Community perspectives on vitamin D and bone health in three at-risk populations

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# Contents

Abstract .......................................................................................................................... 4  
Declaration ...................................................................................................................... 6  
Statement regarding contributions to jointly authored papers ........................................ 7  
Introduction ................................................................................................................... 10  
Literature Review .......................................................................................................... 12  
  Vitamin D: Physiology & Metabolism ........................................................................... 12  
  Insufficiency & Deficiency .......................................................................................... 17  
  Bone and muscle .......................................................................................................... 17  
  Neoplasia ...................................................................................................................... 23  
  Cardiovascular disease ............................................................................................... 25  
  Diabetes ......................................................................................................................... 28  
  Respiratory disorders ................................................................................................. 32  
Appropriate intake and target levels of vitamin D ......................................................... 34  
Drugs and vitamin D ...................................................................................................... 38  
Intellectual disability and bone health .......................................................................... 43  
Bone Structure & Function ............................................................................................ 45  
  Anatomy & Structural Organisation ........................................................................... 45  
  Bone modelling and remodelling .............................................................................. 51  
  Growth and Ageing ...................................................................................................... 52  
  Genetic determinants of bone health ......................................................................... 54  
Polyunsaturated fatty acids and bone .......................................................................... 56  
Vitamin D and Aboriginal Australians ............................................................................ 58  
Paper 1: “Vitamin D and fractures in people with intellectual disability” ....................... 60  
Paper 2: “Vitamin D insufficiency in Aboriginal Australians” ....................................... 70  
Paper 3: “Efficacy and tolerability of calcium, vitamin D and a plant-based omega-3 oil for osteopenia: A pilot RCT” ................................................................. 78
Conclusion .................................................................................................................. 86
Bibliography ............................................................................................................. 89
Abstract

Background:
Disorders affecting bone health, including osteoporosis and fractures, cause significant morbidity and mortality in Australia. Specific sub-groups within the general population are at increased risk of poor bone health and fracture. Such groups include people with intellectual disability, Aboriginal Australians and people known to have osteopenia. These studies aim to document the extent of this increase in risk, examine the underlying reasons and evaluate possible treatment options.

Methods:
Three studies are described:

a) A 5 year retrospective audit of 280 individuals with intellectual disability examined data including age, gender, mobility, dietary status, incident fractures, medications and 25-hydroxyvitamin D (25D) levels, as well as response to vitamin D supplementation.

b) A cross-sectional study of 58 South Australian Aboriginal people investigating the adequacy of vitamin D status and the relationship between serum 25D levels and biochemical variables of calcium and bone mineral homeostasis.

c) A prospective, randomised, placebo-controlled pilot study of the efficacy, acceptability and tolerability of docosahexanoic acid (DHA) supplementation in addition to calcium and vitamin D₃ in 40 individuals with osteopenia.

Results:

a) 57% of intellectually disabled individuals tested were vitamin D insufficient. Vitamin D insufficiency was strongly correlated with reduced mobility (p<0.001) and difficulty consuming solids (p<0.001). The correlation between 25D levels and fractures was not significant (p = 0.3). Oral supplementation using vitamin D₃ 100,000 IU every 4 months was effective in correcting vitamin D insufficiency. 68 fractures occurred over the audit period in 52 individuals, a rate of 1 fracture every 23.8 person years. Peripheral fractures accounted for 54% of all fractures, being particularly prevalent in the most mobile individuals.

b) Serum 25D levels varied seasonally in South Australian Aboriginal people, being higher in summer (P < 0.001). The overall mean of 56.8 nmol/L (SD, 22.1) is below the
recommended target level of 60 nmol/L. Serum 25D levels correlated significantly with c-terminal telopeptide (CTx) \( (P = 0.03) \), but not with age, body mass index, levels of fasting glucose or PTH. BMI and PTH levels were significantly correlated with each other \( (P = 0.001) \).

c) CTx was suppressed after 12 months for all osteopenic participants \( (p=0.04) \) with no difference in effect size between DHA and control groups \( (p=0.53) \). Changes in CTx at 12 months were significantly correlated with changes in bone density at the lumbar spine \( (p=0.01) \) and total proximal femur (TPF) \( (p=0.03) \). Participants rated the supplements as tolerable and acceptable, with few adverse events.

**Conclusions:**

a) Fractures are common in people with intellectual disability. Vitamin D insufficiency may contribute to this increased risk, although this study did not conclusively establish this. Oral vitamin D\(_3\) supplementation is effective in restoring normal vitamin D levels.

b) Vitamin D insufficiency is highly prevalent in adult Aboriginal Australians, with low mean values found in all seasons other than summer.

c) The combination of oral calcium, vitamin D\(_3\) and DHA was safe, tolerable and acceptable when used for 12 months by osteopenic individuals. Both combinations (i.e. calcium, vitamin D\(_3\) and DHA; and calcium, vitamin D\(_3\) and placebo) had a positive effect on bone health, with no significant effect from the addition of DHA.
Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to the thesis, when deposited in the University library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue, the Australian Digital Thesis Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time. The publications contained within this thesis are as follows:


Signature: ________________________________________________

Date: 01 October 2012
Statement regarding contributions to jointly authored papers

Vitamin D & fractures in people with intellectual disability

Co-author: Dr Michael Nugent
Contribution: Dr Nugent was employed as a medical officer at the Strathmont Centre, a residential facility caring for adults with intellectual disability. I approached Dr Nugent to initiate this study, having worked as a locum tenens at the Centre. I planned the study and took the lead role in obtaining ethical approval, followed by data transcription, analysis and manuscript preparation/submission. Dr Nugent provided comments regarding study design, assisted with recruitment, data collection and analysis, and suggested modifications to the manuscript prior to publication. Without his active participation this study would not have been possible.

I give my permission for the inclusion of the above-named paper in this doctoral thesis.

Signature of co-author: ________________    ______(M. Nugent)
Vitamin D insufficiency in Australian Aboriginals

Co-authors: Prof. Jonathan Newbury, Prof. Howard Morris, Assoc. Prof. Alan Crockett

Contributions: these co-authors are also my PhD supervisors. All have extensive experience in the design, running and reporting of clinical trials. In addition, Prof. Morris is recognised internationally as an expert in the vitamin D field. I initiated this study and proposed the study design, upon which they provided comment. I was responsible for obtaining ethics approval (which included community consultation & engagement), participant recruitment and data collection. I took the lead role in data analysis and manuscript preparation/submission, but received significant support and advice from all three supervisors, particularly Prof. Morris, as this topic fell within his area of specific expertise.

We give our permission for the inclusion of the above-named paper in this doctoral thesis.

Signature of co-author: __________________ (J. Newbury)

Signature of co-author: ____________ (H. Morris)

Signature of co-author: ____________ (A. Crockett)
Efficacy & tolerability of calcium, vitamin D and a plant-based omega – 3 oil for osteopenia: a pilot RCT

Co-author: Dr Karin Ried
Contributions: Dr Ried has a major interest in nutritional and complementary therapies, and I approached her to be my co-investigator because of this expertise. I proposed the study design, upon which she provided comment. I was primarily responsible for obtaining ethics approval and negotiating practical matters such as supply of investigational agents and participant recruitment. Participant research visits, data collection and data analysis were shared. I took the lead role in manuscript preparation/submission, with significant comments, suggestions and revisions being made by Dr Ried.

I give my permission for the inclusion of the above-named paper in this doctoral thesis.

Signature of co-author: _________________________________(K. Ried)
Introduction

The first decade of the 21st century was designated by the United Nations and the World Health Organization as the Decade of Bone and Joint Disease (1). The Decade was initiated to focus attention on the growing burden of musculoskeletal diseases occurring worldwide as the population of the global village ages. This is particularly true in developed countries, but is likely to become a matter of growing importance in developing countries as life expectancy increase. Over 60 countries signed up to the Decade, including Australia, and declarations of support were also provided by the United Nations and the World Health Organization.

The Bone and Joint Decade had four major aims:

- to raise awareness of the growing burden of musculoskeletal disorders on society;
- to promote prevention of musculoskeletal disorders and to empower patients through education campaigns;
- to advance research on prevention, diagnosis and treatment of musculoskeletal disorders; and
- to improve diagnosis and treatment of musculoskeletal disorders.

The Decade also focused internationally on five areas of musculoskeletal problems — osteoporosis, osteoarthritis, rheumatoid arthritis, back pain and musculoskeletal trauma. Osteoporosis affects nearly two million Australians and is responsible for nearly two billion dollars in direct costs each year (2). Another aim of the Decade was to empower patients and the community to take a more proactive role in preventing and self-managing musculoskeletal complaints.

A report issued in April 2002 estimated that about 1% of the Australian population has an intellectual disability (3). Three quarters are classified as having mild intellectual disability, the remainder being classified as either moderate, severe or profound. The diagnosis requires significantly below average intelligence (IQ of 70 or less) and inadequate personal skills when compared with other people of the same age and culture. People with intellectual disability are likely to require institutional care, although many still live in the community (3). They may thus be regarded as something of a ‘hidden’ group within the general population. There is evidence
that health problems in the intellectually disabled often go unrecognised (4), probably due to difficulties with memory and communication (5).

