n=22)</th>
<th>Total (n=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number completed study</td>
<td>15</td>
<td>15</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>Males / Females</td>
<td>11 / 8</td>
<td>11 / 11</td>
<td>9 / 13</td>
<td>31 / 32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>83 ± 6</td>
<td>81 ± 8</td>
<td>81 ± 6</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 4</td>
<td>26 ± 5</td>
<td>26 ± 4</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>Physical activity (hr/wk)</td>
<td>7.0 ± 8.0</td>
<td>6.0 ± 6.5</td>
<td>6.4 ± 6.5</td>
<td>6.4 ± 6.8</td>
</tr>
<tr>
<td>Time outside (hr/wk)</td>
<td>6.5 ± 5.9</td>
<td>4.9 ± 7.2</td>
<td>4.9 ± 4.7</td>
<td>5.4 ± 6.0</td>
</tr>
<tr>
<td>Calcium intake* (mg/day)</td>
<td>1150 ± 480</td>
<td>1400 ± 590</td>
<td>1350 ± 530</td>
<td>1320 ± 540</td>
</tr>
<tr>
<td>Vitamin D intake* (IU/day)</td>
<td>310 ± 260</td>
<td>290 ± 240</td>
<td>250 ± 220</td>
<td>280 ± 230</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>58 ± 25</td>
<td>66 ± 41</td>
<td>50 ± 25</td>
<td>58 ± 32</td>
</tr>
<tr>
<td>PTH (pmol/L)</td>
<td>5.1 ± 2.4</td>
<td>5.7 ± 3.6</td>
<td>5.2 ± 2.7</td>
<td>5.3 ± 2.9</td>
</tr>
<tr>
<td>P1NP (µg/L)</td>
<td>83 ± 79</td>
<td>58 ± 45</td>
<td>87 ± 61</td>
<td>76 ± 62</td>
</tr>
<tr>
<td>Serum adjusted calcium (mmol/L)</td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>80 ± 31</td>
<td>107 ± 110</td>
<td>95 ± 36</td>
<td>95 ± 70</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>48 ± 17</td>
<td>48 ± 17</td>
<td>49 ± 15</td>
<td>49 ± 16</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>24 ± 8</td>
<td>20 ± 10</td>
<td>19 ± 5</td>
<td>21 ± 8</td>
</tr>
</tbody>
</table>

There are no significant differences between groups.
Data are Mean ± SD.
* Includes a mean daily intake of 260 mg (Calcium) and 130 IU (Vitamin D) from prescribed and non-prescribed supplements.
† Reference ranges for PTH: 1.7 – 7.3 pmol/L and P1NP: 20 – 80 µg/L (males), 20 – 116 µg/L (postmenopausal females)

5.4.2 25(OH)D, PTH & P1NP Relationships at Baseline

Baseline 25(OH)D showed a similar variation with month as we have previously described in much larger cohorts [295, 296], though this variation was not significant in this smaller number of subjects (data not shown). There was an inverse correlation between 25(OH)D and PTH for the individual data (Table 5.2), but inspection of the data grouped in 10 nmol/L intervals of 25(OH)D indicates that there was little decline in PTH above 25(OH)D levels of 50 nmol/L (Figure 5.1). Other correlations are shown in Table 5.2. Baseline 25(OH)D was negatively correlated with age and positively with total vitamin D intake. Conversely, baseline PTH was positively correlated with age and negatively correlated with total vitamin D intake and creatinine clearance. Baseline P1NP was not related to either 25(OH)D or PTH.
Figure 5.1 Relationships Between Baseline 25-Hydroxyvitamin D [25(OH)D] and (a) Parathyroid Hormone (PTH) and (b) Procollagen Type I Amino-Terminal Propeptide (P1NP). Data are Mean ± SEM.
Table 5.2 Correlations Between Baseline Variables

<table>
<thead>
<tr>
<th></th>
<th>PTH</th>
<th>P1NP</th>
<th>Creatinine clearance</th>
<th>Age</th>
<th>Body mass index</th>
<th>Vitamin D intake</th>
<th>Calcium intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D</td>
<td>0.37 (0.03)</td>
<td>-0.18 (NS)</td>
<td>0.11 (NS)</td>
<td>-0.28 (NS)</td>
<td>-0.15 (NS)</td>
<td>0.45 (0.001)</td>
<td>0.21 (NS)</td>
</tr>
<tr>
<td>PTH</td>
<td>0.03 (NS)</td>
<td>-0.40 (0.001)</td>
<td>0.36 (0.005)</td>
<td>0.23 (0.07; NS)</td>
<td>-0.29 (NS)</td>
<td>-0.02 (NS)</td>
<td>-0.17 (NS)</td>
</tr>
<tr>
<td>P1NP</td>
<td>0.19 (NS)</td>
<td>0.001 (NS)</td>
<td>0.21 (NS)</td>
<td>-0.02 (NS)</td>
<td>-0.09 (NS)</td>
<td>-0.09 (NS)</td>
<td>-0.09 (NS)</td>
</tr>
</tbody>
</table>

Data are Pearson’s Correlation Coefficient $r$ and Level of Significance ($P$).
Not Significant (NS; $P \geq 0.05$)

Independent variables from Table 5.2 were entered into a regression model to predict baseline 25(OH)D and PTH. When 25(OH)D was analysed as the dependent variable vitamin D intake (positively) and PTH, P1NP, creatinine clearance and age (negatively) were retained in the model and collectively explained 40% of the variation in 25(OH)D ($P < 0.001$). P1NP is likely to be elevated in those with recent bone injuries. The analysis was therefore repeated with data removed from analysis for an additional 11 participants (ten following recent hip or knee replacement and one following hip fracture). In this analysis, only vitamin D intake (positively) and PTH (negatively) were retained in the model, collectively explaining 29% of the variation in 25(OH)D ($P < 0.001$). When baseline PTH was analysed as the dependent variable, body mass index (positively) and 25(OH)D, P1NP and creatinine clearance (negatively) were retained in the model, collectively explaining 34% of the variation in PTH ($P < 0.001$). After removal of P1NP data for those with recent bone injury, only body mass index (positively) and 25(OH)D and creatinine clearance (negatively) were retained in the model, collectively explaining 32% of the variation in PTH.

5.4.3 25-Hydroxyvitamin D Responses to Supplementation (Figure 5.2)

The Stat and Stat+Monthly groups showed similar patterns of change, with rapid increases in 25(OH)D to one month (mean increase 58 nmol/L) followed by gradual declines to respective plateaux of $69 \pm 5$ nmol/L and $91 \pm 4$ nmol/l. The decay half-time from peak to plateau for the Stat group was 1.3 months (95% CI 1.7-5.3). In the Monthly group 25(OH)D rose gradually to a plateau of about 80 nmol/L at 5 months. The ANOVA model confirmed that the treatment ($P = 0.002$), time ($P < 0.001$) and their interaction ($P < 0.001$) effects were all significant. Areas under these curves calculated for those who completed the study were also different between groups ($P = 0.02$) with post-hoc tests showing a difference between the Stat+Monthly and Monthly groups (26 000 ± 1000 and 20 000 ± 1000 nmol.days/L respectively). Response to vitamin D supplementation might be affected by BMI and season, but the groups were comparable with respect to these variables at baseline, and there was no significant interaction between them and study group.
Figure 5.2 Effects of Three Regimens of Supplementation With Vitamin D\textsubscript{3} on Serum Levels of 25-Hydroxyvitamin D [25(OH)D]

Time ($F_{(6,241)} = 58, \ p < 0.001$), treatment ($F_{(2,57)} = 7, \ p = 0.002$) and their interaction ($F_{(10,241)} = 13, \ p < 0.001$) effects were all significant, as was the difference between groups in the areas under the curve for those who completed the study ($F_{(2,38)} = 5, \ p = 0.02$). Data are mean ± SEM.

Because these high dose replacement regimens would usually be used in patients with low vitamin D levels, we re-assessed these data in subjects with baseline 25(OH)D levels below 50 nmol/L. Figure 5.3a (upper panel) demonstrates the efficacy of the loading dose in rapidly correcting low levels of 25(OH)D, mean values increasing from 31 nmol/L (range 12-48 nmol/L) at baseline, to 101 nmol/L (range 46-141 nmol/L) one month later. Levels in the Stat+Monthly group then declined to a plateau of 86 nmol/L, reached at 5 months, whereas levels in the Stat group progressively decreased, reaching 58 nmol/L at 9 months. The Monthly regimen was also effective, but took 3 months to reach a plateau of approximately 70 nmol/L. At 1 month, only one individual who received a loading dose failed to attain a 25(OH)D level of at least 50 nmol/L, compared to three individuals assigned to the Monthly group who did not reach this level at 1 month, and one of these who did not reach it until 7 months. Whilst nobody in the Stat+Monthly group had 25(OH)D levels <50 nmol/L at any month after baseline, by 7 months, 25(OH)D levels had fallen below 50 nmol/L in two individuals assigned to the Stat group. One of these two individuals started with a baseline 25(OH)D level above 50 nmol/L, and also remained below this level at 9 months.
With the more widespread use of vitamin D supplementation, assessment of the response in non-deficient subjects is also of interest to determine the likelihood of hypercalcaemia (Figure 5.3b, lower panel). Following the loading dose, the increase to one month in those with initial 25(OH)D levels of 50 nmol/L or greater was 50 (95% CI, 38 – 63) nmol/L, somewhat less than the increase of 71 (95% CI 58 – 84) nmol/L seen in those with initial levels less than 50 nmol/L ($P = 0.03$). Mean 25(OH)D peaked at ~130 nmol/L, and the highest individual 25(OH)D level at one month was 220 nmol/L, in a subject with a baseline value of 136 nmol/L. At three months the maximum level was 160 nmol/L. Serum calcium (corrected for albumin) was measured at baseline, 1, 5 and 9 months. There was no significant increase in serum calcium concentrations from baseline during the study, and no individual values were above the upper end of the reference range (2.6 mmol/L; Table 5.3).

### 5.4.4 PTH and P1NP Responses to Supplementation as Indicators of Optimal 25(OH)D

Serum PTH concentrations decreased by almost 1 pmol/L (-0.8 ± 0.3 pmol/L) at 1 month in the two groups receiving the 500 000 IU stat loading dose of vitamin D ($P = 0.008$ for change from baseline in the pooled groups). At 3 months, there was a similar decrease (-1.2 ± 0.6 pmol/L) in the group receiving monthly supplementation. These changes were maintained in the groups receiving monthly supplementation over the remainder of the study. We have utilised these data to estimate the optimal serum 25(OH)D concentration, using the method of Malabanan et al. [131]. This involves identifying a level of 25(OH)D above which supplementation with vitamin D causes no further suppression of circulating PTH concentrations. To this end, we have pooled PTH data from all study subjects and identified the visits at which 25(OH)D is 20 nmol/L or more above baseline for that subject. The changes in PTH at these visits are shown in Figure 5.4, in relation to baseline 25(OH)D levels. When baseline 25(OH)D was less than 20 nmol/L, there were substantial reductions in PTH as a result of vitamin D supplementation. For baseline 25(OH)D levels between 20 and 50 nmol/L, PTH decreased by about 1 pmol/L. However, at higher 25(OH)D levels, supplementation with vitamin D did not affect PTH. This suggests that there is no advantage, in terms of minimising PTH, in achieving circulating 25(OH)D concentrations of >50 nmol/L.
Figure 5.3 Effects of Three Regimens of Supplementation With Vitamin D$_3$ on Serum Levels of 25-Hydroxyvitamin D [25(OH)D]

Data are divided according to whether baseline concentrations of 25(OH)D were (a) below, or (b) above 50 nmol/L. In both cohorts, there were significant time x treatment interactions in the 25(OH)D response (a $F_{(10,99)} = 11$, b $F_{(105,130)} = 63$; $P < 0.001$ for both). Data are mean ± SEM.
Table 5.3 Serum Albumin-Adjusted Calcium Values Throughout the Study

<table>
<thead>
<tr>
<th></th>
<th>Stat</th>
<th>Stat+Monthly</th>
<th>Monthly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.32 ± 0.13</td>
<td>2.36 ± 0.09</td>
<td>2.38 ± 0.09</td>
</tr>
<tr>
<td>Month 1</td>
<td>2.35 ± 0.12</td>
<td>2.31 ± 0.08</td>
<td>2.37 ± 0.09</td>
</tr>
<tr>
<td>Month 5</td>
<td>2.28 ± 0.11</td>
<td>2.31 ± 0.08</td>
<td>2.31 ± 0.10</td>
</tr>
<tr>
<td>Month 9</td>
<td>2.24 ± 0.08</td>
<td>2.28 ± 0.09</td>
<td>2.28 ± 0.08</td>
</tr>
</tbody>
</table>

The reference range is 2.10 – 2.55 nmol/L
There are no significant month or group differences.
Data are Mean ± SD.

Figure 5.4 Data From All Subjects and All Visits Showing the PTH Change From Baseline (ΔPTH) in Relation to Baseline 25-Hydroxyvitamin D [25(OH)D]
This analysis is restricted to those visits at which the change in 25(OH)D from baseline was 20 nmol/L or greater. Data are mean ± SEM.
The observed decreases in PTH would be expected to be accompanied by more gradual reductions in bone turnover, assessed here by P1NP. These data from the subjects given the 500 000 IU loading dose were plotted over time, dividing the cohort at cut-points for baseline 25(OH)D of 20 (data not shown), 30, 40, 50 and 60 nmol/L (Figure 5.5a-d). With the 30 nmol/L cut-point (Figure 5.6a), there was no mean change in P1NP in subjects whose baseline 25(OH)D was 30 nmol/L or greater, whereas P1NP declined in those with baseline 25(OH)D less than 30 \( (P = 0.05) \). The fall in P1NP was greater in those with baseline 25(OH)D below the cut-point \( (P = 0.01) \). With higher cut-points, there were no differences in change in P1NP between those above or below the cut-points. P1NP data from 10 subjects were excluded from this analysis: seven with recent orthopaedic procedures, one who was immobilised following a stroke, and in two who had markedly elevated baseline values without apparent bone pathology.

### 5.4.5 Effects of Calcium Intake, Creatinine Clearance & BMI on Optimal 25(OH)D Estimates (Figure 5.6)

Since a number of variables have the potential to alter the relationship between baseline 25(OH)D and subsequent change in PTH, we compared the relationships obtained at each tertile of dietary calcium intake (including that from supplements), creatinine clearance and body mass index. For these analyses we again used pooled PTH data from all study subjects for each visit at which 25(OH)D was 20 nmol/L or more above baseline for that subject. When calcium intake was in the highest tertile (greater than 1552 mg/day) PTH showed a mean reduction following the vitamin D intervention of around 3.5 pmol/L for baseline 25(OH)D of less than 30 nmol/L but did not fall when baseline 25(OH)D was above this level. In contrast, in the lower two tertiles of calcium intake, there was still a mean drop in PTH of over 1 pmol/L with baseline 25(OH)D levels up to 50 nmol/L and little reduction above this level, suggesting a lower threshold for 25(OH)D when calcium intake is high (Figure 5.6a).

In the highest tertile of creatinine clearance (greater than 56 mL/min), there was minimal reduction in PTH following the intervention, even when baseline 25(OH)D was below 30 nmol/L. Substantial reductions (of around 2 pmol/L), on the other hand, were noted when creatinine clearance was less than 42 mL/min and baseline 25(OH)D was below 50 nmol/L (Figure 5.6b).

The greatest post-intervention reductions in PTH (of around 2 nmol/L) were noted when baseline 25(OH)D was less than 50 nmol/L and BMI was greater than 28 kg/m\(^2\) compared with BMI below this level for which PTH reductions were minimal (Figure 5.6c).
Figure 5.5 Change From Baseline in Procollagen Type I Amino-Terminal Propeptide (ΔP1NP) in Subjects Who Received a Loading Dose of Calciferol at Baseline (i.e. Stat & Stat+Monthly groups)

Data are divided according to whether the baseline 25(OH)D was above or below 30 - 60 nmol/L. There was a significant interaction between baseline 25(OH)D and time, with respect to the changes in P1NP ($P = 0.01; F(4,34) = 3$). Data are mean ± SEM.
Figure 5.6 Relationship Between Parathyroid Hormone Change From Baseline (ΔPTH) and Baseline 25-Hydroxyvitamin D [25(OH)D] According to Tertile of Total Calcium Intake Including Supplements
Ca intake, (a); Cockroft Gault creatinine clearance (CrCl, b); and body mass index (BMI, c)
Data (mean ± SEM) are from all subjects and all visits restricted to those visits at which the change in 25(OH)D from baseline was 20 nmol/L or greater. Numbers beside data points indicate the sample size.
5.4.6 Grip Strength

Grip strength in the entire cohort increased 2.6 ± 0.6 kg from baseline to the end of the study ($P < 0.001$), but there were no between-groups differences. Increases in individual subjects were not related to the change in 25(OH)D ($r = -0.1$). For subjects in whom 25(OH)D increased by 20 nmol/L or more, there was no effect of baseline 25(OH)D levels (grouped as above or below 50 nmol/L) on the change in grip strength.

5.5 Discussion

The present study demonstrates the efficacy and safety of three regimens utilizing 50 000 IU vitamin D$_3$ tablets. Individuals with initial 25(OH)D levels <50 nmol/L who received a 500 000 IU loading dose of vitamin D$_3$ showed increases in levels of 25(OH)D at 1 month to around 100 nmol/L. In contrast, with monthly 50,000 IU dosing, it took around 3 months for those with low initial vitamin D status to attain satisfactory levels. Both regimens are potentially useful. A loading dose is likely to be desirable for individuals with a high fracture risk and probability of poor vitamin D status, because it rectifies these low levels promptly, though there is still a 3 – 5 month delay in the bone marker response to the increased 25(OH)D levels. Rapid repletion of vitamin D is mandatory in deficient patients wishing to receive intravenous bisphosphonates post-fracture [548], since these drugs can cause sustained hypocalcemia in such individuals [549]. Individuals with osteomalacia or myopathy secondary to vitamin D deficiency will also require rapid repletion. In contrast, many individuals whose fracture risk is lower or in whom vitamin D status is uncertain can be managed satisfactorily with 50 000 IU monthly. If compliance with a monthly regimen is in question, the present results suggest that single doses of 500 000 IU every 6 – 12 months might provide a similar outcome.

Several other studies have assessed the effects of large intermittent doses of vitamin D. Wu et al., 2003 have previously shown that a single dose of 500 000 IU vitamin D$_3$ is safe with respect to serum calcium levels and effective in older women with serum 25(OH)D levels <25 nmol/L, and that it produces a 15% reduction in PTH concentrations [92]. Dosing with 300 000 IU vitamin D$_3$ leads to a rapid rise in 25(OH)D, peaking at 17 days and declining with a half-life of 90 days [92]. Adams administered 500 000 IU vitamin D$_2$ over 5 weeks to deficient patients, resulting in a mean increase in 25(OH)D of 60 nmol/L [550] and Przybelski et al. gave 600,000 IU of D$_2$ over 4 weeks with an increase of 47 nmol/L [416], both results similar to that found in the present study. There were no safety issues in either study. Diamond et al. administered
600,000 IU of D₃ as a single intramuscular injection to 50 subjects [406]. Primary hyperparathyroidism was subsequently diagnosed in one person and mild hypercalcemia was found in two others at 12 months, raising uncertainty regarding its etiology. Others have shown that 7 000-10 000 IU vitamin D₂ per week will prevent falls [168] and 150 000-400 000 IU vitamin D provided as 4 – 12-monthly doses may prevent fractures [166, 405].

Heaney’s group has investigated the time-course and dose-response to daily oral vitamin D₃ in healthy young men with initial 25(OH)D of approximately 70 nmol/L [88, 93]. After 8 - 20 weeks 25(OH)D increased by 0.014 – 0.015 nmol/L for each IU/day of vitamin D₃ when the change in 25(OH)D was regressed against dose. For individual doses, the range was between 0.012 – 0.029 nmol/L per IU vitamin D₃, encompassing the increase seen with monthly dosing in the present study (equivalent to 0.02 nmol/L per IU/day). Comparing these increments with those after the 500 000 IU loading dose used here, suggests that this loading dose produces an increment at one month equivalent to a long term daily dose of about 4000 IU [93]. Hence, the overall dose response to the loading dose appears to be somewhat lower, which may be because of reduced absorption efficiency of large doses.

The number of subjects in the present study with normal 25(OH)D levels was unexpected, and is likely to indicate that they had been taking vitamin D supplements which they did not report, prior to their hospital admission. This has provided the opportunity to assess the effect of high-dose supplementation in individuals whose levels are 50 nmol/L or greater. In this group, treatment with 50 000 IU monthly had a minimal effect, in contrast to those with baseline 25(OH)D less than 50 nmol/L, in whom 25(OH)D increased by around 40 nmol/L. Providing replete individuals with a 500 000 IU stat loading dose resulted in increases in 25(OH)D of around 50 nmol/L. Though substantial, these increases were again not as great as when baseline 25(OH)D was less than 50 nmol/L. A similar observation was made by Viljakainen et al. who provided vitamin D₃ (200 – 800 IU/day) to elderly women and noted that the response was almost doubled when initial 25(OH)D levels were below the median level of 47 nmol/L [397]. Vieth has suggested that homeostatic control mechanisms, possibly hepatic, prevent large increases in 25(OH)D [123]. Our observation of blunted 25(OH)D response to calciferol supplementation when circulating levels of this metabolite are above 50 nmol/L support this possibility, though uptake into adipose tissue could also contribute to this effect.