Little is known about the vitamin D status of Aboriginal Australians, who comprise about 2.5% of the total Australian population (6). Melanin filters incident UVB light, such that darker-skinned individuals synthesise less vitamin D (7), and so tend to have lower 25D levels than paler-skinned individuals when experiencing the same level of sun exposure (8, 9). The vitamin D receptor (VDR) is virtually ubiquitous, and insufficient vitamin D levels and/or VDR genotype have been implicated in a wide range of health problems (10-15). These problems include diabetes (16, 17), lung disease (11), some cancers (18), and cardiovascular disease (19), all of which are highly prevalent in Aboriginal Australians (20). It is possible that vitamin D insufficiency may play a role in the aetiology of these conditions, suggesting that that correction of insufficiency may have a preventive and/or therapeutic role.

Polyunsaturated fatty acids (PUFAs) are obtained mainly from fish and plants in the human diet (21). A diet rich in omega – 3 fatty acids may have beneficial effects in a wide range of normal developmental processes and disease states (22). Many individuals may be interested to maintain and improve their health status using a dietary approach, but as yet the evidence relating to bone health and PUFAs is incomplete and inconclusive (23).

It is therefore both timely and important to specifically examine the role of vitamin D in the health of these different population groups. This thesis presents a review of the literature regarding vitamin D and bone health, with special reference to people from these groups. Studies reported include one concerning vitamin D and fractures in an intellectually disabled population, one concerning vitamin D in Australian Aboriginals, and a third concerning the role of vitamin D, calcium and an omega – 3 oil in people with osteopenia. Recommendations are made for further study and clinical interventions.
**Literature Review**

**Vitamin D: Physiology & Metabolism**

Although the effects of vitamin D deficiency have probably been recognised since antiquity, the first scientific description of a vitamin D deficiency, namely rickets, was provided in the 1645 by Whistler (24). The major breakthroughs in understanding the causation of rickets occurred during the period 1910 – 1930, with the development of nutrition as an experimental science and the appreciation of the existence of vitamins.

It is in one sense an historical accident that vitamin D was classified as a vitamin. In 1919 Sir Edward Mellanby, working with dogs raised exclusively indoors (i.e. in the absence of sunlight and hence ultraviolet light), devised an oatmeal diet that allowed him to unequivocally establish that the bone disease, rickets, was caused by a deficiency of a trace component present in the diet (25). In 1921 he wrote, "The action of fats in rickets is due to a vitamin or accessory food factor which they contain, probably identical with the fat-soluble vitamin." Furthermore, he established that cod liver oil was an excellent antirachitic agent (26).

Shortly thereafter McCollum and associates observed that by bubbling oxygen through a preparation of the "fat-soluble vitamin" they were able to distinguish between vitamin A (which was thus inactivated) and vitamin D (which retained its activity) (27). In 1923 Goldblatt and Siamese clearly identified that when a precursor of vitamin D in the skin (7-dehydrocholesterol) was irradiated with sunlight or ultraviolet light, a substance equivalent to the fat-soluble vitamin was produced (28). Hess and Weinstock verified the assertion that "light equals vitamin D" (29). They excised a small portion of skin, irradiated it with ultraviolet light, and then fed it to groups of rachitic rats. The skin that had been irradiated provided an absolute protection against rickets, whereas the unirradiated skin provided no protection whatsoever; clearly, the irradiated skin was able to produce adequate quantities of "the fat-soluble vitamin", suggesting that it was not an essential dietary trace constituent. In parallel studies, Steenbock and Nelson found that food which was irradiated with ultra violet light and subsequently fed to rats also acquired antirachitic activity (30). However, because of the rapid rise of the science of nutrition and the discovery of the families of water-soluble and fat-soluble vitamins it rapidly became firmly established that the antirachitic factor was to be classified as a vitamin.
The chemical structures of the vitamins D were determined by Windaus and his colleagues at the University of Göttingen in Germany, beginning in 1928 (31). Vitamin D$_2$, which could be produced by ultraviolet irradiation of ergosterol, was chemically characterized in 1932 (32). This work led to Windaus receiving the Nobel Prize in chemistry. Vitamin D$_3$ was not chemically characterized until 1936 when it was shown to result from the ultraviolet irradiation of 7-dehydrocholesterol (33). These results clearly established that the antirachitic substance vitamin D was chemically a steroid, more specifically a secosteroid.

As alluded to above, the term ‘vitamin’ D is something of a misnomer. Strictly speaking, vitamin D$_3$ (cholecalciferol) is a fat-soluble steroid pre-pro-hormone formed from 7-dehydrocholesterol (itself produced by the liver) by the action of ultraviolet light (wavelength 280-320 nanometres) on the basal layers of the skin. It is then hydroxylated at the 25 position, probably by the liver, becoming 25-hydroxyvitamin D$_3$ (25D). This in turn is carried to the kidneys where further hydroxylation occurs at the 1 position, resulting in 1,25-dihydroxyvitamin D$_3$ (1,25D), which is generally regarded as the active form of the vitamin (see Figure 1). The hydroxylation process is regulated by enzymes from the cytochrome P450 (CYP) group. The enzyme 25-hydroxyvitaminD$_3$-1α- hydroxylase is also known as CYP27B1. Recent evidence has shown that CYP27B1 is also present in multiple tissues outside the kidney (34) (35). This allows the paracrine activities of 1,25D in extra-renal tissues. 25D is the major circulating form of the vitamin, which directly reflects the body’s vitamin D status. The relatively long half-life of 25D and its fat solubility allow that which is accumulated over the summer months to sustain the individual through the winter (36). Dietary vitamin D is absorbed in the small intestine via the lymphatic system, and requires the presence of bile acids for absorption (36). In the small intestine, vitamin D receptor (VDR) activation upregulates calcium and phosphate absorption.
1,25D is recognised by target tissues that possess specific VDRs. Best understood is the VDR located in the cell nucleus, which acts at the level of nuclear DNA as a classical nuclear receptor/transcription factor. The binding of 1,25D to the nuclear VDR results in changes of gene transcription of a number of messenger RNA (mRNA) species and subsequent de novo protein synthesis (38). The more recently discovered membrane-bound VDR induces so-called ‘rapid responses’ leading to formation of second messengers and phosphorylation of intracellular proteins (39).

With regard to the musculoskeletal system, in muscle the effects of vitamin D include changes in mRNA that lead to de novo protein synthesis, regulating cell proliferation and induction of terminal differentiation (40). The absence of nuclear VDR has been shown in ‘knock out’ mice lacking the gene for this receptor to inhibit muscle development (41). The non-genomic actions of vitamin D increase
active transport of calcium into the sarcoplasmic reticulum. This increases the calcium pool, which is essential for muscle contraction (42). In bone, VDR activation induces the transcription of a number of genes, and thus the expression of several proteins in osteoblasts, regulating cellular differentiation, function and survival (41). Non-genomic effects include the opening of calcium and chloride channels, raising the levels of calcium storage in the endoplasmic reticulum and enhancing osteoblast mobility and ability to change cellular conformation (43).

For most people at most times the action of sunlight upon skin is the principal source of vitamin D. However, with decreased levels of sun exposure dietary sources become more important, especially fish oils. In this setting, the term ‘vitamin’ may be less misleading. Thus there is a gradient of population 25D levels which follows hours of sunlight and hence latitude quite closely, disturbed only occasionally by regional dietary habits (such as the higher consumption of oily fish in northern Norway compared with the south, for example). With increasing latitude there is a more pronounced seasonal variation in 25D levels in ambulant subjects. The peak and trough levels lag one to two months behind the average daily hours of sunlight (44), reflecting the time taken to synthesise 25D and its biological half-life once produced. Not surprisingly, housebound or institutionalised populations have low serum 25D levels year round, with little or no seasonal variation (45, 46), as shown in Figure 2. There is some evidence that men show a much less pronounced seasonal variation in 25D levels than women (47). Individuals with darker skin pigmentation tend to have lower serum 25D levels (7, 9, 48-50) due to melanin absorption of UV light reducing UV irradiation of the basal layers of the skin.

Vitamin D has effects in numerous tissues throughout the body in addition to its effects outlined above. Expression of the VDR is virtually ubiquitous, being detected widely in a very large number of cell types (14, 51). Insufficient vitamin D levels and/or VDR genotype have thus been implicated in a wide range of health problems, although effects of bone and calcium metabolism are the best understood. Other disease processes or areas in which a lack of vitamin D has been implicated include neoplasia at several sites (colon, prostate, breast, ovary, pancreas) (10, 14, 52, 53), multiple sclerosis (51), mental health disorders (54), respiratory disease (11, 55), impaired glucose metabolism (56) and diabetes mellitus (35, 57, 58). These issues are discussed in greater detail below. From being thought of as an endocrine hormone principally affecting bone, calcium and phosphate, vitamin D has now become the subject of broad-ranging speculation and investigation in relation to a great variety of physiological processes and disease states, as indicated in reviews such as those by Mason (59), Zitterman (37) and Holick (60).
Figure 2
Seasonal variation of serum 25D concentrations and daily hours of sunshine for Adelaide, South Australia, during 1982. The lower (1983) line refers to nursing home subjects, and shows the lack of seasonal variation compared with ambulant subjects. Values indicated by · and ·· have significance of differences of <0.05 and <0.01 respectively, when compared with the February value (from Morris et al, 1984 (45))
**Insufficiency & Deficiency**

**Bone and muscle**

Osteomalacia and rickets are caused by a delay in mineralisation of osteoid, and can only be definitively diagnosed from bone histology (61). This shows a reduction in mineral apposition rate, mineralization surface, and bone formation rate, which can be measured after the administration of double tetracycline labels before the bone biopsy (62). There is a marked reduction in the ratio of mineral to osteoid, compared to healthy bone, with labelling also demonstrating that the average time between the synthesis of osteoid and its mineralisation, known as the mineralisation lag time, is increased (63). In severe vitamin D deficiency, rickets/osteomalacia develops in association with hypocalcaemia and/or hypophosphataemia, and often severe secondary hyperparathyroidism (64). It has been shown that correction of the hypocalcaemia, hypophosphataemia and secondary hyperparathyroidism through dietary manipulation also corrects the skeletal pathology (65, 66). The role of vitamin D in regulating plasma calcium and phosphate homeostasis therefore explains its effects upon the mineralisation rate.

In osteoporosis, the overall amount of mineralized tissue in the skeleton is reduced, but with a normal ratio of mineral to osteoid (67). In addition to changes in mineralisation, there are also changes in bone microarchitecture. These are detailed later in this thesis, and may be summarised here as decreased trabecular number and trabecular bone volume, with loss of connectivity and increased porosity. Unlike osteomalacia, osteoporosis can be diagnosed using imaging techniques such as dual-energy x-ray absorptiometry (DEXA). There is evidence that vitamin D status modulates the development and progression of osteoporosis, particularly in the institutionalised elderly. A recent meta-analysis of clinical observational studies amongst the elderly with a depleted but not severely deficient vitamin D status has demonstrated increased risk of hip fracture (68). A mean serum concentration of 25D around 40 nmol/L has been reported for hip fracture patients (45, 69), and meta-regression analyses of data from randomised clinical trials of vitamin D supplementation indicate that a serum 25D concentration of approximately 75 nmol/L or greater is required to significantly reduce hip and non-vertebral fracture risk (70).

Early reports suggested that the increased risk of fracture associated with low vitamin D status was due to osteomalacia (71, 72). However, further research showed that most hip fracture patients did not have osteomalacia, with one review documenting the proportion with osteomalacia to vary from 0 – 37% (64). The majority of such cases are found to demonstrate osteoporotic bone histology (73, 74). Population
studies indicate that BMD increases with increasing serum 25D until a plateau is reached at serum concentrations of approximately 75 nmol/L (75). Data from randomised clinical trials of vitamin D supplementation of the institutionalised elderly, particularly when administered in combination with calcium, show a reduction in fracture incidence, clearly implicating reduced vitamin D status as a cause of osteoporosis and increased risk of fracture (76-78).

Further evidence for a mechanism by which a depleted vitamin D status can increase the risk of fracture is provided by dietary studies in rodent models (79). Trabecular bone volume was reduced in a dose dependent manner at a number of skeletal sites by reducing serum concentrations of 25D from above 100 nmol/L to either 20 or 45 nmol/L in young, adult Sprague-Dawley rats for 3 months. Mineralisation lag time was not increased, nor was there any disruption to plasma calcium or phosphate homeostasis in these animals, which is consistent with osteoporosis but not osteomalacia. No relationship was found between serum PTH or 1,25D concentrations and trabecular bone volume, suggesting that an adequate serum 25D is critical for maintaining bone mineral homeostasis and thus reducing the risk of osteoporosis.

Increasing vitamin D activity in mature osteoblasts through over-expression of the gene for human VDR in transgenic mouse models increases trabecular and cortical bone volumes (80), acting via both an increase in bone formation and a decrease in bone resorption. As described previously, the CYP27B1 gene encodes a member of the cytochrome P450 superfamily of enzymes. The protein encoded by this gene localizes to the inner mitochondrial membrane where it hydroxylates 25D at the 1α position to form 1,25D. A transgenic mouse line over-expressing the human CYP27B1 gene only in osteoblasts also resulted in increased trabecular and cortical bone volumes (81). In both instances these manipulations will increase the effects of a given level of serum 25D, either by increasing VDR responsiveness to its active metabolite, 1,25D, or by increasing absolute local levels of 1,25D. This demonstrates the role of 25D as the substrate for the enzymatic production of 1,25D, which is the final mediator of the cellular effects of vitamin D.

Effects of a depleted vitamin D status on osteoporosis and increased fracture risk have previously been thought to be due to a decrease in intestinal calcium absorption (64). However this attribution must be questioned since the relationship between serum 25D and calcium absorption disappears when serum 1,25D concentrations are included in the analysis (82, 83). In contrast intestinal calcium absorption is strongly related to serum concentrations of 1,25D (82). Clinical studies on patients with serum 25D concentrations of 40 nmol/L or lower identified that ionised calcium, serum 1,25D and intestinal calcium
absorption were not clinically reduced until serum 25D concentrations fell below 20 nmol/L (84). These clinical data indicate that with average dietary calcium intakes plasma calcium homeostasis is maintained at serum 25D concentrations of 20 nmol/L or greater, a level which is also supported by data from rodent studies (79).

Depleted serum 25D concentrations have been shown to affect bone histology in clinical studies. Need et al. (85), found that serum 25D was significantly, inversely related to unmineralised osteoid thickness in ambulant patients presenting to an osteoporosis clinic. Increased osteoid thickness is not diagnostic of osteomalacia, and has been documented when bone turnover is increased, for example in hyperparathyroidism (61, 86). Mineralization lag time in the Need et al. study patients indicated that they did not exhibit osteomalacia. No bone parameters were related to serum 1,25D concentrations, and the concentration of serum 25D required to minimise osteoid width was between 80 and 100 nmol/L. A large cohort of Northern European road accident victims provided similar results when bone histology and serum vitamin D metabolites were analysed. Osteoid thickness was inversely related to serum 25D with an optimal serum concentration of approximately 75 nmol/L necessary to minimise this variable (87). Once again no relationship was found between serum 1,25D and any of the bone variables. Rodent studies involving dietary manipulation of vitamin D status indicate the critical serum concentration of 25D to abrogate any loss of trabecular bone volume is 75–80 nmol/L or greater (79).

The actions of vitamin D depend not only on vitamin D status but also on dietary calcium intake (88, 89). When dietary calcium intake is inadequate and vitamin D status is also deficient it has been demonstrated that osteomalacic bone develops with increased mineralization lag time (90). Under these conditions plasma calcium and phosphate homeostatic mechanisms are activated which include the endocrine vitamin D system through plasma 1,25D. Serum PTH concentrations may be increased, elevating the activity of CYP27B1 enzyme in the kidney to allow maintenance of serum 1,25D concentrations even though serum 25D concentrations may fall below 40 nmol/L (91). Under these conditions intestinal calcium and phosphate absorption are maintained and osteoclastogenesis is stimulated through the interaction between PTH and 1,25D acting on osteoclasts to increase bone resorption. When serum 25D concentrations fall below 20 nmol/L there is insufficient substrate for the renal CYP27B1 enzyme, and serum 1,25D concentrations fall with a consequent fall in intestinal calcium absorption and the development of hypocalcaemia and osteomalacia (84). When dietary calcium intake is sufficient but vitamin D status is low, or vice versa, plasma calcium and phosphate homeostasis is maintained (90). Depending on the concentrations of dietary calcium or serum 25D, the plasma calcium homeostatic mechanism can minimize serum PTH and 1,25D concentrations, acting to
increase osteoclastic bone resorption. Mineralization lag times are normalized and the bone histology demonstrates osteoporosis rather than osteomalacia (79). When dietary calcium is sufficient to meet the needs of calcium balance and serum 25D concentrations are 75 nmol/L or greater, changes to serum PTH and 1,25D concentrations are minimized. Serum 25D and bone tissue expression of CYP27B1 are maximized. Australian data suggest that calcium intake is often insufficient, with one large, community-based study finding that 76% of women consumed less than the recommended daily intake, even when calcium supplementation was included (92). Disturbingly, 14% of participants in this study had intakes less than the minimal requirement of 300 mg/day, and thus would be in severe negative calcium balance.

In summary, vitamin D modulates bone health by at least two actions. Firstly, it helps maintain plasma calcium and phosphate homeostasis, preventing the development of osteomalacia. It is the plasma 1,25D which contributes to maintaining plasma calcium homeostasis through multiple actions including on osteoblasts to stimulate osteoclastogenesis and bone resorption enhancing the flow of calcium and phosphate into the plasma compartment. The critical concentration of serum 25D to maintain adequate plasma concentrations of 1,25D and intestinal calcium absorption is 20 nmol/L.