Levels of 25(OH)D which constitute a risk of toxicity are not clear, and safety issues associated with high levels cannot be ignored. Vieth has pointed out that studies which document both
vitamin D dose and 25(OH)D levels, have demonstrated toxicity at intakes greater than 10 000 – 40 000 IU/day which are usually associated with 25(OH)D levels greater than 500 nmol/L [123, 418, 419]. Despite this, upper reference limits for 25(OH)D are often set at much lower levels of 150 – 250 nmol/L. The highest level observed in any individual in the present study was 220 nmol/L at 1 month, which appears to be well within the safe range, particularly since this peak was transient. This contention is supported by the normal serum calcium levels throughout this period. However, we did not complete 24-hour urinary calcium measurements which may better reflect peak renal calcium loads and, in any case, we would not recommend loading doses in individuals who are vitamin D sufficient. In contrast, in individuals with low vitamin D status, the loading dose was efficient in normalising 25(OH)D and again did not result in values above the reference range.

The present data provide the opportunity to assess prospectively optimal concentrations of 25(OH)D, by identifying levels above which supplementation does not further suppress PTH and indices of bone turnover. The PTH data from the present study suggest that optimal levels of 25(OH)D are around 50 nmol/L, and the P1NP analyses suggest the optimal value might be even lower. Both these parameters might be affected by the participants’ mobility, though only 11 – 19% had significant mobility impairment during the study period and their exclusion did not affect this analysis. The optimal 25(OH)D level is a matter subject to considerable controversy [13, 33, 551, 552] and much of the evidence supporting higher levels is from cross-sectional studies. Such estimates are problematic because of the considerable variability in PTH levels in these studies, meaning that the levels of 25(OH)D associated with a PTH nadir are not evident from simple inspection of scatter-plots of PTH versus 25(OH)D. As a result, arbitrary statistical assumptions are necessary to develop models which define the relationship between PTH and 25(OH)D [17]. The nature of these assumptions will impact substantially on the results found.

Only three studies have used a longitudinal design to assess optimal 25(OH)D, as in the present study. All have arrived at optimal levels for 25(OH)D of around 50 nmol/L even though they have varied in age-group, race, country and duration [17, 130, 131]. Malabanan et al. assessed responses to 50 000 IU/week vitamin D2 over 8 weeks in 35 subjects with a mean age of 76 years [131]. They found that PTH was not further suppressed in those whose baseline 25(OH)D concentrations were greater than 50 nmol/L. Lips utilised data from the placebo group of a multinational clinical trial in osteoporotic women, and found that in response to calcium (500 mg/day) and vitamin D (400-600 IU/day) over 6 months, subjects only showed a significant decrease in PTH if their baseline 25(OH)D was less than 50 nmol/L [130]. Aloia et al. studied healthy,
postmenopausal African American women given vitamin D₃ (800 – 2000 IU/day) over 3 years, and found that the threshold for PTH suppression was a 25(OH)D level between 40 and 50 nmol/L [17]. Thus, there is substantial consistency between the present study and the others which have used a similar robust design in identifying a threshold of 25(OH)D for bone health. This does not mean that this is the optimal 25(OH)D level for the other tissues on which vitamin D is active [66], nor does it take into account the seasonal variation in 25(OH)D levels. Bolland et al. have recently shown that to ensure a nadir level of 25(OH)D in winter greater than 50 nmol/L, the summer peak must be 60 – 70 nmol/L in women, and 70 – 90 nmol/L in men [487].

It is possible that a slightly higher than average dietary calcium intake might have altered the 25(OH)D threshold estimate in the present study. Here, we have found some evidence of a lowered threshold with higher calcium intakes, although some caution is needed in interpreting these data because some groups comprise small numbers of individuals. Cross-sectionally, calcium intake influences the relationship between 25(OH)D and PTH. Both Aloia et al. [17] and Steingrimsdottir et al. [391] noted interactions between calcium intake and 25(OH)D on PTH levels in healthy adults, with a larger effect of 25(OH)D on PTH and greater elevation of PTH when both 25(OH)D and calcium intake were low (less than 800 mg/day for calcium intake). The same interaction was not observed in a cohort of young Chinese women with a much lower mean daily calcium intake of 480 mg [447] compared to 760 mg [17] and 1240 mg [391]. In a meta-analysis of mainly cross-sectional data which compared estimated 25(OH)D thresholds based on plateaux in PTH levels, Aloia et al. confirmed that calcium intakes below 1 g/day were more likely to result in reported 25(OH)D thresholds above 50 nmol/L [17]. In the present study approximately one third of participants were taking calcium from supplements, and total calcium intake was over 1 g/day for almost two thirds of participants. Although indeed higher than some of the available data, intakes are likely to be comparable to the Lips et al. study [130] in which 500 mg/day supplemental calcium was provided.

Impaired renal function, assessed by estimated glomerular filtration rate, has also been associated with additional elevation of PTH when 25(OH)D levels are less than 50 nmol/L [387]. Correspondingly, in the present study we have shown substantial post-intervention reductions in PTH when baseline 25(OH)D levels below 50 nmol/L accompany creatinine clearance levels of less than 56 mL/min. These data tentatively indicate that the high-dose vitamin D regimens used in this study are helpful in lowering the secondary hyperparathyroidism of renal insufficiency without the risk of hypercalcaemia associated with activated vitamin D therapies.
Since fat mass, independent of 25(OH)D, is a determinant of PTH in healthy postmenopausal women [521], adiposity could also influence the relationship between 25(OH)D and PTH and hence PTH-based estimates of 25(OH)D threshold. Although, they are limited by a small sample size to address this question, our data provide some evidence that PTH reductions may be largest for low initial 25(OH)D levels when BMI is greater than 28 kg/m². This suggests the possibility of additional effectiveness of these regimens in lowering elevated PTH when individuals with low vitamin D status are also overweight.

A limitation of this study is that there is no placebo-only group. When planning this study, we felt that it would be unethical to fail to provide any vitamin D treatment to frail elderly people who were likely to be deficient. It is therefore possible that some of the changes in 25(OH)D were due to factors other than the interventions provided, such as altered behaviour relating to sun exposure or dietary modification. If this were the case, it is likely that the effect would be consistent across all treatment groups and small, given the clear differences in between-group responses. Furthermore, it is unlikely that increased ultraviolet exposure would have affected our results relating to changes in PTH and P1NP since these changes would have occurred irrespective of the cause of the 25(OH)D increases. In addition, we were not able to control calcium intake nor fluctuations in renal function, but these are likely to be lesser confounders in a longitudinal study than they would be using a cross-sectional design. The generalisability of these findings to less frail or younger individuals remains to be determined, though the studies of Lips et al. [130] and Aloia et al. [17] were in much younger individuals and yet found similar relationships.

In conclusion, our results show that a large single dose of vitamin D₃ rapidly normalises 25(OH)D levels in frail elderly people, and is safe in terms of circulating calcium levels. Monthly 50 000 IU dosing is similarly effective and safe, with respect to hypercalcaemia, but takes 3 – 5 months for plateau 25(OH)D levels to be reached. The single high-dose regimen provides a convenient option for the rapid correction of vitamin D deficiency, and monthly dosing is likely to increase compliance in this population group.

These data have been published in Osteoporosis International [553].
CHAPTER 6: THE EFFECT OF 25-HYDROXYVITAMIN D LEVELS ON FIVE-YEAR HEALTH OUTCOMES IN POSTMENOPAUSAL WOMEN

6.1 Abstract

Vitamin D status has been linked to a wide range of health benefits, though most data are from cross-sectional studies and many do not correct for confounding variables such as age, physical activity, body fat or season of blood collection. We investigated the relationship between baseline 25-hydroxyvitamin D [25(OH)D] levels and adverse events from a 5-year calcium intervention study in 1471 healthy postmenopausal women. Half of the women had seasonally adjusted 25(OH)D <50 nmol/L. Compared to those with levels of 50 nmol/L or above, this group of women was older, had greater body mass, fat mass (all \(P < 0.001\)), was less active (\(P = 0.003\)), had lower high-density lipoprotein cholesterol level (\(P = 0.004\)), was more likely to smoke (\(P = 0.09\)), and more likely to have a past history of ischaemic heart disease (\(P = 0.003\)), any heart disease (\(P = 0.008\)), stroke or transient ischaemic attack (\(P = 0.05\)) and dyslipidaemia (\(P = 0.02\)). Prospectively, women with season-adjusted 25(OH)D <50 nmol/L at baseline, compared to above this level, were at greater risk of stroke [hazard ratio and 95% confidence interval [HR (95% CI)] = 1.7 (1.0-3.0), \(P = 0.04\)], composite endpoint of myocardial infarction, stroke or sudden death [HR = 1.5 (1.0-2.2), \(P = 0.03\)] and at lesser risk of breast cancer [HR = 0.4 (0.2-1.0), \(P = 0.05\)]. The effect of 25(OH)D on stroke and composite events was evident in those women allocated to calcium supplementation (\(P = 0.01\)) but not in those allocated to placebo. However, following adjustment for age, fat mass, current smoking, physical activity and past history of diabetes, ischaemic heart disease, and hypertension, the inverse relationships between baseline 25(OH)D and risk of cardiovascular outcomes were removed. In contrast, the positive association of 25(OH)D level with breast cancer risk remained after adjustment for age, fat mass, current smoking and physical activity (\(P = 0.02\)). Those women who were allocated to calcium supplementation and had 25(OH)D levels <50 nmol/L, compared to those in the calcium group with levels \(\geq\)50 nmol/L, also experienced greater reduction in grip strength over the 5-year trial (0.7 ± 0.2 kg versus 1.6 ± 0.2 [mean ± sem], \(P = 0.05\)) after adjustment for age, physical activity and fat mass. In conclusion, mean annual levels of 25(OH)D are associated with reduced 5-year risk of cardiovascular conditions which is due to pre-existing differences in risk factors for these conditions in those with low vitamin D status. Vitamin D status is also positively associated with changes in grip strength in women with high calcium intake and also with an increased risk of breast cancer.
6.2 Introduction

High vitamin D status has been suggested to reduce the risk of a wide range of medical conditions including osteoporosis, coronary heart disease, cancer (particularly colorectal and breast), diabetes, arthritis, and infectious illnesses [33, 136, 175, 262]. Both high levels of 25(OH)D and vitamin D intervention have also been linked to a reduced mortality rate [37, 38]. Some evidence for a modest skeletal benefit accrues from meta-analyses of randomised controlled trials showing that vitamin D supplementation reduces hip and non-vertebral fracture risk, however this benefit appears to be restricted to studies providing higher doses [127] and those conducted in institutionalised elderly when calcium is also provided [12, 306, 351]. Evidence from prospective cohort studies is less clear. One such study noted that a 25-hydroxyvitamin D [25(OH)D] level <50 nmol/L was associated with an increased fracture risk [158], although three other studies found no association when statistical adjustment was made for age and body mass [303, 324, 349]. Evidence of an effect of vitamin D on falls risk is also equivocal with two meta-analyses of interventional studies reaching opposing conclusions [23, 25].

Suggestions of a beneficial effect of vitamin D on the risk of other conditions are based largely on cross-sectional data and confounding variables, such as age, body fat, physical activity, smoking and prior medical history, are often not adjusted for. A small number of randomised controlled interventions show a protective effect of vitamin D supplementation or ultraviolet B radiation on cancer risk when calcium was also provided [249], blood pressure [292, 293], and weight-loss induced changes in low-density lipoprotein cholesterol [554]. Vitamin D intervention has also been demonstrated to attenuate increases in proinflammatory cytokines [555] and prevent reduction in arterial elasticity when combined with vitamin K [556], which may beneficially interrupt the pathogenesis of congestive heart failure and vascular disease. However, other studies have found no effect of vitamin D on blood pressure [557] or colorectal cancer [248], the latter in data obtained from over 36 000 participants in the Women’s Health Initiative. A few prominent prospective cohort and nested case-control studies have also found inverse associations between 25(OH)D and colon or colorectal cancer [235-237], breast cancer [238], prostate cancer [239, 240], incident hypertension [40], and progression of hip osteoarthritis [287]. In contrast, similar studies have not found an effect of 25(OH)D on risk of cancer of the colon [241], prostate [243] or ovaries [245], and one study reported a higher risk of prostate cancer in the highest compared to the lowest quintile of baseline 25(OH)D [246]. Some very large prospective studies have developed 25(OH)D algorithms from correlates. Two such
studies have reported associations between predicted vitamin D status and cancer incidence in 47,000 men [41] and incident hypertension in 115,919 adults [40]. In the former of these studies, Giovannucci et al. found that an increment of 25 nmol/L was associated with reductions in total cancer incidence and mortality, and almost half the risk of digestive-system cancer [41].

In prospective studies, important differences between those with higher and lower 25(OH)D levels may exist at baseline. Many studies investigating an association of initial 25(OH)D with subsequent disease risk have controlled for some confounders either by matching cases and controls nested within a larger prospective study [235, 238, 239, 245, 246], by performing separate analyses [236] or by statistically adjusting for confounding variables including age, reported physical activity levels, prior health status, smoking, body mass and composition, or menopausal status of older women [40, 241, 243, 246, 255, 287]. However, levels of 25(OH)D depend on the amount of ultra-violet radiation within the wavelength range that stimulates vitamin D production hitting an individual’s skin [470] and dietary vitamin D intake [18, 481, 520]. Many factors influence both of these; geographical, seasonal, phenotypic and behavioural correlates of 25(OH)D are numerous, interrelated and often likely to factor in the aetiology of the disease outcomes.

In temperate regions, vitamin D status varies throughout the year and substantial differences in 25(OH)D of between 21 and 40 nmol/L between summer peak and winter nadir have been reported [296, 298, 375, 487, 490], although in women >65 years seasonal variation may be less than in other age groups [558]. Although some prospective investigations of the association between 25(OH)D and disease outcomes have attempted to match cases and controls for the time of measurement or distinguished between winter and summer measurements in their analyses, very few have adequately adjusted for the time of the year of measurement.

In a cohort of postmenopausal women residing independently in the Auckland region of New Zealand (latitude around 37°S) who took part in a calcium intervention we individually-adjusted 25(OH)D levels for day of the year of blood collection. We then investigated whether this season-adjusted level of circulating serum 25(OH)D was prospectively associated with five-year risk of mortality, fracture, chronic diseases or their related medical events.
6.3 Methods

6.3.1 Participants

The study aims were addressed in a cohort of postmenopausal women who participated in a 5-year randomised controlled trial of calcium supplementation on fracture incidence and bone mineral density [559]. The study methods have been previously described [559-561]. In summary, women who were >5 years postmenopausal and >55 years of age were recruited to the study via advertisement and mail-outs using electoral rolls. They were excluded from participation if they had a major ongoing disease, life expectancy ≤5 years, were taking steroids, calcium, bisphosphonates or vitamin D supplements in doses >1000 IU/day, had lumbar bone density below the age-appropriate normal range (z-score <-2), elevated serum creatinine (>0.2 mmol/L) or had 25(OH)D levels <25 nmol/L. One hundred women had 25(OH)D levels ≤25 nmol/L at the initial pre-study screening and, of these, 78 elected to be treated with 500 000 IU vitamin D3, taken as 10 daily 50 000 IU tablets, and rescreened for the study within 3 months. Of these women, 71 subsequently entered the study and for this subgroup the earlier measurement of 25(OH)D is reported and they are categorised in analyses as having received vitamin D supplementation during the follow-up period. A total of 1471 eligible women provided written informed consent and were randomised, 1255 completed follow-up and 839 were still taking the study tablets at five years. Participants received 1 g calcium (as citrate) daily or identical placebo and compliance was 85% for those who continued to take tablets throughout the study. The study was approved by the local area ethics committee.

6.3.2 Measurements

Medical history and lifestyle information were collected at a pre-study visit, and included an assessment of calcium intake [547] and physical activity [562] using adaptations of validated instruments. Blood biochemistry was from a fasting baseline sample. Serum 25(OH)D was measured by radioimmunoassay (DiaSorin, Stillwater, MN). All samples were measured in one laboratory which takes part in, and meets the performance targets for, the Vitamin D External Quality Assessment Scheme (DEQAS) [563]. The interassay CV for the Diasorin assay was 7.6% at 46 nmol/L. Cholesterol and other assays were measured as previously described [559, 564].

Participants were reviewed every six months during the 5-year follow-up. At each visit, height was measured using a Harpenden stadiometer (Holtain Ltd., Crosswell, UK), body mass using Ohaus electronic scales (Ohaus Corp., Pine Brook, NJ, USA), and blood pressure using a Dinamap automatic monitor (Johnson & Johnson, Tampa, FL) [560]. Grip strength was measured in triplicate in the dominant hand annually using hand dynamometer (Lafayette
Instrument Company, Lafayette, IN, USA). BMD of the lumbar spine, proximal femur and total body were measured at baseline, 30 and 60 months and vertebral morphometry at baseline and 60 months using a Lunar Expert dual-energy x-ray absorptiometer, software version 1.7 (GE Lunar, Madison WI, USA). Body composition was derived from the whole body analysis.

6.3.3 Adverse Event Assessment

Adverse events were recorded at each visit but questions about specific symptoms were not asked. All participants kept a falls diary. All fractures were confirmed on x-ray. Osteoporotic fractures comprised all fractures except those of the head, hands, feet, ankles, and those arising from major trauma. Standard definitions of myocardial infarction and stroke were used and all self-reported cardiovascular events were independently verified using medical records and adjudicated by a cardiologist or neurologist as previously described [561]. Death was confirmed on medical records or from information by the participant’s family. All other adverse events including new diagnoses of cancer, diabetes, polymyalgia rheumatica, and any joint replacements, loss of teeth or infections that occurred during follow-up were self-reported.

6.3.4 Seasonal Adjustment of 25(OH)D

Levels of 25(OH)D were adjusted for season by fitting a sine curve to a plot of all measurements for the cohort against day of the year the blood sample was taken. Each individual was assumed to have a seasonal fluctuation equal in amplitude to that calculated for the cohort. Seasonally adjusted 25(OH)D was the baseline of the sine curve calculated for each individual and represents the estimated average 25(OH)D over the entire year. This approach has previously been described in detail [487] and validated in an independent cohort [558].

6.3.5 Statistical Analysis

Data from all women randomised to take part in the trial were used in the analysis. The cohort was divided by vitamin D status (above and below season-corrected 25(OH)D of 50 nmol/L) and treatment allocation and baseline characteristics were compared between the groups using t-tests for continuous variables with a Satterwaite correction applied when variances were unequal and Fisher’s exact test for dichotomous outcome variables. Continuous outcome data were analysed with a mixed models approach to repeated measures (ANCOVA) to examine the time course of the changes in the outcome variables in the cohort by vitamin D status. Significant main and interaction effects were further explored using the method of Tukey to preserve an overall 5% Type I error rate. Cox proportional hazards models were used to compare the incidence of outcome data by vitamin D status and the hazard ratio (HR) with 95% confidence intervals (CI) were reported. The assumption of proportional hazards was explored by performing a test for
proportionality of the interaction between variables included in the model and the logarithm of time. All these analyses were performed separately in the women allocated to calcium, the women allocated to placebo, and then in the entire cohort. The effects of vitamin D supplements were assessed by including an interaction term for supplement use, vitamin D status, and time in the models. Similarly, corrections for other confounding variables were applied by including them in the models also. Further analyses graded season-adjusted baseline 25(OH)D as tertiles. All analyses were performed using the SAS software package (SAS Institute, Cary, NC version 9.1). All tests were two-tailed and P < 0.05 was considered significant. Standard error of the mean (SEM) is reported unless otherwise stated.

6.4 Results

6.4.1 Participants

Almost half (47.5%) of the women entering the study had initial 25(OH)D levels below 50 nmol/L. When 25(OH)D was seasonally adjusted, the lowest third of women fell below 43 nmol/L and the highest above 58 nmol/L. Half the women also had seasonally adjusted 25(OH)D levels of below 50 nmol/L (mean ± SD was 50.5 ± 17.7 nmol/L) and the baseline characteristics above and below this level are shown in Table 6.1. Women with season-adjusted levels < compared to ≥50 nmol/L were older (P < 0.001), less active (P < 0.01), had greater body mass, fat mass and percent fat (all P < 0.0001), lower high-density-lipoprotein-cholesterol (P < 0.01) and lower high to low-density-lipoprotein-cholesterol ratio (P = 0.02). In addition, more women <50 nmol/L compared to ≥50 nmol/L reported a pre-existing heart condition or heart disease (P = 0.008), or a prior history of ischaemic heart disease (P = 0.003), dyslipidaemia (P = 0.02), or stroke or transient ischaemic attack (P = 0.05). During the study 371 women began taking supplements containing vitamin D, including 30.2% of women with season-adjusted 25(OH)D <50 nmol/L and 20.3% of women ≥50 nmol/L (P = 0.001). The mean duration of follow-up in women with season-adjusted 25(OH)D <50 nmol/L and ≥50 nmol/L was 4.6 years and 4.7 years respectively (P = 0.1).