Secondly, vitamin D also acts to maintain mineralised bone tissue volume as assessed by bone mineral density and trabecular and cortical bone volumes preventing the development of osteoporosis and reducing the risk of fracture. In this case increased risk of fracture, bone histology and bone mineral density all relate to serum 25D concentrations and not to serum 1,25D. An interaction between vitamin D status and dietary calcium intake is probable as the most consistent data from randomised controlled trials for fracture risk reduction is demonstrated when these nutrients are combined.

Generally the negative effect of vitamin D deficiency on bone has been attributed to a decrease in calcium absorption (36, 61, 64, 93), although little evidence has been produced to support this assumption. A seasonal change in calcium absorption aligned with the seasonal changes in serum 25D was described by Heaney et al (94), but calcium absorption measurement in this study was very imprecise, and serum 1,25D was not measured. Nordin et al have shown that any correlation between calcium absorption and 25D is lost when serum 1,25D is allowed for (95). Because there is malabsorption of calcium in severe vitamin D deficiency (96, 97) it has been proposed that hypovitaminosis D of any degree will causes calcium malabsorption, but this is not supported by the available data, which instead suggest a threshold effect. As described above, both clinical studies and rodent studies have identified that ionised calcium, serum 1,25D and intestinal calcium absorption were not reduced until serum 25D concentrations fell below 20 nmol/L (79, 84). The presumed fall in ionised
calcium associated with hypovitaminosis D is not due to malabsorption of calcium but to a lack of the 
“calcaemic effect” of vitamin D on bone, first described by Carlsson and Lindquist in 1955 (96). These
authors gave graded doses of vitamin D to vitamin D deficient rats and showed that serum calcium rose 
with the dose of vitamin D. However, once calcium absorption was normalised by a small dose of 
vitamin D, it rose no further, consistent with the threshold described above.

Fragility fractures are defined by the World Health Organization as being “caused by injury that would
be insufficient to fracture a normal bone...the result of reduced compressive and/or torsional strength of 
bone”(98). Clinically, a fragility fracture may be defined as one “...that occurs as a result of a minimal 
trauma, such as a fall from a standing height or less, or no identifiable trauma” (99). Bone strength is 
affected by the process bone turnover, requiring a balance between osteoblast and osteoclast activity. 
The absence of sufficient vitamin D ultimately increases osteoclastic activity. Normal vitamin D levels 
are thus critical for correct regulation of bone turnover, and decreased levels will lead to reduced bone 
strength.

There is ample evidence from observational studies that vitamin D deficiency is associated with femoral 
near fractures in the elderly (100) and probably other fractures also (101). Correcting this deficiency 
has generally been shown to significantly reduce the risk of fractures of the neck of femur, wrist, 
forearm and vertebrae, particularly in the institutionalised elderly (101, 102), although this has not been 
uniformly demonstrated (68). Both trial level and meta-analyses demonstrate beneficial effects of 
vitamin D and calcium supplementation in the institutionalised elderly to reduce the risk of falls and 
premature mortality (101, 103). Reduction in fractures, and therefore a reduction in post-fracture 
mortality, which is of the order of 25 – 30% in the elderly during the first year following a fractured neck 
of femur, is likely to account for some of this reduction in mortality.

As would be expected from the diverse effects of vitamin D on many cell types, the musculoskeletal 
consequences of vitamin D deficiency are not confined to bone. The term osteomalacic myopathy has 
been used to describe the most pronounced effect that vitamin D deficiency has on muscular function 
and strength (41). Case reports in both young and older adults have described how vitamin D 
deficiency was associated with severe muscle weakness, often leading to marked disability. Other 
symptoms may include diffuse skeletal pain, diffuse muscle pain and a waddling gait. Rapid 
 improvement was seen with vitamin D supplementation (104). Electromyography has been shown to be 
abnormal (105), and muscle biopsies in such patients have shown an atrophy of type 2 muscle fibres 
(106). Treatment with a vitamin D analogue has been shown to increase the relative number and size
of type 2 muscle fibres in elderly women within three months of treatment (107). Type 2 fibres are strong and fast-acting, and are the first to be recruited to avoid falling (41). Vitamin D plus calcium compared with calcium alone improved body sway by 9% within two months in elderly ambulatory women (108). Vitamin D supplementation of older people in residential care for 2 years has also been shown to reduce their incidence of falls, even if they are not initially classically vitamin D deficient (109).

Vitamin D deficiency has been reported to be associated with reduced muscle strength, and also with falls and fractures that are not explained by reduced bone density alone (110). Supplemental vitamin D in the dose range 700 – 1000 IU per day was found in a 2009 meta-analysis to reduce the risk of falling in older individuals by 19% (111). Vitamin D deficiency has also been associated with muscle pain, both in the general population, and specifically in Australian Aboriginals (112). A 2006 review concluded that supplemental vitamin D could improve muscle strength, and could also decrease muscle pain, particularly in the back and lower limb (113).

One study has emphasised that there may be adverse effects from large doses of vitamin D. Sanders et al (114) gave an annual vitamin D₃ dose of 500,000 IU or placebo to 2256 community-dwelling women aged 70 years or older. Women in the vitamin D group had 171 fractures, compared with 135 in the placebo group; 837 women in the vitamin D group fell 2892 times (rate, 83.4 per 100 person-years) while 769 women in the placebo group fell 2512 times (rate, 72.7 per 100 person-years; incidence rate ratio [RR], 1.15; P = .03). The incidence RR for fracture in the vitamin D group was 1.26 (P = .047) when compared with the placebo group (rates per 100 person-years, 4.9 vitamin D vs 3.9 placebo). A temporal pattern was observed in a post hoc analysis of falls. The incidence RR of falling in the vitamin D group when compared with the placebo group was 1.31 in the first 3 months after dosing and 1.13 during the following 9 months. This study is unusual in the literature by virtue of its having demonstrated an increase in the risk of falls and fractures, and the mechanism is not clear. However, it did involve an unusually large bolus dose of vitamin D, and may serve as a reminder that using supra-physiological doses of vitamin D may cause harm.
Neoplasia

Garland & Garland (115) proposed in 1980 that vitamin D was a protective factor against colon cancer, describing an ecologic study of the association between colon cancer mortality rates and mean daily solar irradiation in the USA. They suggested that this protection was mediated by increased concentrations of intracellular calcium. The next step by Garland et al. was to test the observations from the ecologic analysis in an observational study carried out in the Western Electric Cohort (116). This prospective study of oral vitamin D intake and colon cancer risk followed a cohort of 1,954 men for 19 years. The main finding was that men who consumed at least 150 IU per day of vitamin D had only half the risk of developing colorectal cancer than men who consumed less.

The Harvard cohort studies (the Nurses’ Health Study, the Health Professionals’ Follow-Up Study, and the Physicians’ Health Study) studied cancer risk and actual circulating 25D level, dietary and supplementary vitamin D intake, and predicted circulating 25D level (117). These cohorts strongly supported an inverse association with colorectal cancer, and also a 30% reduction in risk for breast cancer comparing the highest with lowest quintiles of 25D levels. Vitamin D intake also was associated with a lower risk of pancreatic cancer. Results from the Health Professionals Follow-Up Study also suggested that the poor vitamin D status generally in African-Americans contributes to their higher incidence and mortality from various malignancies.

Few randomised trials of vitamin D for cancer prevention have been performed. However, Lappe et al (118) found that supplementation with calcium (1400 mg daily) and vitamin D (1000 IU daily) substantially reduced all-cancer risk in postmenopausal women. In comparison to the placebo group, the relative risk of developing cancer at study end was 0.402 (CI: 0.20, 0.82; P = 0.013) for the calcium plus vitamin D group and 0.532 (CI: 0.27, 1.03; P = 0.063) for the calcium-only group. In multiple logistic regression models, both treatment and serum 25-hydroxyvitamin D concentrations were significant, independent predictors of cancer risk. The Women’s Health Initiative (WHI) also examined vitamin D and cancer, but used a lower daily dose of vitamin D (400 IU) and a sample of women with substantially lower baseline vitamin D status and much poorer treatment adherence than the Lappe study [median serum 25D: 42 nmol/L] (119). The WHI reported no significant effect of the vitamin D intervention on colorectal cancer incidence but did note a highly significant inverse relation between baseline 25D and incident cancer risk (119, 120), just as Lappe found for all cancers. Other cancers to be associated with vitamin D status include those of the prostate (121), pancreas (122) and non-
melanotic skin cancer (123). These associations have not been found in all studies, and in general terms are not as strong as the associations for breast and colon cancer.