To investigate the effect of seasonally adjusted 25(OH)D levels on outcome variables, we first examined the interaction between season-adjusted 25(OH)D above and below 50 nmol/L on risk for dichotomous outcomes (results shown in Table 6.2) and change across study visits for continuous outcome variables (Table 6.3). Secondly, we adjusted analyses for relevant confounding variables (Tables 6.2 and 6.3). Thirdly, we completed analyses using tertile of season-adjusted 25(OH)D both without and then with further adjustment for confounders. The results of tertile analyses are shown in Table 6.4 for dichotomous outcomes and in Table 6.5 for continuous variables.
## Table 6.1 Baseline Characteristics of Study Participants by Vitamin D Status

<table>
<thead>
<tr>
<th>Seasonally-adjusted serum 25-hydroxyvitamin D</th>
<th>&lt;50 nmol/L (N = 736)</th>
<th>≥50 nmol/L (N = 735)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>74.5 ± 4.4</td>
<td>73.6 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D (nmol/L)</td>
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<td></td>
<td></td>
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<tr>
<td>seasonally-adjusted</td>
<td>38 ± 9</td>
<td>65 ± 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>unadjusted</td>
<td>38 ± 11</td>
<td>66 ± 15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin-adjusted calcium (mmol/L)</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>86 ± 14</td>
<td>87 ± 14</td>
<td>0.8</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>86 ± 22</td>
<td>85 ± 20</td>
<td>0.3</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>42 ± 2</td>
<td>42 ± 2</td>
<td>0.2</td>
</tr>
<tr>
<td>Calcium intake</td>
<td>874 ± 408</td>
<td>841 ± 361</td>
<td>0.1</td>
</tr>
<tr>
<td>Bone mineral density (g/cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Total hip</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Total body</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Previous fracture (%)</td>
<td>40.9</td>
<td>39.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>68 ± 12</td>
<td>66 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>36 ± 4</td>
<td>36 ± 4</td>
<td>0.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>28 ± 10</td>
<td>26 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent fat (%)</td>
<td>43 ± 8</td>
<td>41 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>18 ± 5</td>
<td>19 ± 5</td>
<td>0.2</td>
</tr>
<tr>
<td>Physical activity (MET.h/day)</td>
<td>33 ± 4</td>
<td>34 ± 5</td>
<td>0.003</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>137 ± 23</td>
<td>135 ± 22</td>
<td>0.2</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70 ± 11</td>
<td>71 ± 10</td>
<td>0.5</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>1.5 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>0.004</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>4.3 ± 1.1</td>
<td>4.2 ± 1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>High:low density lipoprotein ratio</td>
<td>0.39 ± 0.17</td>
<td>0.45 ± 0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>6.5 ± 1.1</td>
<td>6.6 ± 1.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6 ± 0.8</td>
<td>1.5 ± 0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1 ± 0.7</td>
<td>5.1 ± 0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Current lipid lowering medication (%)</td>
<td>8.3</td>
<td>9.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Past history of (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ishaemic heart disease</td>
<td>9.8</td>
<td>5.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Any heart disease or condition</td>
<td>13.5</td>
<td>9.0</td>
<td>0.008</td>
</tr>
<tr>
<td>Stroke/TIA</td>
<td>1.2</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>29.1</td>
<td>29.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.9</td>
<td>2.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>10.1</td>
<td>6.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Cancer</td>
<td>5.0</td>
<td>4.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Smoking status (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>3.8</td>
<td>2.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Previous</td>
<td>38.6</td>
<td>38.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data are mean ± SD
P values are from t-tests for continuous variables and Fisher’s exact test for dichotomous outcome variables.
6.4.2 Musculoskeletal Outcomes

There were no differences in fractures and falls (Table 6.2) or changes in bone mineral density (Table 6.3) between those women with seasonally adjusted 25(OH)D levels < and ≥50 nmol/L. Results were similar for unadjusted levels of 25(OH)D, although non-symptomatic vertebral fracture risk was higher in those below, compared to above, this level (P = 0.03). Following adjustment for baseline age, fat mass, current smoking and physical activity level, there was no effect of vitamin D status on any of the fracture types analysed. When analysis was completed for the entire cohort using tertile of season-adjusted 25(OH)D, a significant interaction between tertile and risk of non-symptomatic vertebral fractures was noted (P = 0.02), with a higher risk observed with season-adjusted 25(OH)D levels in the lowest tertile (<43 nmol/L, Table 6.4). In contrast to the protective effect of vitamin D status noted for risk of non-symptomatic vertebral fractures, an opposite effect was noted for osteoporotic fracture risk. Although the interaction term was not significant (P = 0.09), the HR (95% CI) of the lowest compared to highest (>58 nmol/L) tertile of season-adjusted 25(OH)D showed a trend for a higher risk of osteoporotic fractures with low 25(OH)D levels (P = 0.05 for HR, Table 6.4), and this effect attained significance when analysis was restricted to those women allocated to calcium supplementation [HR (95% CI) = 1.7 (1.0 – 2.8); P = 0.03]. The significance of tertile effects remained unaltered after adjustment for confounding variables.

There was a tendency for a greater reduction in grip strength over 5 years for those women assigned to calcium supplementation in those with season-adjusted 25(OH)D <50 nmol/L compared to ≥50 nmol/L (P = 0.06 for interaction term) and this effect attained significance following adjustment for confounding variables (P = 0.047, Table 6.3). A similar effect, of borderline significance, was found when analyses were conducted by tertile of season-adjusted 25(OH)D (P = 0.05 with our without adjustment for confounders, Table 6.5). Levels of season-adjusted 25(OH)D were not related to changes in bone mineral density at any site (Tables 3 & 5).

6.4.3 Body Composition Outcomes

There were no significant effects of season-adjusted 25(OH)D above and below 50 nmol/L on 5 year changes in body composition variables for the entire cohort. However, when analysis was restricted to those women allocated to calcium, body mass decreased more in those with season-adjusted 25(OH)D <50 nmol/L compared to ≥50 nmol/L (P = 0.04), although this effect was no longer significant after adjusting for confounders (P = 0.06). For the tertile analysis within the entire cohort (Table 6.5), a decreasing reduction in 5-year fat mass with increasing tertile of season-adjusted 25(OH)D was found (P = 0.03 for interaction term), and the interaction between
season-adjusted 25(OH)D tertile and change in fat mass remained after adjustment for confounders ($P = 0.01$). A similar effect was noted for lean mass in those women allocated to calcium supplementation ($P = 0.03$ with or without adjustment for confounders).

### 6.4.4 Cardiovascular Outcomes

Amongst the cardiovascular event outcomes, there was an increased risk of stroke ($P = 0.04$) and composite events (stroke, myocardial infarction or sudden death, $P = 0.03$) in those initially below compared to above season-adjusted 25(OH)D of 50 nmol/L (Table 6.2). The protective effect of higher vitamin D status was retained when analysis was limited to those allocated to calcium ($P = 0.01$ for both variables). However, all effects of season-adjusted vitamin D status disappeared when the analysis was adjusted for age, fat mass, current smoking, physical activity and prior history of ischaemic heart disease, hypertension and dyslipidaemia (Table 6.2). A similar effect on composite risk of stroke, myocardial infarction or sudden death was noted across tertiles of season-adjusted 25(OH)D ($P = 0.02$, Table 6.4). Confidence intervals of the HR showed that the independent risks of myocardial infarction and stroke were also greater for those women in the lowest compared to the highest tertile of season-adjusted 25(OH)D ($P = 0.02 – 0.03$), although the interaction effect for tertile by risk of these events did not attain significance (Table 6.4).

No effects of season-adjusted 25(OH)D above or below 50 nmol/L on continuous cardiovascular variables were noted, whether or not these were further adjusted for confounders (Table 6.3). When tertiles of season-adjusted 25(OH)D were analysed, there was a significant interaction with change over time in high density lipoprotein cholesterol ($P = 0.02$), which remained following adjustment for confounders ($P = 0.01$, Table 6.5). Although 5-year differences between tertiles in the change in high density lipoprotein cholesterol were minimal, those with season-adjusted 25(OH)D in the middle tertile (43 – 58 nmol/L) experienced greater increases in high density lipoprotein during the middle stages of the study (12 – 36 months) than those in the lowest or highest tertile (data not shown).
<table>
<thead>
<tr>
<th>Event</th>
<th>Allocated to calcium (n=732)</th>
<th>Allocated to placebo (n=739)</th>
<th>Entire cohort (n=1471)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D (nmol/L)*</td>
<td>HR** (95%CI)</td>
<td>P†</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Fractures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td>7</td>
<td>6</td>
<td>1.1 (0.4-3.2)</td>
</tr>
<tr>
<td>Vertebral</td>
<td>16</td>
<td>11</td>
<td>1.4 (0.7-3.1)</td>
</tr>
<tr>
<td>Non-symptomatic vertebral</td>
<td>62</td>
<td>57</td>
<td>1.1 (0.8-1.6)</td>
</tr>
<tr>
<td>Distal forearm</td>
<td>68</td>
<td>66</td>
<td>1.1 (0.8-1.5)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>19</td>
<td>12</td>
<td>1.7 (0.8-3.4)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>6</td>
<td>4</td>
<td>1.6 (0.4-5.5)</td>
</tr>
<tr>
<td>Stroke</td>
<td>24</td>
<td>10</td>
<td>2.5 (1.2-5.3)</td>
</tr>
<tr>
<td>Transient ischaemic attack</td>
<td>13</td>
<td>12</td>
<td>1.1 (0.5-2.5)</td>
</tr>
<tr>
<td>Composite event†</td>
<td>39</td>
<td>21</td>
<td>2.0 (1.2-3.4)</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bowel</td>
<td>3</td>
<td>3</td>
<td>0.5 (0.1-2.8)</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
<td>10</td>
<td>0.4 (0.1-1.3)</td>
</tr>
<tr>
<td>Any cancer</td>
<td>19</td>
<td>27</td>
<td>0.6 (0.3-1.2)</td>
</tr>
<tr>
<td>Mortality</td>
<td>21</td>
<td>13</td>
<td>1.2 (0.6-2.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>8</td>
<td>6</td>
<td>1.5 (0.5-4.2)</td>
</tr>
<tr>
<td>Tooth loss</td>
<td>7</td>
<td>13</td>
<td>0.5 (0.2-1.4)</td>
</tr>
<tr>
<td>Polymyalgia rheumatica</td>
<td>9</td>
<td>10</td>
<td>0.9 (0.4-2.3)</td>
</tr>
<tr>
<td>Joint replacement</td>
<td>22</td>
<td>23</td>
<td>1.0 (0.5-1.7)</td>
</tr>
<tr>
<td>URTI infection</td>
<td>38</td>
<td>49</td>
<td>0.8 (0.5-1.2)</td>
</tr>
<tr>
<td>Non URTI infection</td>
<td>168</td>
<td>163</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>Any infection</td>
<td>181</td>
<td>186</td>
<td>1.0 (0.8-1.2)</td>
</tr>
</tbody>
</table>
Legend for Figure 6.2 from previous page.
Alternate shading separates musculoskeletal, cardiovascular, cancer, and mortality and other outcomes as reported in the text.
* 25(OH)D is seasonally adjusted 25-hydroxyvitamin D.
** HR is Cox’s proportional hazards ratio of the lower 25(OH)D level compared to the higher with 95% confidence intervals (95% CI).
† Level of significance for the difference using Cox’s proportional hazards model.
‡ Level of significance when adjusted for relevant confounding variables: fractures and incident cancers corrected for age, fat mass, current smoking and physical activity; cardiovascular/cerebrovascular events and mortality corrected for age, fat mass, current smoking, physical activity and prior history of ischaemic heart disease, hypertension and dyslipidaemia; incident cancers corrected for age, fat mass, current smoking and physical activity; falls, incident diabetes and joint conditions corrected for age, fat mass and physical activity; tooth loss and infections corrected for age.
§ Composite event includes myocardial infarction, stroke or sudden death.
¶ URTI are reported upper respiratory tract infections
Table 6.3 Changes from Baseline at Five Years for Continuous Variables in Postmenopausal Women by Treatment Allocation and Vitamin D Status

<table>
<thead>
<tr>
<th></th>
<th>Allocated to calcium (n=732)</th>
<th>Allocated to placebo (n=739)</th>
<th>Entire cohort (n=1471)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25(OH)D (nmol/L)*</td>
<td>P**</td>
<td>P adjusted*</td>
</tr>
<tr>
<td></td>
<td>&lt;50</td>
<td>≥50</td>
<td>Change from baseline</td>
</tr>
<tr>
<td>Bone density (g/cm²)</td>
<td>0.14</td>
<td>0.016</td>
<td>0.8</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>±0.004</td>
<td>±0.004</td>
<td>±0.004</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>-0.017</td>
<td>-0.013</td>
<td>0.6</td>
</tr>
<tr>
<td>Total femur</td>
<td>±0.003</td>
<td>±0.003</td>
<td>±0.003</td>
</tr>
<tr>
<td>Total body</td>
<td>-0.018</td>
<td>-0.017</td>
<td>0.9</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>±0.002</td>
<td>±0.002</td>
<td>±0.002</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>-1.3 ± 0.2</td>
<td>-0.6 ± 0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.7 ± 0.1</td>
<td>-0.5 ± 0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-0.9 ± 0.3</td>
<td>-0.3 ± 0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Percent fat (%)</td>
<td>-0.2 ± 0.3</td>
<td>-0.4 ± 0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>8 ± 1</td>
<td>12 ± 1</td>
<td>0.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.17 ± 0.05</td>
<td>0.19 ± 0.04</td>
<td>0.7</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>-0.82 ± 0.13</td>
<td>-0.80 ± 0.11</td>
<td>0.6</td>
</tr>
<tr>
<td>HDL-LDL-cholesterol ratio</td>
<td>0.14 ± 0.02</td>
<td>0.06 ± 0.04</td>
<td>0.5</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>-0.65 ± 0.14</td>
<td>-0.61 ± 0.12</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>-0.05 ± 0.09</td>
<td>-0.1 ± 0.07</td>
<td>0.1</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>-2.2 ± 0.8</td>
<td>-3.7 ± 0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.01 ± 0.03</td>
<td>0.07 ± 0.03</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Legend for Figure 6.3 from previous page.
Alternate shading separates musculoskeletal, body composition, cardiovascular, and mortality and other outcomes as reported in the text.
Data for change from baseline are least squares means ± SEM.
* 25(OH)D is seasonally adjusted 25-hydroxyvitamin D.
** Level of significance for mixed model ANOVA.
# Level of significance when adjusted for relevant confounding variables: bone mineral density corrected for age, fat mass, physical activity and current smoking; grip strength and glucose corrected for age, fat mass and physical activity; body mass and composition variables corrected for age, fat mass, current smoking and physical activity; blood pressure and cholesterol corrected for age, fat mass, current smoking, physical activity and prior history of ischaemic heart disease, hypertension and dyslipidaemia; creatinine corrected for age.
Table 6.4 Incident Events in Postmenopausal Women by Tertile of Seasonally Adjusted 25-Hydroxyvitamin D

<table>
<thead>
<tr>
<th>Event</th>
<th>Fractures</th>
<th>Mortality</th>
<th>Diastolic Torsion</th>
<th>Obesity</th>
<th>Cancer</th>
<th>Any Infection</th>
<th>Myocardial Infarction</th>
<th>Congestive Heart Failure</th>
<th>Stroke</th>
<th>Transient Ishaemic Attack</th>
<th>Composite Event‡</th>
<th>URTI Infection</th>
<th>URTI Infection §</th>
<th>Any Infection</th>
<th>URTI Infection §</th>
<th>URTI Infection §</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (nmol/L)*</td>
<td>&lt;43</td>
<td>43-58</td>
<td>&gt;58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip Fracture</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>3.4</td>
<td>(0.95-12.4)</td>
<td>0.2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertebral Fracture</td>
<td>22</td>
<td>25</td>
<td>18</td>
<td>1.2</td>
<td>(0.6-2.2)</td>
<td>0.5</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-symptomatic vertebral Fracture</td>
<td>3</td>
<td>14</td>
<td>8</td>
<td>0.3</td>
<td>(0.1-1.2)</td>
<td><strong>0.02</strong></td>
<td>0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal Forearm Fracture</td>
<td>23</td>
<td>28</td>
<td>23</td>
<td>1.0</td>
<td>(0.6-1.8)</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Osteoporotic Fracture</td>
<td>81</td>
<td>83</td>
<td>60</td>
<td>1.4</td>
<td>(1.0-2.0)</td>
<td>0.09</td>
<td>0.3</td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Symptomatic Fracture</td>
<td>89</td>
<td>82</td>
<td>81</td>
<td>1.1</td>
<td>(0.8-1.5)</td>
<td>0.6</td>
<td>0.8</td>
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<tr>
<td>Any Fracture</td>
<td>96</td>
<td>97</td>
<td>90</td>
<td>1.1</td>
<td>(0.8-1.5)</td>
<td>0.8</td>
<td>1.0</td>
<td></td>
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<tr>
<td>Falls</td>
<td>353</td>
<td>383</td>
<td>365</td>
<td>1.0</td>
<td>(0.9-1.2)</td>
<td>0.5</td>
<td>0.6</td>
<td></td>
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<tr>
<td>Myocardial Infarction</td>
<td>23</td>
<td>19</td>
<td>10</td>
<td>2.4</td>
<td>(1.1-5.1)</td>
<td>0.07</td>
<td>0.2</td>
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<tr>
<td>Congestive Heart Failure</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>2.1</td>
<td>(0.7-6.0)</td>
<td>0.4</td>
<td>0.4</td>
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<tr>
<td>Stroke</td>
<td>27</td>
<td>18</td>
<td>14</td>
<td>2.0</td>
<td>(1.1-3.8)</td>
<td>0.08</td>
<td>0.2</td>
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<tr>
<td>Transient Ishaemic Attack</td>
<td>13</td>
<td>14</td>
<td>18</td>
<td>0.7</td>
<td>(0.4-1.5)</td>
<td>0.6</td>
<td>0.5</td>
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<tr>
<td>Composite Event‡</td>
<td>48</td>
<td>36</td>
<td>26</td>
<td>2.0</td>
<td>(1.2-3.2)</td>
<td><strong>0.02</strong></td>
<td>0.1</td>
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<tr>
<td>Cancer</td>
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<tr>
<td>Bowel</td>
<td>3</td>
<td>4</td>
<td>6</td>
<td>0.5</td>
<td>(0.1-2.1)</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Breast</td>
<td>5</td>
<td>8</td>
<td>10</td>
<td>0.4</td>
<td>(0.1-1.3)</td>
<td>0.3</td>
<td>0.1</td>
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<tr>
<td>Any Cancer</td>
<td>29</td>
<td>24</td>
<td>35</td>
<td>0.8</td>
<td>(0.5-1.3)</td>
<td>0.1</td>
<td>0.09</td>
<td></td>
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<tr>
<td>Mortality</td>
<td>23</td>
<td>24</td>
<td>16</td>
<td>1.2</td>
<td>(0.6-2.3)</td>
<td>0.8</td>
<td>0.9</td>
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<tr>
<td>Diabetes</td>
<td>13</td>
<td>5</td>
<td>11</td>
<td>1.1</td>
<td>(0.5-2.6)</td>
<td>0.2</td>
<td>0.3</td>
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<tr>
<td>Tooth loss</td>
<td>16</td>
<td>24</td>
<td>11</td>
<td>1.5</td>
<td>(0.7-3.2)</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Polymyalgia Rheumatica</td>
<td>7</td>
<td>20</td>
<td>12</td>
<td>0.6</td>
<td>(0.2-1.5)</td>
<td>0.05</td>
<td>0.06</td>
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<tr>
<td>Joint replacement</td>
<td>31</td>
<td>33</td>
<td>21</td>
<td>1.6</td>
<td>(0.9-2.7)</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
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<tr>
<td>URTI Infection</td>
<td>52</td>
<td>52</td>
<td>54</td>
<td>1.0</td>
<td>(0.7-1.4)</td>
<td>1.0</td>
<td>0.9</td>
<td></td>
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<tr>
<td>Non URTI Infection</td>
<td>225</td>
<td>228</td>
<td>196</td>
<td>1.2</td>
<td>(1.0-1.5)</td>
<td>0.07</td>
<td>0.07</td>
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<tr>
<td>Any Infection</td>
<td>243</td>
<td>254</td>
<td>220</td>
<td>1.2</td>
<td>(1.0-1.4)</td>
<td>0.1</td>
<td>0.1</td>
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</tbody>
</table>

Alternate shading separates musculoskeletal, cardiovascular, cancer, and mortality and other outcomes as reported in the text.

* 25(OH)D is seasonally adjusted 25-hydroxyvitamin D.

** HR is Cox's proportional hazards ratio of the lowest 25(OH)D tertile compared to the highest with 95% confidence intervals (95% CI).

† Level of significance for an interaction between tertile of seasonally adjusted 25(OH)D and risk of event using Cox's proportional hazards model.

§ Level of significance when adjusted for relevant confounding variables: fractures and incident cancers corrected for age, fat mass, current smoking and physical activity; cardiovascular/cerebrovascular events and mortality corrected for age, fat mass, current smoking, physical activity and prior history of ischaemic heart disease, hypertension and dyslipidaemia; incident cancers corrected for age, fat mass, current smoking and physical activity; falls, incident diabetes and joint conditions corrected for age, fat mass and physical activity; tooth loss and infections corrected for age.