The mechanism by which vitamin D status may affect cancer development is still to be fully determined, but some elements are widely agreed upon in the literature. In particular, many of the human genes containing vitamin D response elements encode for proteins important in the regulation of cell proliferation, differentiation, and apoptosis. When vitamin D status is suboptimal, these activities are impaired. For example, mice rendered vitamin D deficient exhibit enhanced cancer development and cancer growth (124), as do VDR knockout mice (125). Further laboratory and clinical research is required to properly determine both the mechanism(s) and the magnitude of any effects of vitamin D on cancer.
Cardiovascular disease

Scrugg (126) hypothesised in 1980 that vitamin D was protective for cardiovascular disease (CVD), citing the seasonality of CVD mortality and proposing an effect on thrombosis as one possible mechanism. Further research has provided evidence for effects on vascular calcification, cardiac muscle and vascular endothelium (127).

Several large nonrandomised prospective studies with respect to CVD morbidity and mortality have been conducted in Europe and North America. These include the 1739 Framingham Offspring Study (128), the Health Professionals Follow Up Study (129), the LURIC (Ludwigshafen Risk and Cardiovascular Health) study (130), and the Hoorn study (131). In summary, these have shown an association between CVD morbidity, CVD mortality and vitamin D levels, with lower levels increasing CVD events and higher levels appearing to be protective. However, few randomised intervention studies have been performed in this area, making it difficult to establish causation and make firm conclusions.

In the WHI study, combined daily supplementation with calcium (1000 mg) and vitamin D (400 IU) did not alter the risk for coronary events or stroke (132). As previously noted, the supplemental vitamin D dose is low. It is also possible that the use of supplemental calcium may increase the risk of CVD events, thus masking the potential beneficial effects of vitamin D. This possibility was raised by Bolland et al (133), and has generated significant debate within the scientific community. They reported a secondary analysis of a randomised controlled trial of calcium supplementation in healthy postmenopausal women, primarily designed to assess the effects of calcium on bone density and fracture incidence over five years, concluding that calcium supplementation in healthy postmenopausal women is associated with upward trends in cardiovascular event rates. It should be noted that this study involved the use of a high dose of a less common form of supplemental calcium (1 g of elemental calcium as the citrate), and that many of the study's key findings did not reach statistical significance, and that even when they did, they reached only borderline statistical significance. Other studies have generally not replicated the findings of Bolland et al. Locally, a five-year, double-blind, placebo-controlled study in which 1460 women aged over 70 years were given daily supplemental calcium carbonate 1200 mg or placebo showed a hazard ratio for ischemic heart disease of 1.12, with a CI of 0.77-1.64, for calcium supplementation compared to placebo (134). Whilst this is reassuring, Bolland et al (135) also published a meta-analysis in 2010 which concluded that calcium supplements (without coadministered vitamin D) are associated with an increased risk of myocardial infarction. As calcium
supplements are widely used these modest increases in risk of cardiovascular disease might translate into a large burden of disease in the population. A reasonable approach might therefore be to attempt to optimise calcium intake via dietary means, whilst continuing to provide supplemental vitamin D. It is theoretically possible that high peak levels of calcium may be important, with such peaks being minimised by avoiding high supplemental doses of calcium and administering it as a divided dose where higher dosing is shown to be necessary.

Elamin et al (136) performed a systematic review and meta-analysis of 51 prospective, interventional trials of vitamin D supplementation, focusing on cardiovascular outcomes and all-cause mortality. Vitamin D was associated with non-significant effects on the outcomes of death [RR, 0.96; \( P = 0.08 \)], myocardial infarction (RR, 1.02; \( P = 0.64 \)), and stroke (RR, 1.05; \( P = 0.59 \)). Pooled participant numbers were 62,231 for studies of mortality, 39,879 for myocardial infarction and 39,739 for stroke. In all these groups the ratio of female to male participants was approximately 12:1. There were no significant changes in the surrogate outcomes of lipid fractions, glucose, or diastolic or systolic blood pressure. The latter analyses were associated with significant heterogeneity, and the pooled estimates were trivial in absolute terms. They concluded that trial data available to date were unable to demonstrate a statistically significant reduction in mortality and cardiovascular risk associated with vitamin D. They noted that the quality of the available evidence is low to moderate at best, and that recommending vitamin D to patients to reduce cardiovascular risk was not consistent with the available evidence.

The authors do note that many of the included studies were not designed to evaluate cardiovascular outcomes. What is not outlined in any detail are the substantial design limitations of many of the included studies. According to table 1, the mean vitamin D concentration was documented as being in the range generally accepted as deficient at study entry (< 50 nmol/L) in 14 of the 51 studies. In the remaining 37 studies participants were replete at entry, or did not have vitamin D concentrations measured. Supplementation provided to participants was less than the generally recommended value of 800 IU daily in 16 of the 51 studies. End of study vitamin D concentrations were not recorded in 20 studies, in 3 there was no significant difference in concentrations between study end and entry, and in 28 there was a significant change. Of these 28 studies, 2 reported that the mean vitamin D concentration remained below 50 nmol/L. It is therefore difficult to know what the clinical value of these studies is, as it is likely that treating replete individuals and the use of inadequate supplemental doses of vitamin D will tend to drive results towards a null outcome.
Whilst careful examination of this paper and the source trials must lead any reviewer to conclude that there is insufficient evidence to recommend vitamin D for prevention of cardiovascular disease, it also emphasises the need for properly designed and conducted studies which can provide higher quality evidence.

Several mechanisms have been proposed by which vitamin D may mediate prevention of hypertension and cardiovascular disease. Vitamin D deficiency has been associated with markers of subclinical atherosclerosis such as intima-media thickness and coronary calcification as well as with cardiovascular events such as myocardial infarction and stroke as well as congestive heart failure (137). Vitamin D deficiency may contribute to the development of CVD through its association with risk factors, such as diabetes and hypertension (see below), and/or via direct effects the cardiovascular system. Vitamin D receptors are expressed in a variety of tissues, including cardiomyocytes, vascular smooth muscle cells and endothelial cells, and vitamin D has been shown to affect inflammation and cell proliferation and differentiation (138). The renin–angiotensin system plays an essential role in regulating blood pressure, and Li et al (139) have shown that 1,25D is a potent suppressor of renin biosynthesis. Vitamin D deficiency has been associated with elevation of matrix metalloproteinases, which in turn has been shown to cause hypertrophy of cardiac and smooth muscle (140). Direct suppression of PTH may also play a role (141).
**Diabetes**

*Type 1 diabetes*

Insulin is secreted by β-cells, located within the pancreatic Islets of Langerhans. It has been known for several decades that insulin secretion responds to vitamin D (37). More recently, this has been shown to be at least partially mediated by nuclear VDRs present within the β-cells (35). In addition, CYP27B1 is expressed within the Islets, allowing local production of 1,25D (142). Islet cells respond rapidly to treatment with exogenous 1,25D, the speed of which suggests that a non-genomic effect is also likely to be operating, in which 1,25D activates the intracellular signalling pathway through a VDR (142, 143). Animal models with mutated VDRs which are functionally inactive show impaired oral glucose tolerance (144). Non-obese diabetic (NOD) mice are considered a good model for human type 1 diabetes. When they are rendered vitamin D deficient in early life, they show an increased incidence of type 1 diabetes, which has an earlier onset than that seen in vitamin D replete animals (145, 146). Human studies have also showed that vitamin D status affects β-cell function. In one such study involving 126 healthy, glucose-tolerant subjects, insulin sensitivity and first- and second-phase insulin responses were assessed by using a hyperglycaemic clamp technique (56). In this type of study, plasma glucose concentration is acutely raised to 7 mmol/L above basal levels by a continuous infusion of glucose. This hyperglycaemic plateau is maintained by adjustment of a variable glucose infusion, based on the rate of insulin secretion and glucose metabolism. Because the plasma glucose concentration is held constant, the glucose infusion rate is an index of insulin secretion and glucose metabolism. The hyperglycaemic clamp may thus be used to assess insulin secretion capacity. A positive correlation was found between serum 25D concentrations and insulin sensitivity. There was a negative correlation between serum 25D concentration and β-cell function.

Vitamin D deficiency has been observed to be more common in families, suggesting that genetic factors may be responsible for variations in 25D levels. The VDR gene has been studied extensively. Polymorphisms of the VDR gene have been associated with type 1 diabetes (147), fasting glucose levels in healthy young men (148), Addison’s disease (35), Graves’ disease (35) and Hashimoto’s thyroiditis (149). It appears that there may be racial differences in the linkage between VDR polymorphisms and type 1 diabetes, with studies showing an association in Germans (150), Bangladeshi Indians (147) and Japanese (151), but not in Finns or combined large scale analyses from the UK and Romania (35).
Type 1 diabetes is an autoimmune disease, probably triggered by exposure to one or more environmental agents in susceptible individuals. It is thought that the subsequent failure of tolerance leads to autoreactive T-cell induction and thus insulitis and β-cell destruction (35). The VDR has now been found in many cell types of the immune system, including dendritic cells, macrophages and activated T cells (35). VDR agonists have been shown to possess immunoregulatory properties and to have a pronounced pro-tolerogenic effect (152). VDR agonists can act directly on T cells. However, dendritic cells, which are known to be antigen-presenting cells, appear to be their primary targets (152) (153). Chronic administration of 1,25D reduces the incidence of both insulitis and type 1 diabetes in NOD mice (146) and improves streptozotocin-induced diabetes in rats (154). Vitamin D deficiency in early life accelerates the development of type 1 diabetes in NOD mice, even when the deficiency is so subtle that serum calcium and bone turnover markers are not altered (145). Glucose levels are known to rise in response to inflammatory cytokines (155). Both 25D and calcitriol are inversely related to cytokine levels, especially TNF-α (37).

Epidemiological studies have also shown an association between vitamin D status and diabetes. Cod liver oil is a rich source of vitamin D, and also contains vitamin A and long-chain fatty acids. Stene et al (156) showed that the offspring of mothers taking cod liver oil during pregnancy had a lower risk of diabetes than controls (OR 0.30, 95% CI: 0.12 – 0.75). Breast milk is quite a poor source of vitamin D (157), and is probably insufficient to supply infant needs. Infants who receive formula (which is fortified with vitamin D) are less likely to be vitamin D deficient. A birth cohort study followed the offspring of 12,058 Finnish mothers for 31 years (13). Data were collected during the first year of life with regard to vitamin D supplementation and the presence of suspected rickets. The primary outcome was the diagnosis of type 1 diabetes. Vitamin D supplementation was associated with a decreased frequency of type 1 diabetes, both for regular and irregular supplementation. Children who regularly took 200 IU of vitamin D daily had a Relative Risk (RR) of 0.22 (95% CI 0.05 – 0.89) compared with those who did not. Children suspected of having rickets in the first year of life had a RR of 3.0 (95% CI 1.0 – 9.0) compared with those in whom there was no such suspicion. The authors concluded that ensuring adequate vitamin D supplementation for infants could help reverse the increasing trend in the incidence of type 1 diabetes. In Germany, the incidence of type 1 diabetes in adolescents is higher in autumn and winter than in spring and summer (158), coinciding with the observed troughs in serum vitamin D levels. A EURODIAB (European Community Concerted Action Program in Diabetes) subgroup multicentre study of cases and controls found that the risk for type 1 diabetes was significantly reduced in countries with vitamin D supplementation during childhood (159).
In summary, evidence from human and animal studies, both in vitro and in vivo, suggests that vitamin D has beneficial effects on β-cell function and the immune system. Vitamin D insufficiency in early life is a risk factor for type 1 diabetes. Some effects of vitamin D may be as a result of direct action on β-cells, others are mediated via promotion of immune tolerance. Although the optimal dose regimen is not known, it is clear that avoidance of vitamin D deficiency is essential for β-cell function and that it appears to provide protection against the development of type 1 diabetes in later life. Prospective cohort studies in which biomarkers of vitamin D are measured at various times whilst monitoring for the onset of type 1 diabetes would be required in order to resolve issues of timing and dose. This information would then allow the proper design of an intervention trial.

**Type 2 diabetes**

As outlined above, insulin secretion by the pancreatic β-cell is responsive to vitamin D. VDR gene polymorphisms in humans have been shown to be associated with insulin secretion in Bangladeshi Asians, a population known to be at risk of type 2 diabetes, independent of vitamin D levels (160) (147). VDR gene polymorphisms have also been shown to be associated with altered fasting glucose levels in young, healthy, male aircrew with low physical activity (148). A study of 142 elderly Dutch men found that the 1 hour glucose level and the area under the glucose curve during a standard 75g oral glucose tolerance test (OGTT) were inversely associated with the serum concentration of 25D ($r = -0.23$, $p < 0.01$; $r = -0.26$, $p < 0.01$, respectively) (161). These associations were independent of potential confounding factors including month of sampling, physical activity, BMI, alcohol intake and smoking. After excluding newly-diagnosed diabetic patients, total insulin concentrations during the OGTT were also inversely associated with the 25D concentration. These studies suggest that vitamin D status and VDR genotype may be significant risk factors for impaired glucose tolerance (IGT) and type 2 diabetes. It has been suggested that TNF-α plays a key role in the insulin resistance of obesity and non-insulin-dependent diabetes mellitus, and that levels of this inflammatory cytokine are inversely related to 25D levels (162, 163).

A positive relationship between serum 25D and insulin sensitivity was reported in healthy, young adults (56). A study of older subjects reported an association between IGT in both Caucasian and Polynesian New Zealanders (164). The authors noted that low concentrations of 25D in New Zealand Polynesians may partly explain their increased prevalence of diabetes/IGT compared with Caucasians. A cross-sectional population study of 45 older Swedish subjects found a positive correlation between 25D and insulin sensitivity ($r = 0.54$, $p < 0.001$), although it should be noted that 7 of the subjects were known to have type 2 diabetes at the time of their entry into the study (165). The blood level of glycated
haemoglobin (Hb A1c) is a marker of glycaemia over a period of approximately 3 months in a given individual. It has been reported to be inversely related to serum 25D in Canadian aboriginal women (166). A study of 753 postmenopausal, white Australian women attending a menopause clinic showed that fasting glucose was inversely related to serum 25D ($p = 0.0006$) (167). A similar relationship was described in 1726 Mexican-Americans of both genders, aged 20 years and over, although the relationship was not found to be significant in non-Hispanic whites (16). In postmenopausal Australian women, it appeared that a major increase in fasting glucose occurs as 25D levels fall below 40 nmol/l, which would generally be accepted as established vitamin D insufficiency. This study was cross-sectional, and did not measure physical activity, an important contributor to insulin sensitivity, which are significant shortcomings.

High serum 25D concentrations predicted a reduced risk of type 2 diabetes over an 8 – 10 year follow-up period in a study of 2,378 middle-aged individuals with prediabetes (impaired glucose tolerance or impaired fasting glucose), but were not predictive in individuals who had normal glucose tolerance at entry into the study (168). The authors concluded that vitamin D should be evaluated for the prevention of type 2 diabetes in prediabetes individuals. Similarly, data from the AusDiab study have shown that lower 25D concentrations are associated with increased risk of metabolic syndrome, higher fasting blood glucose levels and increased insulin resistance in 4,164 individuals followed over a 5 year period (169), as well as an increased risk of being diagnosed with type 2 diabetes (170). The consistent recommendation from these studies and from review papers is that large, prospective vitamin D supplementation studies will be required to determine whether these associations are causal. There has been some supportive evidence from small studies of this type, such as that reported by von Hurst et al (171). Supplementation with vitamin D$_3$ 4000 IU daily in insulin resistant women was found to decrease insulin resistance but did not change insulin secretion. Mitri et al (172) also found that short-term supplementation with vitamin D improved β cell function and had a marginal effect on attenuating the rise in Hb A1c. However, these studies alone are not sufficient to permit more generalised recommendations to be made.

In summary, the available evidence suggests that vitamin D plays a role in the prevention of IGT and type 2 diabetes, and may also moderate glycaemia in patients with established disease. It appears that the role of vitamin D may vary between different ethnic groups, with the suggestion that it may be particularly important in dark-skinned and aboriginal populations.
Respiratory disorders

With respective to plausible mechanisms for the effects of vitamin D, it has already been documented that the principal active metabolite of 25 D, 1,25D, has antiproliferative, antidiifferentiative and immunomodulatory properties. Airway inflammation is known to be a central process in the pathogenesis of both asthma and chronic obstructive pulmonary disease (COPD) (173). Hypovitaminosis D has also been shown in animals to lead to increased oxidative stress, which is thought to play a role in the pathogenesis of COPD (174). VDR variants have been shown by some investigators to affect the rate of decline of lung function in smokers, although overall, findings have been inconsistent (175, 176).

The immunomodulatory role of vitamin D is supported by the presence of VDRs and the hydroxylation of 25D in relevant cell types, including macrophages and dendritic cells (177). In experimental studies, vitamin D has been shown to inhibit proliferation of CD41 T cells (27) and to reduce the production of Th1 cytokines and IL-17 (178). One way that vitamin D may influence asthma pathogenesis is through modulation of T regulatory cells (179). Vitamin D inhibits matrix metalloprotease (180), inhibits fibroblast proliferation and influences collagen synthesis (181). Although the lungs cease to grow in early adult life (182), remodelling and repair are life-long processes, and it is possible that vitamin D exerts its effects via these processes.

A cross-sectional study of Finnish adults found that vitamin D supplementation during infancy was associated with decreased risk of asthma (183). However, 25D levels were not measured at any point and follow-up data were incomplete. A case-control study of serum 25D and asthma in British adults (184) failed to show an association between vitamin D and asthma, whilst a similar study involving African American children and young adults (185) found a strong association between vitamin D insufficiency or deficiency and asthma. Both of these studies were limited by lack of data on vitamin D status in early life and potential selection bias.