‡ Composite event includes myocardial infarction, stroke or sudden death.

§ URTI are reported upper respiratory tract infections.
Table 6.5 Changes from Baseline at Five Years for Continuous Variables in Postmenopausal Women by Tertile of Seasonally Adjusted 25-Hydroxyvitamin D

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;43</th>
<th>43-58</th>
<th>&gt;58</th>
<th>P†</th>
<th>P_adjusted#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone density (g/cm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>-0.005 ± 0.004</td>
<td>0.010 ± 0.003</td>
<td>0.004 ± 0.003</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Femoral neck</td>
<td>-0.024 ± 0.002</td>
<td>-0.020 ± 0.002</td>
<td>-0.020 ± 0.002</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Total femur</td>
<td>-0.029 ± 0.002</td>
<td>-0.028 ± 0.002</td>
<td>-0.029 ± 0.002</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Total body</td>
<td>-0.025 ± 0.002</td>
<td>-0.022 ± 0.002</td>
<td>-0.024 ± 0.001</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>-1.0 ± 0.2</td>
<td>-0.7 ± 0.2</td>
<td>-0.6 ± 0.2</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>-1.4 ± 0.2</td>
<td>-0.9 ± 0.1</td>
<td>-0.6 ± 0.1</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.7 ± 0.1</td>
<td>-0.5 ± 0.1</td>
<td>-0.5 ± 0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-1.1 ± 0.2</td>
<td>-0.7 ± 0.2</td>
<td>-0.2 ± 0.2</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Percent fat (%)</td>
<td>-0.7 ± 0.3</td>
<td>-0.6 ± 0.2</td>
<td>-0.1 ± 0.2</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>8 ± 1</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>3 ± 0.5</td>
<td>3 ± 4.0</td>
<td>4 ± 4.5</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>0.16 ± 0.04</td>
<td>0.20 ± 0.03</td>
<td>0.18 ± 0.03</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>-0.68 ± 0.12</td>
<td>-0.72 ± 0.11</td>
<td>-0.85 ± 0.09</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>HDL-LDL-cholesterol ratio</td>
<td>0.13 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>-0.54 ± 0.13</td>
<td>-0.55 ± 0.12</td>
<td>-0.70 ± 0.10</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>-0.03 ± 0.07</td>
<td>-0.14 ± 0.07</td>
<td>-0.13 ± 0.06</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>-2.8 ± 1.1</td>
<td>-3.3 ± 1.0</td>
<td>-1.7 ± 1.0</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.04 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>0.04 ± 0.03</td>
<td>0.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Alternate shading separates musculoskeletal, body composition, cardiovascular, and mortality and other outcomes as reported in the text.

Data for change from baseline are least squares means ± SEM.

* 25(OH)D is seasonally adjusted 25-hydroxyvitamin D.

** Level of significance for the interaction between tertile of seasonally adjusted 25(OH)D and the change in variable from a mixed model ANOVA.

† Level of significance when adjusted for relevant confounding variables: bone mineral density corrected for age, fat mass, physical activity and current smoking; grip strength and glucose corrected for age, fat mass and physical activity; body mass and composition variables corrected for age, fat mass, current smoking and physical activity; blood pressure and cholesterol corrected for age, fat mass, current smoking, physical activity and prior history of ischaemic heart disease, hypertension and dyslipidaemia; creatinine corrected for age.
6.4.5 Cancer Outcomes
Risk of breast cancer in those with season-adjusted 25(OH)D below 50 nmol/L was 40% of those with levels ≥50 nmol/L (\(P = 0.045\), Table 6.2). The positive association between 25(OH)D and breast cancer risk strengthened when age, fat mass, current smoking and physical activity were included in the model (\(P = 0.02\)) and the effect was maintained after adjusting for any one of these variables except age, adjustment for which resulted in a borderline level of significance (\(P = 0.07\)). No effects of tertile of season-adjusted 25(OH)D on cancer risk were noted.

6.4.6 Mortality & Other Outcomes
There was no effect of season-adjusted 25(OH)D, above and below 50 nmol/L or by tertile, on death from any cause, whether or not additional confounding variables were included in the model (Tables 2 & 4). Similarly, with or without adjustment for confounders, season-adjusted 25(OH)D was not associated with the incidence of joint replacement, tooth loss, polymyalgia rheumatica or diabetes (Tables 2 & 4) or change throughout the 5-year follow up in glucose or creatinine (Tables 3 & 5).

Whether or not age was included in models, there was no effect of seasonally adjusted 25(OH)D above and below 50 nmol/L on infection risk (Table 6.2). When tertiles of season-adjusted 25(OH)D were analysed, there were no significant interactions between tertile and infection risk for the whole cohort (Table 6.4). However, in only those women allocated to placebo, there was an interaction between tertile of season-adjusted 25(OH)D and risk of any infection (\(P = 0.01\)) and infections other than those of the upper respiratory tract (\(P = 0.02\)), those with levels in the lowest [HR (95% CI) = 1.5 (1.1 – 1.9), \(P = 0.004\)] or the middle [HR (95% CI) = 1.4 (1.0 – 1.8), \(P = 0.02\)] compared to the highest tertile, being at increased risk. The protective effect against infections of levels of season-adjusted 25(OH)D above 58 nmol/L, in those women not subsequently supplemented with calcium for the study duration, remained after adjustment for age.

6.4.7 Effect of Vitamin D Supplement Use
Because more women with initial levels of season-adjusted 25(OH)D < 50 nmol/L took vitamin D supplements during the study, and this supplementation may have masked the effect of low vitamin D status, we first repeated analyses for season-adjusted 25(OH)D above and below 50 nmol/L excluding supplement users. There were no statistically significant differences between women above or below this level for any outcome variable. Secondly, we repeated all the analyses to determine an interaction between supplement use, season-adjusted vitamin D status
and time for all dichotomous outcomes and continuous variables. When the entire cohort was included in the analysis, only four such interactions were statistically significant.

Two significant 3-way interactions between vitamin D supplement use, season-adjusted vitamin D status and time were found for skeletal outcomes: the risk of any symptomatic fracture and total femur bone mineral density ($P = 0.04$ for both). Despite the significant interaction for risk of symptomatic fracture, the HR for those women below compared to above season-adjusted 25(OH)D levels of 50 nmol/L was not different from unity after controlling for vitamin D supplement use [HR (95% confidence interval) was 0.8 (0.6-1.2)]. Total femur bone mineral density reduction was attenuated by vitamin D supplement use in women with season-adjusted 25(OH)D levels <50 nmol/L ($P = 0.04$), and this effect was more apparent when only those women allocated to placebo were included in the analysis ($P = 0.007$, Figure 6.1).

Vitamin D supplement use also interacted with vitamin D status [season-adjusted 25(OH)D < and ≥50 nmol/L] and time for systolic ($P = 0.003$) and diastolic ($P = 0.03$) blood pressure (Figure 6.2). These interactions were retained in the group allocated to calcium ($P = 0.006$ for systolic and $P = 0.01$ for diastolic) but not in the placebo group (data not shown). However, visual inspection of the relationships in Figure 6.3 suggests that vitamin D status had no consistent effect on blood pressure changes throughout the 5 years in either those women who took vitamin D during the study, or in those who did not.

In addition, for only those women allocated to placebo the interaction of vitamin D supplementation and season-adjusted status was also significant for risk of upper respiratory tract infections ($P = 0.02$), although correction for supplement use did not alter the HR (95% confidence intervals) from unity [1.6 (0.9 – 2.7)].

### 6.4.8 Effect of Non-Seasonally-Adjusted 25(OH)D

Because many investigations of vitamin D status and health outcomes fail to adequately adjust results for important confounding variables, including season, we also repeated analyses for levels of 25(OH)D above and below 50 nmol/L that were not seasonally adjusted. Results were similar to those of season-adjusted 25(OH)D that was not adjusted for other confounders. For the entire cohort, four additional variables attained significance: risk of non-symptomatic vertebral fractures ($P = 0.03$) and myocardial infarction ($P = 0.03$), and changes in grip strength ($P < 0.0001$) and systolic blood pressure ($P = 0.01$). Inspection of HRs (95% CIs) for dichotomous variables and changes in least squares means for continuous variables showed that levels of
25(OH)D < 50 nmol/L, compared to ≥50 nmol/L, were associated with reduced risk of non-symptomatic vertebral fracture [0.4 (0.2 – 0.9)], increased risk of myocardial infarction [2.4 (1.4 – 4.3)], greater 5-year reductions in grip strength (-1.1 ± 0.2 kg compared to -0.4 ± 0.1 kg), and a faster rise in systolic blood pressure culminating in reduced 5-year increases (-9 ± 1 mmHg compared to -12 ± 1 mmHg). In addition, risk of breast cancer was no longer significant when non-seasonally-adjusted 25(OH)D levels were used in the analysis.

Figure 6.1 The Effect of Season-Adjusted Baseline 25-Hydroxyvitamin D [25(OH)D] Below & Above 50 nmol/L by Vitamin D Supplement Use on Change in Total Femoral Neck Bone Mineral Density (BMD)
An interaction exists between study visit, vitamin D status and supplement use for those randomised to placebo (P = 0.007; upper two panels, a & b) and for the entire cohort (P = 0.04; lower two panels, c & d). The graphs on the left (a & c) include those women who did not take vitamin D supplements during the study and on the right (b & d) those who did. Data are changes in least squares means ± SEM.
Figure 6.2 The Effect of Season-Adjusted Baseline 25-Hydroxyvitamin D [25(OH)D] Below & Above 50 nmol/L by Vitamin D Supplement Use
An interaction exists between study visit, vitamin D status and supplement use for systolic blood pressure ($P = 0.003$; upper two panels, a & b) and diastolic blood pressure ($P = 0.03$; lower two panels, c & d). The graphs on the left (a & c) include those women who did not take vitamin D supplements during the study and on the right (b & d) those who did. Data are changes in least squares means ± SEM.
6.5 Discussion

In this 5-year prospective study of postmenopausal women, we have found no association between seasonally adjusted levels of 25(OH)D above and below 50 nmol/L and musculoskeletal, joint or cardiovascular variables after adjustment for confounding variables. There was no interaction of any of these effects with vitamin D supplement use during the study, and removing participants who started vitamin D from analyses made no difference to these results. Although vitamin D status has been cross-sectionally linked to a wide range of health benefits [33], 25(OH)D levels correlate with many lifestyle, medical and behavioural variables which may confound observed effects. Statistical adjustment for covariates (including the time of year when measurements were made, age, prior medical history and reported lifestyle) has been inconsistent across the literature.

Many studies report cross-sectional associations between 25(OH)D and musculoskeletal outcomes, including fractures [565], bone mineral density [130, 147, 156, 310, 313, 566], bone turnover markers [29, 348] though not universally for all of these variables [156, 312, 318, 320, 324]. Some interventional studies have also reported independent effects of initial levels of 25(OH)D, or the interaction of these with treatment, on subsequent changes in bone mineral [128, 329]. Cross-sectional investigations of 25(OH)D and fracture history in elderly are confounded by pre-fracture frailty, which may both limit time outside and precipitate falls and fractures, and inconsistent periods of post fracture immobility and hospitalisation. In the present study, it is possible that more frequent vitamin D supplement use amongst those with low vitamin D status may have masked effects of vitamin D status on outcomes. Nonetheless, only two interactions between vitamin D supplement use and skeletal outcomes were identified. The negative findings in the present investigation in relation to vitamin D status and falls or skeletal outcomes when statistical adjustments for age and fat mass were made, suggest that previously observed effects may also result from confounding variables.

Prospective cohort studies of the association between 25(OH)D and fractures are few and have used two different approaches. One approach is to compare the 25(OH)D levels of all or randomly selected cases compared to controls and studies employing this approach have consistently failed to find an effect [303, 350, 567]. By far the largest of these, a nested case-control analysis within the Women’s Health Initiative (WHI) study with over 36 000 participants, reported no difference in baseline 25(OH)D levels between those who did or did not
sustain hip fractures after an average of 7 years follow-up, whether or not the adjunct calcium/vitamin D intervention was adjusted for [350]. In the present study, using another approach, we have analysed the risk ratios of those with seasonally adjusted baseline 25(OH)D levels above and below threshold levels and not shown an effect on subsequent fractures, change in bone mineral density or falls. Of other prospective cohort studies reporting the relative risk of incident fractures for 25(OH)D levels below a specified threshold [158, 324, 349], only one reported a significant effect [158]. This Swedish study (Malmö Osteoporosis Prospective Risk Assessment: OPRA) reported an increased three-year risk of low-trauma fracture in women with 25(OH)D initially below 50 nmol/L, which was sustained after adjustment for season of measurement and after removal from analysis of those taking vitamin D supplements [158]. In an analysis of randomly selected fracture cases and controls from another United States study (Study of Osteoporotic Fractures: SOF), 25(OH)D below 47.5 nmol/L was not associated with increased fracture risk after adjustment for age and body mass and with or without additional adjustment for season or vitamin D supplement use [349]. Similarly, although analysis of data from a French cohort study (OFELY), with an average 11 year follow-up, found a difference in fracture risk above and below 25(OH)D levels of 30 nmol/L and 50 nmol/L, these effects disappeared following adjustment for age [324]. The same study showed no association between initial 25(OH)D levels and bone loss (longitudinally) or cross-sectionally with bone turnover markers and grip strength [324].

Here, we have found a prospective association of season-adjusted 25(OH)D with the change in grip strength over the study duration which attained significance after adjusting for both age and fat mass. Whilst this is in contrast with the aforementioned cross-sectional data from the OFELY study [324], it does support results from another prospective cohort analysis from Longitudinal Aging Study Amsterdam (LASA) of sarcopenia [159]. In that study, after a three-year follow-up, 25(OH)D levels less than 25 nmol/L were associated with a 2.6 fold increased risk of sarcopenia (grip strength decrease greater than 40%) following adjustment for physical activity level, season of blood collection, creatinine, chronic disease, smoking and body mass index.

Following adjustment for confounding variables in the present study, we found no association between season-adjusted 25(OH)D (either by using 50 nmol/L as a cut-point or between tertiles) and any cardiovascular event, total mortality or change in cardiovascular continuous variable except for mid-study effects on high density lipoprotein cholesterol which are difficult to interpret. Without adjustments for season and/or other confounding variables, we did find beneficial effects of higher vitamin D status on prevention of myocardial infarction and strokes
and in a delayed rise in systolic blood pressure, especially when a calcium supplement was taken. Since none of these remained significant when the effects of confounding variables were removed, it seems likely that all arose from confounding. Nonetheless, vitamin D interventional studies in adults do show short-term (8 week or less) reductions in systolic blood pressure of 6-8 mmHg [292, 293], though not in all studies [557] and these could also affect the rate of increase in blood pressure over the study duration. A prospective analysis of data from two large United States cohorts with 4 to 8 year follow-ups has also reported an increased risk of incident hypertension in adults with 25(OH)D levels less than 37.5 nmol/L compared to those with levels of 75 nmol/L or greater. In the present cohort, there was a lack of women with high seasonally adjusted levels of 25(OH)D to provide sufficient statistical power for this test, only a third of participants had levels above 58 nmol/L, and it may be that higher vitamin D status is associated with small beneficial changes in blood pressure.

Most prospective studies that have reported a beneficial effect of vitamin D on cancer risk have investigated colon or colorectal cancer [235-237]. In an earlier large study with over 25 000 adult participants and an eight-year follow-up, Garland et al. [235] reported that those with initial 25(OH)D levels >50 nmol/L had one third the risk of developing colon cancer compared to those below this level. An effect of 25(OH)D of a similar magnitude on the risk of death from colorectal cancer was also reported in a recent analysis of data from the Third United States National Health and Nutrition Examination Survey (NHANESIII) [236]. In the NHANESIII study, those with levels ≥80 nmol/L had close to one quarter the risk (relative risk = 0.28) and those with levels 50 – 80 nmol/L had a relative risk of 0.44 compared to those with levels <50 nmol/L [236]. The same study, however, reported no association between 25(OH)D levels and total cancer mortality risk of the risk of death from cancer at any other site examined [236]. In a randomised placebo-controlled trial of calcium, baseline 25(OH)D levels >73 nmol/L were associated with a reduced risk of colorectal adenoma recurrence, but only for those randomised to 1200 mg/day calcium [255], suggesting a possible interaction between calcium intake and 25(OH)D levels on cancer risk. The calcium interventional study and all three previous prospective studies report results adjusted for race (when relevant), age and sex. All except for the Grau et al. study also adjusted for season of measurement, although this study additionally adjusted for smoking and alcohol use [255]. In contrast to the results of these studies, in the present study we found no difference between women randomised to calcium and placebo in the effect of 25(OH)D on cancer risk and no evidence of an association of initial 25(OH)D levels with bowel cancer risk whether or not adjustment was made for season, age, fat mass or smoking.
Because 25(OH)D levels in a recent nested prospective cohort study were 5% lower in cases compared to matched controls [238], our findings of an increased risk in breast cancer with higher levels of 25(OH)D was unexpected. Some caution is warranted in the interpretation of this observation amongst the analysis of a sizeable number of outcomes. Nonetheless, these results may be consistent with the results of a recent Finnish prospective nested case control study of vitamin D status and prostate cancer [246]. Analysing data from a large (almost 30 000 men) trial of β-carotene and α-tocopherol, these authors reported graded increases in the 16-year risk of prostate cancer from the lowest to the highest quintiles of baseline 25(OH)D. Adjustment for smoking, season of measurement and other variables identified as the strongest confounders (physical activity, education and serum retinol) increased the effect, resulting in a 2.5 to 3 fold increased risk for those in the highest compared to the lowest quintile. The authors postulated two mechanisms that might explain this observation. The first implicates the role of vitamin D metabolites in the synthesis and regulation of growth factors, particularly insulin [568], and these have also been shown to be associated with breast cancer risk [569-571]. Secondly, the authors suggested that vitamin D status might be correlated with organochlorine pollutant intake from diets high in fish, although the protective effect of low vitamin D status was maintained after correction for fish intake [246]. There is also a controversial link between organochlorines and breast cancer [572], however dietary vitamin D intake is low in New Zealand (around 120 IU / day) [573] and therefore would seem an unlikely explanation for the effect observed here.

Observations that upper respiratory tract infections are more common in winter than summer [574, 575] and low sun exposure or vitamin D status is associated with respiratory infections in the young [576-578], which appear less prevalent following vitamin D treatment [579] have sparked hypotheses that infection risk is mediated by vitamin D status [178]. A recent analysis of NHANESIII data provides support for this hypothesis by showing a graded inverse association between 25(OH)D levels and recent self-reported upper respiratory tract infections in adolescents and adults [580]. These effects remained even when analyses were adjusted for season, regional and demographic variables, smoking, body mass index and prior history of asthma or chronic obstructive pulmonary disorder. In the present study, we have found lower risks of participant reports of any infection and infections other than upper respiratory tract between those with season-adjusted 25(OH)D of 58 nmol/L or above compared to below 43 nmol/L, particularly for those women assigned to the placebo group. However, these effects disappeared following adjustment for age. Visits were at six-month intervals and no specific question was asked about colds or influenza. It is therefore likely that these events may have been inconsistently or under
reported. Nonetheless, there is no reason to suspect differences in reporting with differing vitamin D status. Although our results might imply that either a high dose of supplemental calcium or a high vitamin D status is protective against infections, it seems more likely that these effects are modulated by confounders.

A strength of the present study is that adjustment for seasonal differences in ultraviolet exposure has been made on a precise basis. In temperate regions (including New Zealand) there is month to month variation in 25(OH)D [108, 121, 295, 296, 342, 370, 372, 442]. We have adjusted each individual to a predicted mean annual level based on a sine curve generated for the entire sample, who all lived within a narrow range of latitude in regional Auckland, New Zealand.

Outcomes in the current investigation were adverse events and as such were not a priori outcomes. We have determined a 0.05 level of significance, although testing for multiple outcomes, as in this investigation, necessitates many statistical tests. Furthermore, statistical adjustment for some variables might emphasise the effect of an overlooked confounding variable on disease risk or might artefactually remove a real effect of vitamin D status. We therefore do not regard our negative or positive results as definitive, but as generating a pattern that requires further corroboration from controlled trials of vitamin D.