A birth cohort study of British children reported a strong association between serum vitamin D levels in late pregnancy and asthma at 9 years of age, but was limited by inadequate follow-up of participants (186). Birth cohort studies in Boston, Scotland, Japan, and Finland have shown that maternal dietary intake of vitamin D during pregnancy is inversely associated with wheeze (187-189) and asthma (190) in early childhood. All studies were limited by relatively short duration (1.3 to 5 years), significant loss to follow-up, and lack of serum 25D measures during pregnancy or in infancy. An birth cohort study from
New Zealand found that 25D levels in cord blood were inversely associated with wheeze but not with incident asthma by 5 years of age (191). Although a birth cohort study of Australian children found an association between 25D level at age 6 years and asthma in boys at age 14 years, it lacked vitamin D measures in early life and had substantial loss to follow-up, and the analyses were unadjusted for potential confounders (192).

Black & Scragg (11) analysed data from the Third National Health and Nutrition Examination Survey (NHANES III). This included 14,091 individuals aged 20 years and over, who were interviewed at mobile examination centres, underwent spirometry and had their 25D level measured. After adjusting for a large number of confounding variables, they showed a strong relationship between serum levels of 25D and several indicators of lung function. It appeared that a dose-response effect was operating. It has been shown elsewhere that decreased Forced Vital Capacity (FVC) and accelerated decline in Forced Expiratory Volume in one second (FEV₁) are markers of susceptibility to chronic obstructive pulmonary disease (COPD) (193), and that decreased FEV₁ is a risk factor for cardiovascular morbidity & mortality independent of age, gender and smoking history (194). As a cross-sectional study, this study could not confirm a temporal relationship, nor was there any intervention. Only one randomised, prospective study of vitamin D supplementation in COPD patients has been published (195). Participants with moderate to severe COPD received oral vitamin D 100,000 IU at the beginning of winter. The study failed to show any effect on COPD exacerbations. These findings are consistent with data from the Lung Health Study (196), which showed that 25D levels did not determine the rate of decline in FEV₁ in a limited subgroup of the overall study. However, in post hoc analysis, the sub-group with the lowest 25D levels at baseline (<25 nmol/L) did show a significant reduction in exacerbations, suggesting that this group deserves further investigation.

There is also a limited amount of evidence linking maternal vitamin D intake with atopic diseases generally in childhood, not just asthma. Maternal consumption of oily fish during pregnancy has been shown to reduce the incidence of childhood eczema (197) as well as asthma (198), with these effects being shown in a majority of studies, but not all (197). Oily fish is rich in vitamin D and long-chain polyunsaturated fatty acids (PUFAs), both of which may play a role in modulating the development of atopy.
**Appropriate intake and target levels of vitamin D**

There is disagreement in the literature as to what constitutes an appropriate level of serum vitamin D and also what recommendations should be made regarding appropriate vitamin D intake. In part, these differences may arise from the tension between what should be recommended at a population level, as opposed to what a treating clinician may recommend for an individual patient. There is also a discrepancy between published recommendations for bone health (especially prevention of fractures), which has been extensively studied, and non-skeletal health, where the evidence base is not as extensive. Different approaches to intervention studies also make comparisons and conclusions difficult, particularly the issue of whether study participants were given a standard dose of supplemental vitamin D, or if dose was adjusted to attain a target 25D level. The latter approach is considerably more expensive, owing to the need for one or more measurements of serum 25D, which has limited its implementation.

**Bone health and fractures**

25D, the substrate or pro-hormone which is converted to the active 1,25D hormone, is the main circulating vitamin D metabolite. As the biomarker of whole body vitamin D status it is therefore the most commonly measured form of the vitamin in clinical studies (199). However, it is not the most active metabolite, so decisions about what constitutes normality, insufficiency and deficiency need to take account of homeostatic mechanisms such as the synthesis of 1,25D and the increase in PTH level seen with vitamin D deficiency. One method of establishing the required 25D level is to examine the 25D level at which PTH begins to rise. This inflexion point varied in several studies between 30 and 78 nmol/l (199), but is generally around 60 nmol/L. It is likely that some of this variation is due to interlaboratory variation as a result of different assays for 25D. Dietary calcium intake is also known to influence serum PTH, which may in turn influence metabolism of vitamin D. A low calcium intake causes an increase in PTH and 1,25D, thereby decreasing the half-life of 25D. On balance, a 25D level of 50 - 60 nmol/l or higher is generally considered appropriate, levels between 30 and 49 nmol/l mild deficiency, between 12.5 and 29 nmol/l moderate deficiency and below 12.5 nmol/l severe deficiency (200), with the proviso that these levels may be influenced by dietary calcium intake (199). The 2012 Australian position statement on vitamin D and health suggested a target concentration of ≥ 50 nmol/L at the end of winter, and 10–20 nmol/L higher at the end of summer to allow for seasonal decrease, for optimal musculoskeletal health (200).
Trials of the effects of vitamin D supplementation in the elderly have generally shown a reduction in fracture rates and the rate of falls (109), although there is a lack of consistency (201, 202). The lack of significant reduction in fractures in some studies could be due to an insufficient dose of vitamin D, or to a lack of power due to shorter duration of study, insufficient sample size, poor compliance with treatment protocols or a lower number of events. The RECORD study (203), a large randomised trial of participants with a recent low-trauma fracture, failed to show any benefit of calcium or vitamin D on fracture incidence. It should be noted that compliance with medication was only moderate, declining to 63% after 2 years, and might even have been as low as 45% if non-responders to the compliance questionnaire had been included. The vitamin D status of the trial population at the baseline was also poorly defined, being measured in only 60 participants (1.1%). The participants were younger than those in most other studies, were ambulatory and lived in the community. For these reasons they were less likely to have vitamin D deficiency. As a result, there has been considerable debate about how to interpret this study, especially as other studies have reported a therapeutic benefit of vitamin D on fracture. Two randomised controlled trials have found 400 IU/day of supplementation with vitamin D to be ineffective in reducing fracture rates (50, 204) but one study did find evidence of vitamin D deficiency in subjects receiving a 400 IU/day dose (205). However, several randomised controlled trials have demonstrated the efficacy of a higher dose, equivalent to 800 IU/day (101, 102, 108, 206), a conclusion which has been reinforced by the meta-analyses of Tang et al (207) and Bischoff-Ferrari et al (208). There is evidence that this dose is well within the margin of safety with regard to vitamin D toxicity (110). Vitamin D may be given as a daily supplement, or as 100 000 IU orally every 4 months (101), which might be associated with better compliance. However, provision of an annual oral ‘megadose’ of 500,000 IU vitamin D to older women was found to be associated with a short-term increase in the risk of falls and fractures (114), and an annual intramuscular dose of 300,000 IU given to community-dwelling elderly men and women did not alter the rate of non-vertebral fractures (202). The optimal regimen both in terms of dose and frequency thus remains to be precisely determined.

Fracture risk is a product of the risk of falling and bone quality. A 2009 meta-analysis of the effect of vitamin D on falls has concluded that vitamin D supplementation appears to reduce the risk of falls among older individuals by 19% (111). A fall was defined as “unintentionally coming to rest on the ground, floor or other low level”, and specifically excluded coming to rest against furniture, a wall or high trauma events (such as falling from a ladder). More recently, the 2012 U.S. Preventive Services Task Force recommended vitamin D supplementation for prevention of falls in community dwelling older adults (209). It suggested a dose of 600 IU daily in those aged 51 – 70 years, and 800 IU daily in those aged over 70.
Non-skeletal diseases

As outlined above, there is a wide range of disease states which have been shown to be associated with lower vitamin D levels. Although the level of evidence available from randomised trials is generally not as strong as that regarding falls and fractures, as has also been outlined above the evidence of benefit is quite strong in some instances. On recent review concluded that mean serum 25D levels of 75 to 110 nmol/l provide optimal benefits for all investigated endpoints without increasing health risks, and that these levels could be best obtained with oral doses in the range of 1,800 to 4,000 IU vitamin D per day (210). The authors emphasised that further work is needed, including subject and environment factors, to better define the doses that will achieve optimal blood levels in the large majority of the population.

This section would be incomplete without some discussion of the influential 2011 report from the Institutes of Medicine of the National Academies (IOM), “Dietary Reference Intakes for Calcium and Vitamin D” (211). For adults aged 70 and below, a recommended dietary allowance (RDA) of 600 IU was proposed, increasing to 800 IU for those aged over 70. It was suggested that these RDAs would correspond to a serum 25D level of at least 50 nmol/L for at least 97.5% of the population. These RDAs are a little lower than those advocated in the 2010 International Osteoporosis Foundation (IOF) position statement on vitamin D and older adults, which advocated doses of 800 – 1000 IU per day, aiming for a serum 25D level of 75 nmol/L (212). It appears that the IOF placed a greater emphasis on Bischoff-Ferrari’s 2009 meta-analysis (111) than did the IOM. The US Endocrine Society commissioned a Task Force to produce a Clinical Practice Guideline regarding the evaluation, treatment and prevention of vitamin D deficiency, with an emphasis on the care of those at risk of deficiency (213). The intent of this Task Force was to provide guidance for clinicians caring for patients, not to make recommendations for normal, healthy populations. This is in contrast to the IOM report, which took a population-based approach. The Clinical Practice Guideline also does not specifically address the issue of calcium intake, unlike the IOM report. For adults, the Clinical Practice Guideline advocates a daily vitamin D requirement of 1500 – 2000 IU, with an upper limit of 10000 IU. This is recommended with the target 25D level of 75 – 250 nmol/L in mind.