Overall, we have shown that vitamin D status, when adjusted to a predicted annual mean, is not associated with skeletal or joint effects, body composition changes, total incidence of cancer or mortality, although there may be a small protective effect on the risk of osteoporotic fractures or a reduction in weight loss for those also taking a substantial dose of calcium. Higher levels of 25(OH)D are associated with beneficial cardiovascular effects and reduced risk of infection, but these effects disappear when confounding variables including age, fat mass, smoking, physical activity and prior health history are adjusted for. When calcium intake is high, we have noted an attenuation in the reduction in grip strength in older women with initial vitamin D status of 50 nmol/L or greater. Our results also suggest that levels of 25(OH)D below 50 nmol/L might be protective against breast cancer. Both these findings warrant further investigation in randomised controlled trials.
7.1 Abstract

Elderly people are known to have poor vitamin D status but recent studies have also reported prevalent deficiency and insufficiency in younger adults and children. At very high risk are migrants from tropical and equatorial regions living in urban areas or in the West. We analysed data from all people assayed for 25-hydroxyvitamin D [25(OH)D] over a 15-month period in the Auckland region. When children and men were compared by age and ethnicity categories, mean levels of 25(OH)D were consistent with New Zealand national survey results. In women, levels of 25(OH)D were higher here than national survey data for ethnicity categories and age categories ≥45 years but lower for age categories <45 years (all \( P < 0.05 \)). Mean annual levels of 25(OH)D differed between age groups (\( P = 0.001 \)), with adults 50 years or older having the highest levels (55 nmol/L), followed by children and adolescents <18 years (50 nmol/L), and adults 18 – 49 years lowest (46 nmol/L). Mean levels <35 nmol/L, were observed in people of Indian ethnicity and in Middle Eastern and African females. Those who were predicted to fall below 25 nmol/L or 50 nmol/L at some time during the year were classed as vitamin D deficient and insufficient respectively. The majority of vitamin D deficient (65%) and insufficient (60%) people were <65 years of age and there were approximately three times more females than males in both groups. Indian, Middle Eastern and African adults, and Māori, Pacific Island, African and Middle Eastern children were substantially over-represented in the group of those who were insufficient. Detection of insufficiency as a proportion of all tests undertaken was high for children and young people who were assessed by community general practitioners (80%) and for people of ethnic groups other than New Zealand European (86% compared to 55% for New Zealand Europeans). We conclude that prevalence of vitamin D deficiency and insufficiency is likely to be high in these groups and suggest that interventions to increase vitamin D status in Pacific Island, Māori and migrant communities might be a more cost-effective strategy than blood screening.
7.2 Introduction

It is well established that low vitamin D status is common in the elderly, especially when they are in residential care [122, 130, 146, 295, 296]. Skin reduces its capacity for vitamin D production with aging [435]. In addition, elderly people are likely to spend less time outside as mobility decreases, and they may wear more outdoor clothing. However, a number of recent surveys of otherwise healthy children and adults of all ages living in both temperate and tropical regions of the world have also reported a high prevalence of vitamin D deficiency or insufficiency, using definitions based on circulating 25-hydroxyvitamin D [25(OH)D] levels ranging from <17.5 nmol/L to <28 nmol/L for deficiency and <30 nmol/L to <75 nmol/L for insufficiency [121, 379, 392, 426, 442, 444, 455, 481, 581, 582].

Determinants of vitamin D status are numerous. The amount of vitamin D synthesised in the skin is influenced by environmental, physiological and behavioural factors [470]. Dark skinned people are more likely to have low levels of 25(OH)D because the melanin molecules responsible for skin pigmentation also screen ultraviolet radiation of wavelengths within the action spectrum for skin vitamin D production [460, 461, 470]. Data from United States studies show that African Americans have lower 25(OH)D levels that their Caucasian counterparts, with Hispanic Americans often reported as having intermediate levels [375, 383, 437, 440, 448, 462]. Very low levels have also been reported in Southern Asian adults [314]. Darker skinned migrants to western countries are also at risk of poor vitamin D status. Low levels of 25(OH)D have been reported in immigrants from Africa, the Middle East, South Asia or South East Asia living in Northern Europe, North America and Australia [430, 583-585]. Other factors that may contribute to low status in young people include long working hours, air pollution, sun avoidance for cosmetic reasons, or covering of the face for religious or cultural reasons [379, 445, 456-459].

There is also substantial seasonal fluctuation in 25(OH)D levels in temperate regions due to increased filtration of ultraviolet radiation in winter compared to summer by increased cloud and the thicker atmospheric layer it passes through when the sun is lower in the sky [470, 586]. Differences between summer peak and winter nadir of 25 nmol/L to 41 nmol/L have been reported in areas ranging in latitude from 35° to 51° N or S [296, 298, 370, 375, 490]. Despite this, few studies reporting the prevalence of vitamin D insufficiency or deficiency have adjusted for the time of year that measurements were made.
Some might assume that New Zealand, having a temperate climate and outdoor lifestyle, would be exempt from problems of vitamin D deficiency. Nonetheless, studies of independently living elderly women have found that, depending on the month of measurement, up to 16% are deficient [25(OH)D <25 nmol/L] and between 30% and 74% are insufficient [25(OH)D <40 to 50 nmol/L] [296, 587]. Up to 20% of older New Zealand men are insufficient [295]. Recent data suggest that levels in the broader population are similar. Just under half (48%) of the New Zealand adult population were estimated to be below 50 nmol/L in the 1997 National Nutrition Survey (NNS97) [121] and this prevalence may range from around 40% to 70% depending on the time of year [121, 586]. Many New Zealand children may also have low vitamin D status and data from 2002 National Children’s Nutrition Survey (CNS02) noted that around 30% of children aged 5 to 14 years old had levels of 25(OH)D below 27.5 nmol/L [442].

Ethnicity characteristics of people who are vitamin D deficient or insufficient in New Zealand have not been well characterised. Immigration to New Zealand has increased rapidly in recent years, especially in the major cities. Statistics New Zealand record 90 000 long stay immigrants in the year to May, 2009, of which 5700 were from Africa and the Middle East, 27 000 from Asia, and 6000 from the Pacific Islands and a net migration of 11 000 [588]. Indications are that vitamin D status in some of these groups could be very poor. Screening of a Wellington urban cohort of multiethnic women under antenatal care recruited over a 12 month period reported 87% with 25(OH)D levels below 50 nmol/L and over 60% below 25 nmol/L.

The main purpose of this study was to describe the demographics of people who were predicted to be vitamin D deficient or insufficient at some time during the year, from results of all assays undertaken in the Auckland region over a 27-month period, and using a validated method for seasonal correction of 25(OH)D levels. In order to determine whether levels of 25(OH)D grouped by gender, age and ethnicity identified from these assays were likely to be comparable to the population as a whole, we also compared our results with New Zealand national survey data. Two further aims were to compare hospital doctors and community general practitioners (GPs) in terms of the rates of detection of vitamin D deficiency or insufficiency relative to the number of tests requested and also to determine whether there were differences in the demographic distributions of those people identified as deficient or insufficient between the two types of referring doctor.
7.3 Methods

7.3.1 Participants
All tests for serum 25(OH)D conducted at the Auckland Labplus regional laboratory covering the area North of the Bombay Hills, over 26 months between 1 July, 2001 and 1 October, 2003 inclusive, for which date of birth, gender, ethnicity and referring doctor could be established were analysed. Only the first measurement for any individual during this time period was included in the analysis. Ethnicity data were obtained from the National Health Index (NHI) database.

7.3.2 25-Hydroxyvitamin D Measurement
Serum 25-hydroxyvitamin D was measured in duplicate using the Diasorin radioimmunoassay. Labplus takes part in, and meets the performance targets for, the Vitamin D External Quality Assessment Scheme (DEQAS) [563]. The interassay coefficient of variation for the Diasorin assay was 7.6% at 46 nmol/L.

7.3.3 Statistical Analysis
The methods for seasonal adjustment have been described in detail [487] and previously validated in this cohort [558]. In summary, the seasonal trend for each demographic group was determined by fitting a sine curve to the relationship between 25(OH)D against day of the year. Using the amplitude and phase-shift characteristics of this sinusoidal relationship, an annual nadir was predicted for each individual, which represented the lowest serum level of 25(OH)D expected for that person at some time during the year. In order to determine which demographic groups required separate sine curves, ANOVA models that included month of blood collection were used to determine whether seasonal fluctuation in 25(OH)D levels differed between age groups, ethnicities or genders. Post-hoc pairwise comparisons for significant effects were made using Tukey’s Least Significant Difference test. The existence of a statistically significant interaction between a demographic variable and month of collection meant that separate sine curves were employed for different categories of that variable.

Chi-square goodness-of-fit tests were used to compare proportions within the current data and population statistics and Chi-square tests of independence between two variables were undertaken to determine differences between observed and expected proportions.

For all tests, the level of significance was set at 0.05.
7.4 Results

7.4.1 Participants

A total of 23,391 tests for 25(OH)D were reported by the laboratory during the study period. Removal of 4,543 duplicate tests and 29 tests for which the date of birth or gender of the patient were unknown left a total of 18,819 tests. Ethnicity data were unavailable for a further 6,268 tests. Three outlying test results above 250 nmol/L were also removed from analysis because they were highly likely to be non-physiological [123] and it was deemed that rare cases of very high 25(OH)D might be overrepresented in this sample due to deliberate assessment for vitamin D toxicity with prior knowledge of poisoning. Hence, 12,548 measurements were included in the main analyses and their frequency distribution is shown in Table 7.1.

<table>
<thead>
<tr>
<th>Table 7.1 Age, Gender and Ethnicity Distribution of the Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children and Adolescents (&lt;18 years)</td>
</tr>
<tr>
<td>NZ European</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Māori</td>
</tr>
<tr>
<td>Pacific Islander</td>
</tr>
<tr>
<td>SE/East Asian</td>
</tr>
<tr>
<td>Indian</td>
</tr>
<tr>
<td>Middle Eastern</td>
</tr>
<tr>
<td>African</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Adults (18-49 years)</td>
</tr>
<tr>
<td>NZ European</td>
</tr>
<tr>
<td>Māori</td>
</tr>
<tr>
<td>Pacific Islander</td>
</tr>
<tr>
<td>SE/East Asian</td>
</tr>
<tr>
<td>Indian</td>
</tr>
<tr>
<td>Middle Eastern</td>
</tr>
<tr>
<td>African</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Older Adults (≥50 years)</td>
</tr>
<tr>
<td>NZ European</td>
</tr>
<tr>
<td>Māori</td>
</tr>
<tr>
<td>Pacific Islander</td>
</tr>
<tr>
<td>SE/East Asian</td>
</tr>
<tr>
<td>Indian</td>
</tr>
<tr>
<td>Middle Eastern</td>
</tr>
<tr>
<td>African</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Data are frequencies with percent of age group in parentheses.
When compared to 2006 New Zealand Census data [589] Māori and Pacific Island ethnicities were under-represented, although the proportion of European and Asian ethnicities was similar to that expected from the population statistics (P < 0.001 for chi-square analyses of females and males; Table 7.2).

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Female (%)</th>
<th>NZ Census 2006</th>
<th>Male (%)</th>
<th>NZ Census 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ European*</td>
<td>63.9</td>
<td>64.6</td>
<td>60.5</td>
<td>64.7</td>
</tr>
<tr>
<td>Māori</td>
<td>2.6</td>
<td>11.2</td>
<td>3.2</td>
<td>11.0</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>3.9</td>
<td>14.3</td>
<td>5.7</td>
<td>14.3</td>
</tr>
<tr>
<td>Asian</td>
<td>16.7</td>
<td>19.0</td>
<td>18.4</td>
<td>18.6</td>
</tr>
<tr>
<td>Middle Eastern / Latin American / African</td>
<td>0.03</td>
<td>1.4</td>
<td>0.04</td>
<td>1.6</td>
</tr>
<tr>
<td>Other</td>
<td>10.3</td>
<td>0.1</td>
<td>8.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*New Zealand (NZ) European includes those who indicated that their ethnicity was New Zealander in the 2006 NZ Census.

The distribution by ethnicity for both females and males was different to that expected from NZ census data (P < 0.001 for both) and was also different when “NZ European” and “Other” categories were omitted from the analysis (P < 0.01 for both). Data were obtained from Statistics New Zealand Website Available from: [http://www.stats.govt.nz/census/census-outputs/quickstats](http://www.stats.govt.nz/census/census-outputs/quickstats) [589].

### 7.4.2 Vitamin D Status Comparison With National Nutritional Survey Data

Because people referred by a doctor for measurement of 25(OH)D do not represent a random sample from the population at large, we sought to determine the representativeness of the cohort in relation to vitamin D status. Levels obtained from all tests were compared with those reported in data from two recent national surveys which employed random sampling techniques: the 2002 National Children’s Nutrition Survey (CNS02) [442] and the 1997 National Nutrition Survey (NNS97) [121].

For children under 15 years, mean 25(OH)D levels obtained in the present investigation were generally consistent with national survey means (Table 7.3). The 95% confidence intervals for levels of 25(OH)D encompassed CNS02 means in all ethnicity- and age-by-gender categories except for young boys (aged 5 – 6 years). Mean levels in this group were lower in the present study compared to corresponding CNS02 data, though numbers were low here and only represented by 39 samples.
Another way of establishing the degree of bias in 25(OH)D levels from the present cohort relative to the general population is to compare proportions falling below a particular level of 25(OH)D. For children, a level of 37.5 nmol/L was selected, as this was the definition of insufficiency for which data were presented in the CNS02 report [442] (Table 7.4). Once again, the proportion of children in each ethnicity-by-gender category who had 25(OH)D levels <37.5 nmol/L was similar to CNS02 data. Exceptions were for children categorised as “New Zealand European or Other” and for young (5 – 6 year old) children, more of whom were identified as being below this level compared to CNS02 data ($P < 0.05$ for chi-squared analyses for girls and boys in both ethnicity and age categories).

In adults, levels of 25(OH)D compared with the NSS97 data were similar for men but not for women (Table 7.5). Here, levels (including 95% confidence intervals) were higher in women in each ethnicity category than corresponding means from the NNS97, but only in Māori men ($P < 0.05$). Similarly for women, 95% confidence intervals for 25(OH)D did not encompass NNS97 means in any age group, however in women <45 years, mean levels here were almost 10 nmol/L lower, whilst in those ≥45 years they were higher by a similar margin. Levels reported here for each age group of men were not different from NNS97 means, except for those aged between 25 – 44 years, in which they were lower.

More variation from NNS97 data was observed when proportions of adults with 25(OH)D levels <50 nmol/L were compared (Table 7.6). Lesser proportions of Pacific Island women and all women in age groups ≥45 years fell below this level compared to expected proportions from NNS97 ($P < 0.05$). In contrast, greater than expected proportions were observed for women in age groups <45 years, “New Zealand European or Other” men, and all men in age groups ≥25 years.
Table 7.3 Levels of 25-Hydroxyvitamin D in Children by Gender, Age and Ethnicity Compared with Means from the 2002 National Children’s Nutritional Survey (CNS02) for Each Category

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Female Present Cohort</th>
<th>Female CNS02</th>
<th>Male Present Cohort</th>
<th>Male CNS02</th>
<th>Total (Both Genders) Present Cohort</th>
<th>Total (Both Genders) CNS02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Māori</td>
<td>&lt;15 43 (36-50)</td>
<td>40</td>
<td>54 (45-64)</td>
<td>47</td>
<td>48 (43-54)</td>
<td>43</td>
</tr>
<tr>
<td>Pacific Island</td>
<td>&lt;15 34 (28-41)</td>
<td>34</td>
<td>33 (27-40)</td>
<td>38</td>
<td>34 (29-38)</td>
<td>36</td>
</tr>
<tr>
<td>NZ European &amp; Other</td>
<td>&lt;15 49 (46-52)</td>
<td>50</td>
<td>53 (50-56)</td>
<td>56</td>
<td>51 (49-53)</td>
<td>53</td>
</tr>
<tr>
<td>Total (All Ethnicities)</td>
<td>&lt;5 48 (43-52)</td>
<td>51 (45-54)</td>
<td>49 (46-52)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5-6 48 (39-57)</td>
<td>43 (38-49)*</td>
<td>57</td>
<td>46</td>
<td>52 (48-56)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7-10 50 (43-56)</td>
<td>51</td>
<td>55 (49-60)</td>
<td>53</td>
<td>52 (48-56)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-14 43 (38-47)</td>
<td>42</td>
<td>55 (49-62)</td>
<td>50</td>
<td>47 (44-51)</td>
<td></td>
</tr>
<tr>
<td>Total (&lt;15)</td>
<td>47 (44-50)</td>
<td>47</td>
<td>51 (48-54)</td>
<td>52</td>
<td>49 (47-51)</td>
<td>50</td>
</tr>
</tbody>
</table>

Data are geometric means with 95% confidence intervals for grouped data compared to survey-weighting-adjusted means from the CNS02 [442].
*95% confidence interval does not encompass the corresponding CNS02 mean.

Table 7.4 Proportions (%) of Children Identified as Insufficient [25(OH)D < 37.5 nmol/L] Compared with Those from the 2002 National Children’s Nutrition Survey (CNS02) [442]

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Female Present Cohort</th>
<th>Female CNS02</th>
<th>Male Present Cohort</th>
<th>Male CNS02</th>
<th>Total (All Ethnicities) Present Cohort</th>
<th>Total (All Ethnicities) CNS02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Māori</td>
<td>&lt;15 41</td>
<td>46</td>
<td>32</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pacific Island</td>
<td>&lt;15 56</td>
<td>64</td>
<td>57</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ European &amp; Other</td>
<td>&lt;15 38*</td>
<td>29</td>
<td>34*</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (All Ethnicities)</td>
<td>5-6 45*</td>
<td>29</td>
<td>36*</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7-10 35</td>
<td>31</td>
<td>29</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11-14 44</td>
<td>43</td>
<td>28</td>
<td>33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The observed proportion within the group varies significantly from that expected from the corresponding CNS02 proportion.
Table 7.5 Mean levels of 25-Hydroxyvitamin D in Adults by Gender, Age and Ethnicity Compared With Data From the 1997 National Nutritional Survey (NNS97)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Female Present Cohort</th>
<th>NNS97</th>
<th>Male Present Cohort</th>
<th>NNS97</th>
<th>Total (Both Genders) Present Cohort</th>
<th>NNS97</th>
</tr>
</thead>
<tbody>
<tr>
<td>Māori</td>
<td>≥15</td>
<td>46 (43-50)*</td>
<td>41</td>
<td>54 (44-64)*</td>
<td>43</td>
<td>48 (44-51)*</td>
</tr>
<tr>
<td>Pacific Island</td>
<td>≥15</td>
<td>40 (38-42)*</td>
<td>34</td>
<td>42 (38-46)</td>
<td>40</td>
<td>40 (38-42)*</td>
</tr>
<tr>
<td>NZ European &amp; Other</td>
<td>≥15</td>
<td>53 (52-54)*</td>
<td>48</td>
<td>52 (51-53)</td>
<td>53</td>
<td>53 (52-53)*</td>
</tr>
<tr>
<td>Total (All Ethnicities)</td>
<td>15-18</td>
<td>45 (39-51)*</td>
<td>55</td>
<td>43 (36-50)</td>
<td>49</td>
<td>44 (40-49)</td>
</tr>
<tr>
<td></td>
<td>19-24</td>
<td>38 (34-42)*</td>
<td>49</td>
<td>52 (45-58)</td>
<td>48</td>
<td>41 (38-44)</td>
</tr>
<tr>
<td></td>
<td>25-44</td>
<td>43 (42-44)*</td>
<td>49</td>
<td>43 (41-45)*</td>
<td>52</td>
<td>43 (42-44)</td>
</tr>
<tr>
<td></td>
<td>45-64</td>
<td>53 (53-54)*</td>
<td>45</td>
<td>53 (50-54)</td>
<td>52</td>
<td>53 (52-54)</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>56 (55-57)*</td>
<td>43</td>
<td>55 (53-56)</td>
<td>55</td>
<td>56 (55-57)</td>
</tr>
<tr>
<td>Total (≥15)</td>
<td></td>
<td>52 (52-53)*</td>
<td>47</td>
<td>52 (50-52)</td>
<td>52</td>
<td>52 (52-53)*</td>
</tr>
</tbody>
</table>

Data are geometric means (nmol/L), with 95% confidence intervals for grouped data, from the present study and the NNS97 [121].

*95% confidence interval does not encompass the corresponding NNS97 mean.

Table 7.6 Proportions (%) of adults identified as insufficient [25(OH)D < 50 nmol/L] compared with those from the 1997 National Nutritional Survey (NNS97) [121]

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Female Present Cohort</th>
<th>NNS97</th>
<th>Male Present Cohort</th>
<th>NNS97</th>
</tr>
</thead>
<tbody>
<tr>
<td>Māori</td>
<td>≥15</td>
<td>65</td>
<td>68</td>
<td>59</td>
</tr>
<tr>
<td>Pacific Island</td>
<td>≥15</td>
<td>74*</td>
<td>79</td>
<td>68</td>
</tr>
<tr>
<td>NZ European &amp; Other</td>
<td>≥15</td>
<td>49</td>
<td>49</td>
<td>49*</td>
</tr>
<tr>
<td>Total (All Ethnicities)</td>
<td>15-18</td>
<td>59*</td>
<td>39</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>19-24</td>
<td>71*</td>
<td>52</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>25-44</td>
<td>65*</td>
<td>51</td>
<td>63*</td>
</tr>
<tr>
<td></td>
<td>45-64</td>
<td>47*</td>
<td>52</td>
<td>49*</td>
</tr>
<tr>
<td></td>
<td>65+</td>
<td>45*</td>
<td>58</td>
<td>45*</td>
</tr>
</tbody>
</table>

*The observed proportion within the group varies significantly from that expected from the corresponding NNS97 proportion.

7.4.3 Differences in Seasonally Adjusted 25-Hydroxyvitamin D With Age & Gender

We sought to determine if there were differences between age-by-gender categories in 25(OH)D levels adjusted for season or in seasonal fluctuations of 25(OH)D levels. In order to investigate these questions we defined three age groups: children and adolescents (<18 years); adults (18 – 49 years); and older adults (≥50 years). Levels of 25(OH)D by gender in each of these age groups are shown with sine curves that were fitted separately for each of the age by gender groups in Figure 7.1 a-c. We then performed a 3-way ANOVA on 25(OH)D, including the month of the year the measurement was made, age group and gender in the model. We found
significant differences in 25(OH)D across months and age groups \((P < 0.001)\), but not between genders. Levels observed in adults 50 years or older (55 nmol/L) were higher than those of children and adolescents <18 years (50 nmol/L, \(P < 0.001\)), which were, in turn, higher than levels in adults 18 – 49 years (46 nmol/L, \(P = 0.02\)). There was also a significant interaction between month and age group \((P < 0.001)\) though not between month and gender indicating age-group but not gender differences in the seasonal fluctuation of 25(OH)D. Visual inspection of Figure 7.1 a – c suggests that children and adolescents displayed more seasonal fluctuation than older age groups. Since age group but not gender effects and interactions were significant, results are also presented with data for males and females pooled (Figure 7.2). The age group by gender interaction was also significant \((P = 0.04)\), indicating that the gender differences varied between age groups. We tested this by performing three 2-way (month by gender) ANOVAs for each age group. There were no between gender differences in any of the three age groups. However, for adults 18 – 49 years of age, but not for the other age groups, there was a month by gender interaction \((P = 0.004)\) suggesting that in this age group the seasonal fluctuation in females might be less than in males (Figure 7.1b).

### 7.4.4 Differences in Seasonally Adjusted 25-Hydroxyvitamin D With Ethnicity & Gender

To determine if there were ethnicity-by-gender differences in seasonally adjusted 25(OH)D or in its seasonal fluctuation, similar analyses to that of age groups were performed. Eight categories of ethnicity were defined and 25(OH)D levels separately adjusted for season for each ethnicity category by gender are shown in Figure 7.3. Effects were tested using a 3-way ANOVA on 25(OH)D including month of measurement, ethnic group and gender. Levels of 25(OH)D differed by month, ethnic group and gender \((P < 0.001)\) for all) and the interaction between ethnic group and gender was significant \((P < 0.001)\). The highest levels of 50 – 60 nmol/L (marginal means) were observed for New Zealand European and Other ethnicities and in Māori males. Intermediate levels of 35 – 45 nmol/L were found in people ethnically classified as Pacific Islander or Southeast/East Asian and in Māori females and Middle Eastern and African males. Lowest levels, <35 nmol/L, were observed in people of Indian ethnicity and in Middle Eastern and African females. The ANOVA results also indicate that 25(OH)D levels in males (45 ± 1 nmol/L; marginal mean ± sem) was higher than that in females (41 ± 1 nmol/L) when variation due to ethnicity is accounted for \((P < 0.001)\). None of the interaction effects that included month of measurement (month by ethnicity, month by gender and the three-way interaction effect) were significant, which indicated a lack of difference in the pattern of seasonal variation between ethnicities or genders.
Figure 7.1 Between-Gender Comparisons of Sine Curves of the Best Fit for 25-Hydroxyvitamin D [25(OH)D] Versus Day for Children (a), Adults (b) and Older Adults (c). Data points show monthly means and error bars are SEM. Month by gender interaction was significant for 18 – 49 year age group ($P = 0.004$).
Figure 7.2 Sine Curves of the Best Fit For 25-Hydroxyvitamin D [25(OH)D] Versus Day for Different Age Groups
Data points show monthly means and error bars are SEM. Month, age group and month by age group interaction were all significant ($P < 0.001$). Mean levels in adults 50 years or older were higher than mean levels in both other age groups ($P < 0.001$) and those in under-18s were higher than in adults 18 – 49 years ($P = 0.02$).

Figure 7.3 Mean Annual 25-Hydroxyvitamin D [25(OH)D] by Gender, Using Ethnicity Categories Recorded in the New Zealand National Health Index Database
Numbers of subjects in each category are shown above the bars.
7.4.5 Demographics of People Identified as Vitamin D Deficient or Insufficient

In order to describe the age, gender and ethnicity characteristics of the group of people predicted to be vitamin D deficient or insufficient at some time during the year, from levels reported in diagnostic testing, we first seasonally adjusted individual 25(OH)D measurements to a predicted annual nadir (trough). To do this, independent sine curves were fitted to data from the three broad age categories used for the previous analysis: <18 years, 18-49 years and ≥50 years. For each age group mean seasonally adjusted levels (represented by the baseline of each sine curve), mean change from baseline (represented by the amplitude) and the phase shift relative to a nadir occurring on October 1st (spring in the Southern Hemisphere) were calculated (Table 7.7).

Individual adjustment was made by assuming that each person followed a pattern of seasonal variation with an amplitude and phase shift identical to the mean for their age group. For the purposes of this analysis, individuals whose levels of 25(OH)D were predicted to fall below 25 nmol/L at some time during the year were defined as vitamin D deficient, and those predicted to fall to a minimum level of 25 – 50 nmol/L defined as insufficient [8]. We also report those either deficient or insufficient, who would thus be predicted to fall below 50 nmol/L at some time during the year. To gain more insight into the age distribution of deficient or insufficient individuals we further subdivided age into seven categories defined as follows: Prepubertal were females <11 years or males <13 years; Pubertal were females 11-15 years or males 13-17 years; Young Adults were females 16-29 years or males 18-29 years; Adults were females or males 30-49 years; Middle Aged were adults 50-64 years; Older Adults were 65-79 years; and Elderly were adults ≥80 years.

Table 7.7 Parameters of the Fitted Sine Curves for 25-Hydroxyvitamin D Versus Day of the Year by Age Group.

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>Baseline (nmol/L)</th>
<th>Amplitude (nmol/L)</th>
<th>Phase Shift (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18</td>
<td>50</td>
<td>15</td>
<td>51</td>
</tr>
<tr>
<td>18-49</td>
<td>46</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>≥50</td>
<td>56</td>
<td>8</td>
<td>34</td>
</tr>
</tbody>
</table>

Baseline represents the mean seasonally-adjusted 25(OH)D level, the amplitude the amount of seasonal excursion from the baseline, and the phase shift is the timing of the nadir (trough) shown as the number of days before the 1st October.

Over one quarter (27%) of results from all tests uncovered vitamin D deficiency and a further 37% insufficiency. The demographic distributions are shown by age (Figure 7.4) and ethnicity (Figure 7.5) for the cohorts of individuals who were deficient, insufficient or either deficient or
insufficient. People <65 years comprised a majority of vitamin D deficient (65%) and insufficient (57%) individuals and substantial numbers of young women aged 16 – 29 years (post-pubertal) and women aged 30 – 49 years (premenopausal) were deficient, comprising 12% and 31% of all deficient females respectively. Approximately three times more females than males were identified as being deficient or insufficient, and this ratio was apparent in all age groups of adults. There were approximately double the number of pubertal adolescent females compared to males, and similar numbers of prepubertal girls and boys identified as deficient or insufficient.

Approximately half (48%) of deficient and three quarters (75%) of insufficient individuals were classified as New Zealand European or Other ethnicity, although the proportions of males in these categories were lower than females (58% of all males either deficient or insufficient compared to 65% of all deficient or insufficient females). People of Indian ethnicity, including similar proportions of females and males, constituted 28% of all those deficient and 7% of all those insufficient. Māori and Pacific Islanders made up 9% of all deficient or insufficient people, South East and East Asian ethnicities made up 7%, and those categorised as Middle Eastern or African 2 – 3%.

Comparisons of observed and expected frequencies of deficient or insufficient people were made from a contingency table of age by ethnic group (data not shown) to identify age/ethnicity categories that might have been over- or under-represented. In summary, Māori, Pacific Island and African prepubertal children (and infants), Māori and Middle Eastern pubertal adolescents, African young adults, and Indian and Middle Eastern adults in age groups <50 years all appeared to be substantially over-represented with observed frequencies all more than double that expected. Indian and Middle Eastern prepubertal children were also over-represented and New Zealand Europeans in all age groups <50 years old were under-represented. Observed frequencies were more similar to those expected in age groups >50 years, except that New Zealand European elderly were an over-represented category. Very similar patterns were noted for the distribution by age group and ethnicity of all tests requested suggesting that the over-representation of particular demographic groups resulted from greater numbers of people in these groups being screened for deficiency (data not shown).
Figure 7.4 Individuals Predicted to be Vitamin D Deficient, Insufficient or Either of These at Some Time During the Year, for Each Age Group as a Proportion of the Total Number for Each Gender.
Definitions of deficiency and insufficiency are 25-hydroxyvitamin D <25 nmol/L and <50 nmol/L respectively. Age groups are as follows: Prepubertal, females <11 years or males <13 years; Pubertal, females 11-15 years or males 13-17 years; Young Adults, females 16-29 years or males 18-29 years; Adults, 30-49 years; Middle Aged, 50-64 years; Older Adults, 65-79 years; Elderly, ≥80 years. Numbers above bars are frequencies.

Figure 7.5 Individuals Predicted to be Vitamin D Deficient, Insufficient or Either of These at Some Time During the Year, for Each Ethnic Group as a Proportion of the Total Number for Each Gender.
Definitions of deficiency and insufficiency are 25-hydroxyvitamin D <25 nmol/L and <50 nmol/L respectively. Numbers above bars are frequencies.
7.4.6 Detection of Vitamin D Deficiency or Insufficiency by Hospital and Community Doctors

Because practice with respect to vitamin D screening might differ between hospital doctors and community GPs, we aimed to compare these groups in terms of the rates of detection of vitamin D deficiency or insufficiency [25(OH)D adjusted to annual nadir < 50 nmol/L] relative to the total number of tests requested and also in terms of the overall age, gender and ethnic distributions of the insufficient or deficient people identified. The distribution of vitamin D deficient or insufficient individuals and the corresponding deficiency or insufficiency detection rates are shown by age group in Table 7.8 and ethnicity in Table 7.9.

Hospital doctors requested approximately one quarter (27%) of all tests for 25(OH)D and the remainder were requested by GPs. Vitamin D deficiency or insufficiency was uncovered in 64% of all assays for 25(OH)D, and this detection rate was not different between hospital and community doctors.

The detection rates (Table 7.8) for vitamin D deficiency or insufficiency were higher than the overall rate in those <50 years (76%) and lower at or above this age (58%). Detection rates were particularly high for children and young people who were assessed by GPs (80%). Relatively more tests of elderly (≥80 years) and children (girls <16 years or boys <18 years) compared to other age groups ($P < 0.0001$), and of males compared to females ($P < 0.0001$) were requested by hospital doctors compared to GPs. The resulting age and gender distribution of people identified as deficient or insufficient approximately paralleled that for requests. Consequently hospital doctors compared to GPs also identified relatively larger numbers of vitamin D deficient or insufficient children and adults ≥65 years compared to other age groups ($P < 0.001$), and relatively more deficient or insufficient males ($P < 0.001$).

Rates of detection of vitamin D deficiency or insufficiency were low in New Zealand Europeans and those classified as Other ethnicity (mean across groups 56%), were high for Māori and South East/East Asians (78%) and very high for those ethnically classified as Pacific Islander, Indian, Middle Eastern or African (89%, Table 7.9). For hospital doctors compared to GPs, relatively higher numbers of Māori, Pacific Islanders and New Zealand Europeans and relatively lower numbers of people of all other ethnic categories were tested ($P < 0.0001$) and identified as deficient or insufficient ($P < 0.0001$).
Table 7.8 Distribution of Individuals Predicted to be Vitamin D Deficient or Insufficient at Some Time During the Year According to Age Group, Sex & Referring Doctor Type

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Hospital Doctors</th>
<th>Community General Practitioner</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Prepubertal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11 years female;</td>
<td>77 (71)</td>
<td>110 (68)</td>
</tr>
<tr>
<td>&lt;13 years male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pubertal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-15 years female;</td>
<td>54 (83)</td>
<td>29 (81)</td>
</tr>
<tr>
<td>13-17 years male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Adults</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-29 years female;</td>
<td>93 (78)</td>
<td>40 (58)</td>
</tr>
<tr>
<td>18-29 years male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>263 (76)</td>
<td>94 (64)</td>
</tr>
<tr>
<td>30-49 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle Aged</td>
<td>262 (59)</td>
<td>100 (66)</td>
</tr>
<tr>
<td>50-64 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Older Adults</td>
<td>379 (62)</td>
<td>143 (60)</td>
</tr>
<tr>
<td>65-79 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elderly</td>
<td>389 (55)</td>
<td>139 (64)</td>
</tr>
<tr>
<td>≥80 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1517 (63)</td>
<td>655 (64)</td>
</tr>
</tbody>
</table>

Data represent the number of individuals in each category with levels of 25-hydroxyvitamin D < 50nmol/L followed, in parentheses, by the proportion (%) of individuals found to be deficient or insufficient compared to the total number of tests in each category.

The difference in distribution of deficient or insufficient individuals between genders \( (P < 0.001) \) and across age groups \( (P < 0.001) \) was different between the two types of referring doctor. Age group distribution was also different for males and females \( (P < 0.001) \).

Table 7.9 Frequency of Individuals Predicted to be Vitamin D Deficient or Insufficient at Some Time During the Year by Ethnicity, Sex & Referring Doctor Type

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Hospital Doctors</th>
<th>Community General Practitioner</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Māori</td>
<td>79 (83)</td>
<td>42 (70)</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>88 (91)</td>
<td>56 (70)</td>
</tr>
<tr>
<td>South East / East Asian</td>
<td>80 (75)</td>
<td>22 (76)</td>
</tr>
<tr>
<td>Indian</td>
<td>148 (82)</td>
<td>57 (97)</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>24 (89)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>African</td>
<td>9 (90)</td>
<td>9 (82)</td>
</tr>
<tr>
<td>NZ European</td>
<td>1009 (58)</td>
<td>434 (60)</td>
</tr>
<tr>
<td>Other</td>
<td>80 (57)</td>
<td>24 (40)</td>
</tr>
<tr>
<td>Total</td>
<td>1517 (63)</td>
<td>655 (65)</td>
</tr>
</tbody>
</table>

Data represent the number of individuals in each category with levels of 25-hydroxyvitamin D < 50nmol/L followed, in parentheses, by the proportion (%) of individuals found to be deficient or insufficient compared to the total number of tests in each category.

The difference in distribution of deficient or insufficient individuals across ethnicities was different between the two types of referring doctor \( (P < 0.001) \). Ethnicity distribution was also different for males and females \( (P < 0.001) \).
7.5 Discussion

In this investigation we report the demographics of the group of people identified as vitamin D insufficient or deficient in the Auckland region of New Zealand, using levels of 25(OH)D corrected for the time of year of measurement. We have firmly established that children and adults under the age of 65 years constitute a majority of this group. All age categories are represented and approximately one third of those identified are male. People of Indian ethnicity represent a disproportionately large component of the insufficient and deficient groups. Although absolute numbers are smaller, people of African and Middle Eastern ethnicity may also be over-represented, particularly in those with vitamin D deficiency. Children and adolescents with ethnic classifications other than European or East/South East Asian also appeared to be over-represented in both vitamin D insufficient and deficient groups. The inclusion of substantial numbers of children and young people in these groups is of concern and extends recent data that highlight low vitamin D status in children [31, 374, 583] and younger women [379, 392, 444, 445, 456, 457]

What constitutes an optimal level of 25(OH)D is a matter of ongoing controversy. The lower reference range quoted in earlier studies of vitamin D status varied from 10 nmol/L to 50 nmol/L [8]. More recently, target levels of 75 nmol/L to 80 nmol/L have been suggested by some [115-117] and 50 nmol/L by others [86, 118]. Definitions for vitamin D deficiency and insufficiency of 25 nmol/L and 50 nmol/L have been commonly applied [111, 130]. Few studies investigating vitamin D status of various cohorts have adjusted for the month of the year of measurement, although some have sampled over a 12 month period [14, 146, 356] or collected data in the winter or summer months [379, 457]. Two recent New Zealand surveys have reported data statistically adjusted for season dichotomised as summer or winter [442] or as four seasons [121]. This method of adjustment lacks precision since 25(OH)D has been shown to vary from month to month in temperate regions [108, 121, 295, 296, 342, 370, 372, 442]. In the present study, we have adjusted each individual to a predicted annual nadir level based on a sinusoidal relationship derived for the specific demographic. Our calculation of a nadir or annual trough measurement may slightly overestimate the proportion of vitamin D deficient or insufficient individuals, relative to studies sampling throughout the year.
The proportion of Māori and Pacific Islanders included within the group of vitamin D deficient or insufficient people was less than half the proportion in the regional population as a whole. However, this does not imply a low risk of deficiency or insufficiency because both ethnic groups comprised even smaller proportions of all requested tests for 25(OH)D, just short of 3% for Māori and 5% for Pacific Islanders, compared to 11% and 14% within the Auckland regional population (Table 7.2). Relative numbers of deficient or insufficient Māori and Pacific Island children and Māori adolescents were much higher. However, since Māori and Pacific Island infants, children and adolescents (<18 years) comprised only 10.0% and 9.6% of all people <18 years (Table 7.1), under-representation of these ethnic groups could not fully be explained by a differing age-distribution which included more young people.

A surprising result from the present investigation is that mean levels of 25(OH)D from all tests undertaken in the region in men and children as well the proportion of people in these groups with identified vitamin D deficiency or insufficiency was largely consistent with studies sampling randomly from New Zealand children [442] and adults [121]. In addition, for middle-aged and older women mean 25(OH)D levels here were higher than levels in corresponding age groups in the national survey [121]. Both results suggest that clinical presentation and patient history obtained in typical medical screening is largely ineffective at pre-selecting those likely to have low vitamin D status for most age-by-gender categories.

Overall, two thirds of 25(OH)D tests identified individuals who were predicted to fall below 50 nmol/L at some time during the year and there was no difference in detection rates between hospital and community-based doctors. Detection rates were lower for middle aged, older adults and the elderly. One possible explanation for lower detection rates is that there is a higher degree of uncertainty in the assessment of the amount of outdoor sun exposure in this age group. Alternatively, low rates might reflect a lower level of discrimination by doctors when determining who should be assessed for 25(OH)D in these age groups, perhaps because it is deemed that the potential skeletal consequences of low status with advancing age presents a more serious risk. In contrast, detection rates approaching and exceeding 80% in those of non-European ethnicity might reflect more effective pre-selection of those at risk of low vitamin D status. On the other hand, these rates are consistent with 75% and 87% overall prevalence of vitamin D insufficiency [25(OH)D < 50 nmol/L] noted in two multiethnic cohorts: one of young adults living in Toronto, Canada [585] and the other of pregnant women in a Wellington, New Zealand medical clinic [441] and with 75% insufficiency in a multiracial cohort of healthy young people living in Toronto. The high rates in Aucklanders of non-European ancestry may therefore
simply indicate a very high prevalence in this population. In either case, such high vitamin D insufficiency detection rates in non-Europeans questions the necessity of diagnostic blood screening at all. Indeed, widespread vitamin D supplementation in these populations might be a more cost effective health measure.

Because the sample for this investigation is based on people referred by doctors for vitamin D assessment, it is difficult to establish whether differences in observed rates of insufficiency or deficiency between demographic groups is based on differences in the underlying prevalence, differences in pre-selection of patients for blood screening or differences in access to the health system. Nonetheless, the proportions of people by age and ethnicity with 25(OH)D levels <50 nmol/L and <25 nmol/L observed here, are largely consistent with cohort studies or studies with randomly selected samples.

We have confirmed that vitamin D insufficiency and deficiency in the Auckland region of New Zealand is widespread amongst all age groups and ethnicities. Because of the disproportionately high numbers of younger people of non-European and non-East/South East Asian ethnicity found to have low levels of 25(OH)D, it seems very likely that there is a very high prevalence of insufficiency and deficiency in these groups within the population at large. In these demographic sectors particularly, very high proportions of all blood screens for 25(OH)D undertaken reveal levels below 50 nmol/L. As an alternative to diagnostic blood screening, we suggest that widening public health measures such as recommending modest amounts of regular outdoor skin exposure and or mass vitamin D supplementation to ensure that Pacific Island, Māori and migrant communities are targeted might be more cost effective health strategies.

Some of these data analyses form part of a paper published in the New Zealand Medical Journal [558].
CHAPTER 8: PREVALENCE OF DIETARY SUPPLEMENT USE IN MIDDLE-AGED & ELDERLY NEW ZEALAND MEN

8.1 Abstract

Use of dietary supplements in the United States and Britain has become increasingly prevalent in recent years, especially in women. Little is known about use of these products by middle-aged and older men in New Zealand. In particular, increased profile of fish-oil supplements may have implications for intake of lipid-soluble vitamins A, D and E. In this investigation we surveyed men over 40 years of age who were participating in a trial of calcium supplementation on bone and cardiovascular outcomes. Almost half (47%) reported using at least one type of supplement. Almost a third (30%) of users took more than two different supplements and users spent an average of NZ$35 ± $39 (mean ± SD) each month on these products (median: $20; interquartile range: $10 - $45). The most common supplements used were vitamins or minerals (49%), followed by nutritional oils (22%) (including fish oils, 13%) and glucosamine/chondroitin preparations (13%). Supplements were mainly taken for reasons of non-specific prophylaxis or health maintenance (58% of reasons), although 21% of reasons cited treatment or symptom alleviation for a medical condition. An analysis of nutrient intake from foods and supplements revealed that 60%, 25% and 55% of men exceeded requirements of vitamins A, D and E respectively, and three men exceeded tolerable upper intake levels (two for retinol and one for vitamin D). The amount of money spent on substances with uncertain health benefits and potential risks is concerning. Health professionals should remain alert to supplement use by their patients, including males.

8.2 Introduction

Dietary supplements include a vast quantity and variety of over-the-counter pills, liquids or powders containing vitamins and minerals, herbal and other botanical products, amino acids, a range of other enzymatic or potentially nutritive substances, and mixtures of these. At the turn of the millennium, sales of supplements in the United States (US) were estimated at US$14.7 billion [590] and, at that time, 52% of adults (47% of men and 57% of women) in the Third US National Health and Nutrition Examination Survey (NHANESIII) had taken a vitamin, mineral or other dietary supplement in the last month [591]. Use of supplements in older adults is also prevalent. Data from a longitudinal study of healthy US adults ≥60 years of age suggest sharp
rises in dietary supplement use by this age group in the late 1990s [592]. In 1994, at the time of enrolment to this US study, a relatively small proportion (12% of men and 14% of women) took supplements, but by the end of the study, in 1999, these figures approached one half (41% of men and 46% of women). The increase in use of dietary supplements at this time in the US has been attributed to concurrent congressional changes in legislation [593].

There are less data on supplement use in older adults outside of the US. In a British national survey of people aged 65 years and over undertaken in the mid-1990s, 29% reported regular use of non-prescribed supplements [594]. This figure probably represents the vast majority of all supplement use in the United Kingdom (UK) at that time, because oral dietary supplements, not solely for enteral nutrition, were infrequently prescribed there [595]. A later postal survey in the north-west of England reported 35% of adults indicated that they usually took at least one dietary supplement, suggesting that prevalence of use might have increased slightly since the mid-1990s [596]. What little data exists from Australasia implies that supplement use may be less common. In one study, only 7% and 4% of Australian women in the late 1990s reported using calcium or multivitamin supplements respectively [597]. At around the same time, another survey of 26-year-olds born in Dunedin, New Zealand reported the prevalence of dietary supplement use to be 17% (20% in females and 13% in males) [598] and data from the 1997 New Zealand National Nutritional Survey indicate less regular supplement use by men (21%) than women (34%) and approximately half the prevalence of occasional use in men compared to women over the age of 45 years [599]. A more recent survey of Australians aged 65 years or more reported that 43% (52% of females and 35% of males) used dietary supplements [600].

A number of studies have examined the reasons why people choose to use dietary supplements, what marketing is most influential, and from where users source their information [601-604]. Because the majority of these studies are of women, the reasons for supplement use and sources of information influencing older men are unknown.

Whilst many supplements make claims of improved health or nutrition, there is often a lack of published evidence to support these benefits, except in the case of dietary deficiency [605]. For those taking supplements, intakes of some nutrients may exceed tolerable upper intake levels (UL) [606, 607], a daily intake level judged safe for almost all individuals [608]. Furthermore, potentially harmful interactions may arise from concurrent use of herbal preparations and prescription medications [592, 605] or toxicity from contaminants in supplements, including those derived from fish oils and other marine products [609-612]. The association of omega-3
polyunsaturated fatty acids, often obtained from supplemental fish oils, with primary and secondary cardiovascular disease and event prevention, reduced inflammation and pain in arthritic conditions, and other health benefits has been the subject of recent attention [613-615], although results of randomised controlled trials are inconsistent [616]. Publicity surrounding these benefits may be paralleled by increased use of these supplements in older men. Consequently, it is possible that recommended intakes of lipid-soluble vitamins A, D, and E may be exceeded.

In a recent calcium trial conducted in middle-aged and older men living in the Auckland region [573] we were surprised by the number of men who reported dietary supplement use in a baseline questionnaire of medication use. Therefore, in order to further characterise this use, we asked participants to complete a more detailed questionnaire at a later visit. The purpose of this study is to characterise dietary supplement use in men over 40 years enrolled in a calcium trial in Auckland, New Zealand. We sought to determine the number of different supplements taken, the number of tablets (or similar doses) taken each day and the estimated expenditure on these products. Because of a lack of data in this area, we also investigated the importance of various information sources and the reason for using each product.

### 8.3 Methods

#### 8.3.1 Participants

Men in the study were taking part in a two-year randomised controlled calcium supplement trial investigating skeletal and cardiovascular endpoints, the methods and results for which have been previously detailed [295, 573]. In summary, 909 men responded to newspaper advertisements seeking men over the age of 40 in good general health for a study of the effects of calcium supplementation on bone mineral density, cholesterol level and blood pressure. A total of 570 men returned study questionnaires and 323 were subsequently randomised to the trial. They were excluded if screening 25(OH)D was less than 25 nmol/L, serum creatinine was greater than 0.2 mmol/L, they had severe hypertension (systolic or diastolic blood pressure greater than 200 mmHg and 100 mmHg respectively), or another major ongoing disease, or they were on therapy for hyperlipidaemia, osteoporosis or taking vitamin D supplements at a dose greater than 1000 IU/day. At baseline, 35 men reported taking multivitamins [573]. The study was approved by the local ethics committee and registered with the Australian Clinical Trials Register (ACTRN012605000274673).
8.3.2 Baseline Measurements

Smoking history and a record of physical activity [562] were obtained at a screening visit prior to baseline. At baseline, height was measured in triplicate using a Harpenden stadiometer (Holtian Ltd., Crosswell, UK) and the median recorded, body mass using electronic scales. Blood pressure was measured using a Dinamap automatic monitor (Johnson & Johnson, Tampa, FL) at each visit, according to a well-established protocol which specifies arm position, sphygmomanometer cuff application, and a five minute period of rest before measurement. The device automatically takes three measurements over a three-minute period, during which time subjects remain undisturbed. Analysis of the data showed that the first recording of systolic and diastolic blood pressure was higher than the subsequent measurements (\( P < 0.001 \) for both), as we have noted in another cohort [560]. Therefore, the mean of the second and third readings was reported. Blood samples were collected and serum lipid measurements performed using a Roche Modular autoanalyser (Roche Diagnostics, Basel, Switzerland) as previously described [559]. Serum 25(OH)D was measured by radioimmunoassay (DiaSorin, Stillwater, MI, USA) in the first 212 men and by a chemiluminescent assay (Nichols, San Juan Capistrano, CA, USA) in the last 111 men. Assays were undertaken in a laboratory that participates in and meets the performance targets for the Vitamin D External Quality Assessment Scheme for both assays [563]. Results obtained using the Nichols assay were converted to DiaSorin results using the equation DiaSorin = Nichols x 0.75 + 5.6 as described previously [295]. Total body bone mineral density was measured using a Prodigy dual-energy x-ray absorptiometer (GE-Lunar, Madison, WI, USA). Dietary intake of nutrients from food with dietary supplements excluded was analysed from a 24-hour food diary using food composition data from the Foodfiles 2004© (Revision 18) database (New Zealand Institute for Crop and Food Research Ltd., Christchurch, New Zealand) accessed through FoodWorks Professional Edition 4 (Xyris Software (Australia) Pty. Ltd., Brisbane, Australia). Dietary analysis was also undertaken at the end of the study (24 months). Calcium intake was additionally assessed at baseline using an adapted version of a validated food frequency questionnaire [547].

8.3.3 Supplement Questionnaire & Analysis

Men were asked to complete a questionnaire describing their use of supplements, vitamins or similar, their reasons for taking each supplement and the importance of 11 listed and other participant-specified sources of information in their decision to take them (Appendix I). Importance was rated on a 5-point scale with 0 indicating that the source was of no importance in their decision and 4 indicating that it was very important. This questionnaire was completed between April, 2006 and July, 2007 when participants were in their last year of the study.
Baseline differences between men who were taking at least one supplement and those who were not were investigated using t-tests. The number of different supplements listed and the number of daily doses taken by each man was quantified. A single dose was defined as one tablet, or the recommended dose for liquid preparations. Men were asked to estimate the monthly cost of each supplement.

Intake from supplements of vitamins A, D and E were computed using information provided by manufacturers. Vitamin A was expressed as retinol activity equivalents (RAE), which equates to the amount of precursor or preformed vitamin A that must be consumed to equal 1 µg of retinol, using the following conversion formulae [608]:

\[
\text{Equation 1.} \\
\text{Retinol Activity Equivalent} = \text{International Units (from preformed retinol)} \times 0.3
\]

\[
\text{Equation 2.} \\
\text{Retinol Activity Equivalent} = \text{mg} \, \text{β-carotene} \times 83
\]

\[
\text{Equation 3.} \\
\text{Retinol Activity Equivalent} = \text{International Units (from β-carotene)} \times 0.05
\]

\[
\text{Equation 4.} \\
\text{Vitamin D (µg)} = \text{International Units} / 40
\]

\[
\text{Equation 5.} \\
\text{Vitamin E (mg d-α-tocopherol)} = \text{International Units} \times 0.67
\]

In the situation when the supplement listed preformed vitamin A (retinol) as the ingredient and international units (IU) were provided, equation 1 was used. The listed mass of the ingredient was only used if IU content was not listed due to ambiguity about whether it referred to that of retinol or its salt. In the situation when the supplement listed was β-carotene equation 2 was used or equation 3 when only IU were provided. Vitamin D and E were recorded as the listed mass when this was available or from calculated from IU using equations 4 and 5 respectively. For the purposes of this report, vitamin D has been recomputed to IU for consistency with other chapters of the thesis.

Statistical analyses were performed using SPSS version 15.0 (SPSS Inc. Chicago, IL, USA). Comparisons between age categories were made using ANOVA models with Tukey’s Least Significant Difference post-hoc tests. Errors are expressed as standard errors of the mean (SEMs) unless otherwise indicated.
8.4 Results

8.4.1 Participants and Supplement Use

A total of 305 men (94% of those randomised) returned the supplement questionnaire, and their general characteristics are shown in Table 8.1. Respondents did not differ from the original group in any of these variables. Almost half of respondents (142 men) reported taking supplements. Supplement users took $2.2 \pm 1.4$ (mean $\pm$ SD) different dietary supplements (median: 2; interquartile range: 1-3) or $2.8 \pm 2.2$ doses/day (median: 2; interquartile range: 1-2) and did not differ from those not taking supplements in any respect (Table 8.1). There was a tendency for supplement takers to have a higher dietary food intake of vitamin D ($P = 0.06$). Almost a third of users [42 (30%)] took more than two different supplements, and 10 (7%) took five or six. Only 16 (11%) of the men reported taking any supplements prescribed by their doctor and 4 (3%) took only prescribed supplements. Response to the question assessing the importance of various information sources in supplement-taking decisions was incomplete in returned questionnaires. Between 89 (63% of supplement users) and 103 (73%) responded to individual items within the question. In addition, four men who were not taking supplements answered some part of this question, but are not included in the analysis. Monthly cost data were also incomplete for 21 men who took supplements and, in these cases, were estimated from the cost of purchasing the same or similar products on the Internet. The monthly cost for those who took supplements was NZ$34.70 \pm 38.80$ (mean $\pm$ SD) and a quarter of men spent $45$ or more per month on supplements (median: $20$; lower quartile: $10$). Seventy-six men reported that they started taking these supplements prior to baseline, and 73 before the screening questionnaire.
<table>
<thead>
<tr>
<th>Use of Supplements*</th>
<th>Takers (n = 142)</th>
<th>Non-takers (n = 163)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>57.1 ± 10.5</td>
<td>56.2 ± 9.8</td>
</tr>
<tr>
<td><strong>Height (m)</strong></td>
<td>176 ± 7</td>
<td>177 ± 7</td>
</tr>
<tr>
<td><strong>Body mass (kg)</strong></td>
<td>82 ± 11</td>
<td>84 ± 13</td>
</tr>
<tr>
<td><strong>Fat mass (kg)</strong></td>
<td>19 ± 7</td>
<td>20 ± 8</td>
</tr>
<tr>
<td><strong>Lean mass (kg)</strong></td>
<td>59 ± 6</td>
<td>60 ± 7</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>26.3 ± 3.1</td>
<td>26.7 ± 3.5</td>
</tr>
<tr>
<td><strong>Smoking status (%)</strong></td>
<td>current</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>previous</td>
<td>46</td>
</tr>
<tr>
<td><strong>Physical activity (METS.hours/week)</strong></td>
<td>32 ± 5</td>
<td>32 ± 6</td>
</tr>
<tr>
<td><strong>25-Hydroxyvitamin D (nmol/L)</strong></td>
<td>96 ± 34</td>
<td>90 ± 32</td>
</tr>
<tr>
<td><strong>Albumin (mmol/L)</strong></td>
<td>43.6 ± 2.7</td>
<td>43.7 ± 2.6</td>
</tr>
<tr>
<td><strong>Creatinine (µmol/L)</strong></td>
<td>94 ± 12</td>
<td>94 ± 11</td>
</tr>
<tr>
<td><strong>High density lipoprotein cholesterol (mmol/L)</strong></td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.6 ± 0.9</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>128 ± 14</td>
<td>128 ± 14</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>77 ± 8</td>
<td>78 ± 8</td>
</tr>
<tr>
<td><strong>Total body bone mineral density (g/cm²)</strong></td>
<td>1.253 ± 0.008</td>
<td>1.258 ± 0.095</td>
</tr>
<tr>
<td><strong>Dietary intake (from food)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Energy (MJ/day)</strong></td>
<td>11.5 ± 11.9</td>
<td>10.6 ± 7.7</td>
</tr>
<tr>
<td><strong>Fat (g/day)</strong></td>
<td>96 ± 88</td>
<td>93 ± 71</td>
</tr>
<tr>
<td><strong>Protein (g/day)</strong></td>
<td>130 ± 292</td>
<td>114 ± 75</td>
</tr>
<tr>
<td><strong>Carbohydrate (g/day)</strong></td>
<td>330 ± 522</td>
<td>298 ± 317</td>
</tr>
<tr>
<td><strong>Calcium (mg/day)</strong></td>
<td>984 ± 529</td>
<td>923 ± 574</td>
</tr>
<tr>
<td><strong>Calcium from FFQ (mg/day)</strong></td>
<td>929 ± 481</td>
<td>857 ± 518</td>
</tr>
<tr>
<td><strong>Vitamin D (IU/day)</strong></td>
<td>134 ± 169</td>
<td>100 ± 126*</td>
</tr>
<tr>
<td><strong>Vitamin A (RAE</strong>*/day)**</td>
<td>1233 ± 969</td>
<td>1161 ± 1663</td>
</tr>
<tr>
<td><strong>Vitamin E (mg/day)</strong></td>
<td>13 ± 12</td>
<td>11 ± 9</td>
</tr>
</tbody>
</table>

Data are mean ± SD

* Response to questions asking whether men were currently taking prescription or non-prescription supplements, vitamins or similar.

† The difference between takers and non-takers of supplements was of borderline significance (P = 0.06)

‡ Calcium intake estimated from validated food frequency questionnaire [547]

** Retinol activity equivalent (RAE) is the amount of precursor or preformed vitamin A that must be consumed to equal 1 µg of retinol [608].
8.4.2 Distribution of Supplement Use by Age

To ascertain whether there were differences in supplement use across different ages, men were categorised into ten-year age groups. There were no age group differences in the proportion of men who took supplements (data not shown). Similarly, for those who took supplements, there were no age group differences in number taken, daily dose or monthly cost (Table 8.2).

Table 8.2 Age Group Comparisons of Supplement Use in Middle-Aged Men

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70+</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>33</td>
<td>48</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td>Number of different supplements listed</td>
<td>2.1 ± 0.2</td>
<td>1.9 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Daily dose of supplements</td>
<td>3.0 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Monthly cost of supplements (NZ$)</td>
<td>39.30 ± 7.40</td>
<td>33.80 ± 6.40</td>
<td>34.10 ± 5.20</td>
<td>30.70 ± 6.70</td>
</tr>
</tbody>
</table>

Data are mean ± SEM

8.4.3 Sources of Information

The information sources reported to be of greatest importance in decision making were scientific or medical publications, their own doctor and health professionals other than their doctor, which scored between 2.0 and 2.3 (Table 8.3). Magazines, news articles and people other than health professionals were also rated as moderately important (scoring between 1.7 and 1.8). There were age differences in the importance attributed to various sources. Men over 70 years attributed less importance to fitness establishments and health professionals other than doctors but more importance to people other than health professionals compared to younger groups of men (Table 8.3).
### Table 8.3 Importance or Sources of Information in Supplement-Taking Decisions*

<table>
<thead>
<tr>
<th>Source of Information</th>
<th>All supplement users</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>70+</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magazine articles</td>
<td>1.7 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>News articles</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>1.4 ± 0.3</td>
<td>1.6 ± 0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Advertisements (e.g. TV)</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Scientific or medical publications</td>
<td>2.3 ± 0.1</td>
<td>2.3 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>2.4 ± 0.3</td>
<td>1.3 ± 0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Internet</td>
<td>0.9 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Your own doctor</td>
<td>2.0 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>1.5 ± 1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Other health professionals</td>
<td>2.1 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>1.2 ± 1.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Gym, fitness centre, or health club</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 1.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Pharmacy</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>1.0 ± 1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Health food store</td>
<td>1.4 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>0.9 ± 1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>People other than health professionals</td>
<td>1.7 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.4</td>
<td>2.5 ± 1.5</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Respondents’ rating of importance from 0 = "of no importance" to 4 = “very important”
# Level of significance for difference between age categories.
Data are mean ± SEM

### 8.4.4 Types of Supplements & Reasons For Use

A total of 307 different products were taken. Almost half of the listed supplements (49%) were vitamins or minerals, either a single nutrient or combined multivitamin/multimineral preparation with or without other herbal ingredients. Nutritional oils such as evening primrose or fish oils (22%, with 13% specifically branded as fish oil derivatives), preparations of glucosamine, chondroitin and/or methylsulfonylmethane (13%), and individual herbal products (8%) were also commonly taken. Other products (totalling 8%) included bee pollen (3%), dietary fibre (1%) and co-enzyme Q10 (1%). One man reported taking a multivitamin/multimineral supplement formulated for horses. The most common reason provided for taking supplements was for non-specific health maintenance or prophylaxis (58% of the 286 listed reasons). Many men referred to treatment or relief of symptoms for specific indications (21% of reasons) or countering potential dietary deficiencies (16%), and a few cited athletic performance (2%) as the primary reason for use.
8.4.5 Vitamin Intake From Supplements

Daily intake of lipid soluble vitamins (A, D, and E) from supplements was calculated in the group of men who took any dietary supplement (n=142, Table 8.4).

Supplements containing vitamin A were taken by 80 men (56%). These men took 504 ± 645 RAE (mean ± SD) daily. Most men took preformed retinol, only 12 took supplements which contained β-carotene. Twelve men exceeded the US-determined requirement (Recommended Daily Allowance or RDA) of 900 RAE (two of these from β-carotene). Only one man, who was taking two halibut liver oil tablets per day, equalled the tolerable upper intake level (UL) of 3000 RAE from supplements alone.

Eight-five men (60%) took 226 ± 363 IU (mean ± SD) Vitamin D daily. Eleven men took more than 400 IU per day, and three took more than 1000 IU. These men achieved this by taking multiple doses recommended by the manufacturers of two US brands, which are distributed through network marketing.

Vitamin E-containing supplements were taken by 67 men (47%). Of those who took them, daily intake was 82 ± 138 mg. All except eight men exceeded the US RDA, but none exceeded the UL.

Table 8.4 Daily Vitamin Intakes from Nutritional Supplements in Men Who Took Any Supplement (n=142)

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>75th Percentile</th>
<th>Maximum</th>
<th>US RDA* or AI</th>
<th>US UL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A (RAE)</td>
<td>284 ± 544</td>
<td>9</td>
<td>364</td>
<td>3000</td>
<td>900</td>
<td>3000</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>136 ± 302</td>
<td>5</td>
<td>200</td>
<td>2400</td>
<td>200-400</td>
<td>2000</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>39 ± 103</td>
<td>0</td>
<td>38</td>
<td>826</td>
<td>15</td>
<td>1000</td>
</tr>
</tbody>
</table>

* The US recommended daily allowance (RDA) is defined as the level of an intake sufficient to meet the nutrient need of almost all healthy men in this age group. Insufficient data is judged to exist to set this level for vitamin D. Instead, an adequate intake (AI), defined as the approximate average nutrient intake that appears to sustain a desired indicator of health, has been set. The tolerable upper intake level (UL) is defined as the maximum daily intake by an individual that is unlikely to pose risks of adverse health effects to almost all men in this age group [608].

β Retinol activity equivalent (RAE) is the amount of precursor or preformed vitamin A that must be consumed to equal 1 µg of retinol [608].
8.4.6 Vitamin Intake From Food & Supplements Combined (Table 8.5)

In order to determine the number of men whose total daily intake of vitamins A, D and E were above recommended levels, daily nutrient intake calculated from the 24-month diet recall were combined with nutrient intake from supplements, for those men who took any dietary supplement (Table 8.5).

Over half of the men (60%) had a total intake of vitamin A from food and supplements in excess of the US RDA. Because high intakes of β-carotene are not toxic, the UL for vitamin A (3000 ug) applies to intake of preformed retinol only. Two men exceeded this level of retinol intake. One, the same individual as above, combined the two halibut liver oil tablets with daily retinol intakes from food of 340-530 µg, and the other took supplements manufactured by a US company that are normally distributed in New Zealand through personal contacts.

For vitamin D, one quarter of men exceeded a daily intake of 400 IU vitamin D from food and supplements, although only one exceeded a daily intake of 2000 IU.

More than half (55%) exceeded the US daily requirement (RDA) for vitamin E from food and supplements, although none exceeded the UL.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>75th Percentile</th>
<th>Maximum</th>
<th>US RDA* or Al</th>
<th>US UL*</th>
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</thead>
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<tr>
<td>Vitamin A (RAE)</td>
<td>1328 ± 938</td>
<td>1123</td>
<td>1826</td>
<td>4482</td>
<td>900</td>
<td>none set</td>
</tr>
<tr>
<td>Retinol (ug)</td>
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<td>356</td>
<td>679</td>
<td>3528</td>
<td>none set</td>
<td>3000</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>237 ± 329</td>
<td>113</td>
<td>402</td>
<td>2464</td>
<td>200-400</td>
<td>2000</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>51 ± 104</td>
<td>17</td>
<td>53</td>
<td>839</td>
<td>15</td>
<td>1000</td>
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</tbody>
</table>

* The US recommended daily allowance (RDA) is defined as the level of an intake sufficient to meet the nutrient need of almost all healthy men in this age group. Insufficient data is judged to exist to set this level for vitamin D. Instead, an adequate intake (AI), defined as the approximate average nutrient intake that appears to sustain a desired indicator of health, has been set. The tolerable upper intake level (UL) is defined as the maximum daily intake by an individual that is unlikely to pose risks of adverse health effects to almost all men in this age group [608].

Retinol activity equivalent (RAE) is the amount of precursor or preformed vitamin A that must be consumed to equal 1 µg of retinol [608].
8.5 Discussion

This investigation provides evidence that many older New Zealand men take dietary supplements. Almost half (47%) of men reported taking dietary supplements. Although, this proportion cannot be extrapolated to the population as a whole, since recruitment was not random, it is wholly consistent with data from a large, recent US national survey, in which an identical proportion of men in reported taking a dietary supplement [591]. Because the present study recruited men specifically for a calcium trial, this cohort may have a more favourable than average view of dietary supplementation, and there might consequently be an overestimation of overall prevalence. On the other hand, dietary supplements are often taken irregularly [594, 617], and surveying at a single evaluation point may have resulted in an underestimation of the numbers of men who use them.

In New Zealand, a crown entity (the Pharmaceutical Management Agency of New Zealand or PHARMAC) manages the schedule of subsidised pharmaceuticals. Subsidy applies to a single multivitamin containing vitamins A, B1, B2, B3, C and D, although various subsidised vitamins and minerals may be prescribed individually. Other multivitamin/multimineral products must be purchased over the counter from pharmacies, health food stores, supermarkets, through network marketing schemes or on the Internet. From the present data, it is evident that many men spend substantial sums of money on these products. A quarter of men spent NZ$45 or more each month on these products. Although we do not have individual income data available, this figure represents approximately 2% of the average monthly net income in New Zealand [618].

By far the most commonly used supplements were multivitamin/multiminerals, followed by nutritional oils, over half of which were fish-derived, and glucosamine/chondroitin preparations. When supplement and food intake were combined, over half the men who took supplements exceeded the US requirement for vitamins A and E. Whilst approximately a quarter also exceeded recommendations for vitamin D, recent reports suggest that reevaluation of these 1997 guidelines are necessary [619, 620] and some estimates suggest that daily vitamin D intakes between 700 IU – 1120 IU are necessary to raise levels of 25-hydroxyvitamin D in almost all health adults to ≥50 nmol/L [621, 622].

In general, recommended levels of intake of vitamins and minerals are based on available evidence. Thus, by definition, there is no demonstrated benefit for healthy individuals taking levels of these nutrients in excess of recommendations. Furthermore, there is minimal evidence
for specific benefits of vitamin supplementation in the literature. Meta-analyses of antioxidant interventions (including vitamins A, C and E) for prevention of cancers, particularly gastrointestinal, cognitive impairment and asthma report inconsistent results and no overall effect [623-626]. They may increase mortality [627, 628]. The effects of glucosamine and/or chondroitin are also equivocal. Recent systematic reviews of randomised trials report an effect of glucosamine sulphate, but not other salts, on osteoarthritic pain and disease progression, with most of the existing data for knee osteoarthritis [629-632]. However, effects are considerably greater for trials that are industrially funded, particularly those of a single manufacturer [629]. In contrast, there may be some justification for omega-3 fatty acid supplementation supplied by fish oils in middle-aged men. There is growing evidence of an effect of omega-3 fatty acids on cardiovascular risk, although evidence is conflicting for the numerous other reported benefits of fish oils [614, 633].

Previous studies which combine food and dietary supplement consumption have noted nutrient intakes in excess of tolerable upper intake levels (UL) for a range of nutrients, including vitamins B3, C, iron, magnesium and zinc [606, 607]. The current data raise the concern that high doses of multivitamins or fish-liver oil products may result in potentially harmful intakes of pre-formed vitamin A or other fat soluble vitamins. It is difficult to estimate the content of naturally occurring vitamins in fish oil supplements and manufacturers do not ordinarily report it. For levels of vitamin D in natural fish oils, the indications are that whilst they are low, they are highly variable [506, 634]. Another cause of potentially high intakes are particular supplement brands, distributed by network marketing in New Zealand, which recommend multiple daily doses of tablets. For a few individuals here, their use was associated with sizeable intakes of vitamins A and D. While there is a possibility of harm from these high intakes, the tolerable upper intake levels (UL) set for these nutrients have a substantial safety margin built in to them, and in both cases they have been criticised for being too low [419, 635]. Therefore few, if any, men here were had intakes of vitamin A, D, or E that would be likely to put them at risk.

Although men in the present study claimed that information from scientific or medical publications, their own doctor and other health professionals were the most important factors in their decisions about supplement use, it is notable that few products were actually prescribed by a physician. Past studies report that individuals are unlikely to seek medical advice or inform their doctors of their use of dietary supplements [603, 636]. It seems likely that men here overemphasised the importance of sources which they felt might be the most acceptable to the study investigators.
Regulations prevent dietary supplements being marketed as treatment or alleviation of symptoms of specific medical conditions. It is therefore not surprising that most men noted non-specific prophylactic reasons for taking them. Compensating for possible dietary insufficiencies may also be a justifiable reason, particularly for vitamin D, since data from a national nutrition survey show that 44% of New Zealand men ≥45 years are unable to maintain 25(OH)D levels of >50 nmol/L [121]. The dietary intake of 116 IU/day found here for vitamin D is consistent with expectations since few foods naturally contain more than trace amounts of vitamin D [33] and, in New Zealand, fortification with the nutrient is rare. However, from our data, there is no evidence that men whose food intake was low in vitamins A or E were more likely to take supplements. No differences between supplement users and non-users in baseline dietary food intakes of vitamins A or E were noted here. Moreover, we observed a trend for greater vitamin D intake from food in those men who took dietary supplements compared with those who did not. Previous studies have also found that supplement use is associated with adequate nutrient intakes from food, or higher intakes of some nutrients than for non-users [602, 607]. Over one fifth of reasons for taking a supplement cited symptom alleviation or treatment for an existing specific medical condition. Associations between dietary supplement use and the existence of various medical conditions have been noted previously in a large US postal survey [637]. Nonetheless, given the lack of evidence of a benefit arising from almost any ingredient in these products, it is alarming that so many men in the present study took dietary supplements for this reason.

There is a paucity of data regarding dietary supplement use either in Australasia or more generally in middle-aged and older men. The present results shed some light on the issue, though a more extensive survey in New Zealand is warranted. Of those men volunteering to take part in a clinical trial of calcium supplementation, we note that many reported spending substantial amounts of money on dietary supplements. This is despite a lack of evidence supporting benefits of their use, and some evidence of associated risk. As a whole, this cohort purported to be most strongly influenced by health professionals and the results of scientific studies with respect to their decision to take supplements. Although these factors may not have ultimately driven men’s behaviour, their high ratings suggest that these sources of information are acknowledged to some extent. So that sound and convincing advice may be provided, it is important that health professionals maintain up to date knowledge of available dietary supplements, evidence surrounding their use as well as common claims made by their manufacturers and suppliers.
CHAPTER 9: CONCLUSIONS

This thesis set out to investigate a number of research questions relating to the determinants, optimal levels, and supplementation of vitamin D. One of the key aims was to determine a threshold level of 25-hydroxvitamin D [25(OH)D] at and above which secondarily elevated parathyroid hormone (PTH) is minimised. This question was addressed in young women, for whom few data exist, and in frail elderly individuals, who remain an important group due to the severe consequences of poor bone health. Though some researchers have recently concluded that levels in excess of 75 nmol/L are optimal [42], data within this thesis somewhat temper this message. Cross-sectional data examining the relationship between 25(OH)D and PTH in two cohorts of young Chinese women (Chapters 3 and 4) and in frail elderly New Zealanders (Chapter 5) suggest that there is little further drop in PTH with 25(OH)D levels greater than 40 – 60 nmol/L. Interpretation of the Chinese data should be applied cautiously because of the lack of individuals with baseline levels >50 nmol/L.

Addressing the question of optimal levels of 25(OH)D using cross-sectional data is a commonly employed paradigm, but limited due to substantial variability in individual levels of PTH at a certain level of 25(OH)D. Much of this variability may be reduced using a longitudinal approach in which vitamin D supplementation raises 25(OH)D in each individual, and the corresponding effect on PTH levels are monitored in relation to baseline 25(OH)D. Despite the plethora of cross-sectional data, to the knowledge of the author, only three previous studies have addressed this question longitudinally [17, 130, 131]. Although all three of these studies have arrived at relatively low estimates of optimal levels ~50 nmol/L, their conclusions have seldom been singled out as more pertinent given their superior research design.

In this thesis, we also employed a longitudinal approach to address the question of optimal 25(OH)D in the studies described in Chapters 3 and 5. In Chapter 3, no relationship at all between baseline 25(OH)D and changes in PTH was evident in the 221 young Hong Kong women studied. Because of the modest dose of vitamin D (200 IU/day) and possibly to falling compliance, which was 75% at 1 month in the original milk product intervention [532], the observed 3-month 25(OH)D response in treated women was small and not different from that of the control group. The lack of a relationship between baseline 25(OH)D and post-supplementation PTH reduction was very likely to be due to the small changes in 25(OH)D and hampered an attempt to ascertain optimal levels of 25(OH)D longitudinally. In the study of frail elderly, described in Chapter 5, the effects of three different dosing regimens were investigated. Consequently, 25(OH)D increments
from baseline were also inconsistent between dosing groups. This problem was solved by restricting the longitudinal analysis of the relationship between baseline 25(OH)D and PTH change to visits at which the change in 25(OH)D from baseline was 20 nmol/L or greater. Performing the analysis in this way resulted in an estimate of optimal 25(OH)D of ~50 nmol/L, identical to the results of the three previous longitudinal studies addressing this question [17, 130, 131]. Similar analyses of changes in the bone formation marker procollagen type I amino-terminal propeptide (P1NP) in relation to baseline 25(OH)D, also presented in the Chapter 3 study, suggested that slightly lower levels of ~40 nmol/L might be sufficient.

A secondary aim of the studies described in Chapters 4 and 5 was to investigate determinants of 25(OH)D and potential modulators of the relationship between 25(OH)D and PTH. Because changes in 25(OH)D may have a direct effect on PTH levels, but not the reverse, variables that alter the relationship between the two variables are likely to be ones that are associated with PTH independently of 25(OH)D. In the study of two cohorts of Chinese women (Chapter 4), inverse associations were noted for both body mass and percent body fat with 25(OH)D, suggesting that both adiposity and lean tissue may be associated with vitamin D status in this group of lean, young women. In contrast, there was no evidence that either indices of adiposity or calcium intake modulated the cross-sectional relationship between 25(OH)D and PTH. Therefore, the possibility that either levels of body fat or dietary calcium consumption might influence the estimation of optimal 25(OH)D levels using this relationship were not substantiated in this group. On the contrary, in the study of frail, elderly New Zealanders presented in Chapter 5, data suggest that optimal 25(OH)D levels, estimated from longitudinal assessment of the relationship between baseline 25(OH)D and change in PTH, might be lower when calcium intake is high (>1552 mg/day), and may be influenced by renal function or body composition.

Although optimal levels of 25(OH)D and its determinants were investigated in the study described in Chapter 5, the main research outcome of this study was to provide much needed data on the time-course of responses to high-dose vitamin D₃ supplementation. Vitamin D is frequently prescribed to frail elderly people, but prior to this study, there were a scarcity of data informing decisions about safe and effective use of high-dose vitamin D₃ supplements in this group. The study reported the effects of three dose regimens including supplementation with a loading dose (500 000 IU), monthly dosing (50 000 IU), or a combination of the two. Loading doses may be useful for frail elderly people likely to have poor vitamin D status, for whom compliance with more frequent dosing may be difficult, and for whom fractures are common and can be devastating. The large loading doses (equivalent to 10 x 50 000 IU of the Cal.D.Forte
tablets available in New Zealand) were found to rapidly normalise 25(OH)D levels, with no adverse effect on serum calcium levels. Monthly dosing was also effective and safe, with respect to hypercalcaemia, but it took 3 – 5 months to reach a plateau. These data also provided evidence that vitamin D supplementation is effective at lowering PTH when renal function is impaired and in overweight individuals.

A growing body of data suggest that suboptimal vitamin D status increases the risk of a range of chronic conditions and infectious illnesses [33, 175, 262]. Notwithstanding, almost all of the evidence is cross-sectional and much of it is circumstantial, based on seasonal or geographical differences in disease prevalence. Correlates of vitamin D status such as poor health, low levels of physical activity, obesity, or nutritional deficiencies might confound effects on long-term health outcomes. Adverse events, recorded as part of a large 5-year calcium intervention in postmenopausal women, presented an opportunity to investigate prospectively the effect of baseline 25(OH)D levels on these outcomes. This research is described in Chapter 6. Baseline data confirmed that women with low vitamin D status (season-adjusted 25(OH)D levels < 50 nmol/L) differed in many important respects from their counterparts with levels ≥50 nmol/L. Prospectively, women with season-adjusted 25(OH)D levels < 50 nmol/L had a greater risk of stroke and of a composite event (stroke, myocardial infarction or sudden death). When further adjustment was made for relevant confounders (age, fat mass, current smoking, physical activity, and prior history of cardiovascular disease-related conditions), the effect of vitamin D status on these outcomes disappeared. These results suggest that the purported association of vitamin D status with these outcomes might arise indirectly through the effect of 25(OH)D correlates. Paradoxically, low vitamin D status was also associated with a reduced risk of breast cancer, which remained significant following similar adjustments. The reasons for this association are unclear and warrant further investigation.

Another important aim of this thesis was to characterise populations of vitamin D deficient or insufficient individuals. Within the cohorts of otherwise healthy Chinese women of childbearing age described in Chapters 3 and 4, the prevalence and degree of low vitamin D status were startling. In both Hong Kong and Beijing, 94% of participants had baseline 25(OH)D < 50 nmol/L. In Beijing, almost a half (45%) were also below 25 nmol/L compared with 18% in Hong Kong. The study described in Chapter 7 primarily aimed to describe the group of individuals in the Auckland region of New Zealand who were screened for and detected with vitamin D deficiency [season-adjusted 25(OH)D < 25 nmol/L] or insufficiency [season-adjusted 25(OH)D between 25 and 50 nmol/L]. Data here confirmed that vitamin D insufficiency and deficiency
were common amongst certain groups. In particular, young Māori and Pacific women and children and adults of Middle Eastern, Southern Asian and African ethnicity appeared to be over-represented demographics. A related, subsidiary aim was to compare rates of detection of vitamin D deficiency or insufficiency, relative to the number of individuals screened, between hospital- and community-based doctors. Although the location of the medical referral made no difference to the rates of detection across age and ethnicity groups, detection rates were relatively low (58%) in men and women ≥50 years and very high (89%) amongst those of Pacific Island, Indian, Middle Eastern or African ethnicity. These results suggest that screening based on physical examination and patient history is ineffective at predicting low vitamin D status in older adults but very effective amongst minority ethnic groups. These results concur with experiences of collecting data for the study described in Chapter 5. Whilst the original intention of this study was to select frail hospitalised elderly who were likely to have a low vitamin D status, the observed mean level of 58 nmol/L was much higher than anticipated and differed little from levels in independently living adults of the same age group in the New Zealand population [121].

Although often disregarded, the role of dietary supplement use amongst middle-aged and older New Zealand men could contribute importantly to vitamin D status. The penultimate chapter of this thesis (Chapter 8) surveyed the nature and cost of, reasons for use, and sources of information about prescription and non-prescription supplements taken by a cohort of men participating in a calcium intervention. In addition, dietary intake of lipid-soluble vitamins A, D and E from food and supplements were calculated to determine whether any men were consuming higher than recommended levels of these vitamins. Data from this survey revealed that almost half of men (47%) participating in the trial took a wide range of other mainly non-prescribed supplements and spent $35 ± $39 (mean ± SD) on these. Though few, if any, men who took dietary supplements, consumed levels of vitamins A, D or E likely to put them at risk of toxicity, over half (55 – 60%) exceeded daily requirements for vitamins A and E. Though only a quarter of men taking dietary supplements took in excess of 400 IU vitamin D daily, New Zealand has few foods fortified with vitamin D and intake from supplements is very likely to be a relatively important determinant of vitamin D status in middle-aged and older men.

Following from this thesis, there are still a number of important unanswered questions. There is still more work to be done investigating determinants of circulating PTH and its response to vitamin D supplementation. Larger longitudinal investigations are required and could help shed light on inconsistencies in the literature regarding relationships between vitamin D status and skeletal outcomes or skeletal responses to vitamin D intervention.
More data from well designed long-term randomised controlled interventions of vitamin D on non-skeletal outcomes could settle the question whether vitamin D lowers the risk of the many disease outcomes that it has been associated with. Although, the recently published large Women’s Health Initiative data, has not shown an effect on cancer risk [248], trials of a dose of vitamin D$_3$ greater than 400 IU/day and analysed in relation to starting levels of 25(OH)D might elicit different results. Documentation of adverse events in such trials would also be crucial in determining the possibility of increased risk of harm.

Data presented in this thesis show that whilst a relatively low proportion of European adults (<60%) who are screened for vitamin D insufficiency are detected as being insufficient, the proportion is very high (close to 90%) in some demographic groups. Vitamin D screening is costly to the public purse, compared to vitamin D interventions which are relatively inexpensive, and may be unnecessary for groups in which the rates of detection are so high. More research is required to investigate specific self-reported dietary, behavioural and skin-type-related determinants of vitamin D status in mixed-ethnicity/age populations in order to provide non-invasive predictors or vitamin D status which could indicate treatment without requirement for blood screening. Ideally such studies would be conducted over the course of a year to correct for seasonal differences in predictors and vitamin D status. They would also provide data from countries in which food fortification with vitamin D is common, and those, such as New Zealand, where it is not. In addition to self reports of these variables, if such studies were also to include quantification of variables such as level of skin pigmentation (reflectance measures), time spent outdoors, sun-seeking or avoidance behaviour, clothing cover and sun-screen use, then more precise recommendations regarding effective and safe UV exposure could be provided.

In summary, research presented in this thesis has contributed to the field of knowledge in a number of key ways. Firstly, evidence from studies in frail elderly and young Chinese women suggest that optimal vitamin D status is lower (~50 nmol/L) than some recent recommendations. Secondly, data are presented that indicated safe and effective use of large loading doses and routine monthly high-dose vitamin D supplementation in populations likely to be at risk of deficiency. Thirdly, thesis data reveal that many people in the Auckland region who are not elderly Europeans are vitamin D deficient. Detection rates in some groups are high enough to suggest that broader supplementation interventions are required. Future research to clearly establish prevalence and causes of vitamin D deficiency in these groups is indicated.
APPENDIX I

Dietary Supplement Use Questionnaire
THE EFFECT OF CALCIUM SUPPLEMENTATION ON LIPIDS AND BONE DENSITY IN MEN

Participant Number ___________________________  Today’s Date ___________________________

Surname: ___________________________  First name(s): ___________________________

1. Are you currently taking any supplements, vitamins or similar that were prescribed by your doctor?  YES ☐  NO ☐

2. Are you currently taking any supplements, vitamins or similar that were not prescribed by your doctor?  YES ☐  NO ☐

If you answered ‘NO’ to both questions above, then there are no more questions.
If you answered ‘YES’ to either question, please complete Question 3 below and provide details in the table overleaf:

3. Please indicate the importance of the following sources of information in your decision to use supplements, vitamins or similar.

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<th>SOURCE OF INFORMATION</th>
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<td>Brand Name</td>
<td>Dose Specify whether daily or weekly</td>
<td>Where Purchased Health Food Store, Supermarket etc.</td>
<td>Approximate Monthly Cost</td>
<td>Approximate Date Started Just year is okay</td>
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<td>e.g. 3 tablets / week(average)</td>
<td>e.g. Supermarket (Pak N Save)</td>
<td>e.g. $6</td>
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