Given that there are suggestions that the higher target 25D level (i.e. 75 nmol/L or more) will offer additional health benefits, and that the increased RDA of vitamin D required to achieve this higher level still lies well within even the IOM report’s safe upper limit of 4000 IU per day, a higher target level (i.e. 75 nmol/L or more) and RDA (i.e. 800 – 1000 IU) have been proposed. However, this enthusiasm...
must be tempered by a small number of studies which have suggested that high vitamin D supplemental doses and/or serum 25D levels may be associated with adverse outcomes. In the musculoskeletal area, the study reported by Sanders et al (114) which showed an excess of falls and fractures in the vitamin D group has already been discussed. There are also observational studies which suggest that both very low and very high serum 25D levels are associated with adverse outcomes. Durup et al (214) conducted a retrospective, observational cohort study of 247,574 adults, with a median follow-up of 3 years. A reverse J-shaped association between 25D levels and mortality was observed. A level of 50 – 60 nmol/L was associated with the lowest mortality, with hazard ratios at very low (< 10 nmol/L) levels being 2.13 (95% CI 2.02 – 2.24), and at very high levels (>140 nmol/L) being 1.41 (1.31 – 1.53) respectively. Michaelsson et al (215) prospectively observed a cohort of 1194 elderly men over a median period of 12.7 years. Total mortality rates were approximately 50% higher in men with the lowest 10% (<46 nmol/L) and the highest 5% (>98 nmol/L) 25D levels, which they describe as a U-shaped association. Cancer mortality was higher at both the higher and lower concentrations, but cardiovascular mortality was increased only where lower 25D concentrations were present. These findings are consistent with those reported by Melamed et al (216) from the NHANES III cohort study, who found an increased risk of mortality in women whose levels exceeded 125 nmol/L. However, whilst the association between increased mortality and low 25D levels is very consistent, not all observational studies have found an association between higher levels and increased mortality. Studies in this latter category include those reported by Jia et al (217) in elderly, community-dwelling Scottish men and women, Ford et al (218) in US adults aged 20 and over, and by Hutchinson et al (219) in community-dwelling Norwegians aged 25 years and over. The major shortcoming of these studies is that are almost all observational, which makes determination of causation impossible.

Adequately-powered, purpose-designed, prospective, randomised studies should help resolve these sorts of controversies, once they are reported upon. Three such large studies are known to be approved or to have commenced. These are the ViDA study in New Zealand (220) (principal investigator Robert Scragg), the VITAL study in North America (221) (principal investigator JoAnne Manson) and the DO-HEALTH study in Europe (222) (principal investigator Heike Bischoff-Ferrari). In particular, the ViDA study is measuring 25D levels at entry, which significantly enhances its ability to attribute causation.
**Drugs and vitamin D**

The association between antiepileptic drugs (AEDs) and poor bone health has long been recognised. Drugs such as phenobarbital, phenytoin and primidone were shown in the 1970s to be associated with decreased bone mineral density, and even overt osteomalacia, resulting in fractures (223). The concern was initially limited to institutionalised patients, where other confounding factors such as mobility problems and limited exposure to sunlight may have played a role. However, more recent evidence suggests that clinically significant osteopathies are common in both children and adults with epilepsy, both those in institutions and those living in the community (224, 225).

For both AED users and clinicians the true significance of these biochemical changes in bone and calcium metabolism lies in whether or not AED use increases fracture risk. Studies have been limited by relatively low patient numbers, particularly when assessing subgroups (226, 227), inadequate accounting for potential confounding factors, a the lack of appropriate control subjects (228), varied study methodologies, and relatively few longitudinal studies. Estimates of the increase in fracture rates for those using AEDs when compared with age and sex-matched members of the general population vary from a doubling to a ten-fold increase (229, 230) (224). The overall fracture rate in AED users was estimated by one review to be 1 fracture every 9-14 person-years in AED users (224), compared with approximately 1 every 50 - 286 person-years in unmatched studies of the general population (231, 232). A 2012 paper from Australia reported a cross-sectional study comparing fractures and falls in 150 AED users with 506 matched non-users (233). Users had greater odds of fracture occasions (OR 1.40; 95% CI 1.02 – 1.91). Odds at various sites ranged from 2.34 at the ankle (95% CI 1.01 – 5.42) to 3.92 (95% CI 1.08 – 14.16) at the spine. Bone mineral density testing has shown that AED users have significantly reduced BMD at clinically relevant fracture risk sites (234), with increased odds for developing osteoporosis (233). However, the degree to which the increase in fracture rate can be explained by altered bone density deficit has yet to be precisely established.

The standard explanation for the reduced BMD seen in AED users is that AEDs cause induction of the cytochrome P450 enzyme system in the liver microsomes. This is thought to cause increased catabolism of vitamin D to inactive metabolites, thus causing vitamin D deficiency bone disease (235). Animal models have suggested that AEDs may bind to either the steroid xenobiotic receptor or the pregnane X receptor (236), which leads to up regulation of CYP24 gene expression. CYP24 is a mitochondrial enzyme known to catabolise vitamin D$_3$ (237). Direct effects of phenytoin on calcium metabolism (238) and on bone (239) have also been proposed. Hormonal changes found in some
women with epilepsy result in lower endogenous oestrogens (240), and there have been reports that valproate has an anti-androgenic effect (241) and that carbamazepine increases sex hormone-binding-globulin in both genders (242). It has also been suggested that AED users have a higher fat mass than non-users (234). Given that obesity is associated with lower vitamin D levels, this could also be a contributing factor. The observation that use of non-enzyme inducing AEDs, such as valproate may also affect bone health also shows that enzyme induction cannot be the sole explanation (243).

Regardless of the precise mechanisms, biochemical abnormalities such as hypocalcaemia, hypophosphataemia, reduced vitamin D levels and hyperparathyroidism have all been associated with AED use (244). These may result in bone loss and subsequent fracture. In addition, bone turnover is increased, as measured by markers of bone formation and bone resorption (245). Increased bone turnover is itself a risk factor for fracture, independent of absolute BMD (246).

The clearest association with reduced BMD is seen with the enzyme-inducing AEDs phenobarbital, phenytoin and primidone (234, 244, 245). Another enzyme-inducing drug, carbamazepine, has also shown a less robust association. Valproate, may also adversely affect bone, although reports are less consistent (245) (243). Several other AEDs have become available over the past 10 - 15 years, and few studies have evaluated their effects on bone metabolism and fractures. Table 1 is taken from a 2010 review paper by Verotti (247) and summarises the effects of ‘classic’ and new AEDs on BMD, 25D, calcium, PTH and bone turnover markers. For the new agents there are very limited fracture outcome data.
Table 1: Main effects of ‘classic’ and new AEDs on bone and calcium metabolism (247)

<table>
<thead>
<tr>
<th>Drug</th>
<th>BMD</th>
<th>25D</th>
<th>Ca</th>
<th>PTH</th>
<th>Bone turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>‘Classic’ AEDs</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>–</td>
<td>↑</td>
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<tr>
<td>Primidone</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Valproate</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td><strong>Newer AEDs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gabapentin</td>
<td>↓</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>?</td>
<td>N</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>–</td>
<td>?</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

Another difficulty is that many patients use combination therapy, making it difficult to isolate the effects of any single agent. There is evidence suggesting that polypharmacy (i.e. concurrent use of more than one AED) further increases the risk of fracture (228).

Although there is evidence that vitamin D deficiency is associated with AED-induced bone loss, not all studies that document such loss have shown this association (225). It is possible that vitamin D levels as measured in studies may be related to more immediate serum fluctuations, whereas BMD reflects longer term levels. The definition of what constitutes an adequate vitamin D level also varies a little between studies, and is itself the subject of some ongoing debate. Other factors known to be associated with bone loss independent of vitamin D levels are also more common in people with epilepsy, including reduced physical activity & inadequate calcium intake. Finally, children who participated in reported studies may have higher vitamin D intake than adults, partly through use of supplements, and partly from a high intake of dairy products, particularly in regions such as North America and Northern Europe where these products are fortified with additional vitamin D. This is likely to confound attempts to test the association between AED use and bone loss in children, a group in whom a number of studies have been conducted. It also makes application of such studies to the Australian setting problematic, as food fortification with vitamin D is less widespread.
It has been suggested that trauma occurring during seizures is the principal cause of the increase in fractures seen in AED users. Estimates of the proportion of fractures known to have occurred as a result of seizures vary from 25-43% (224, 232, 248). Generalised seizures seem to be particularly strongly associated with fractures (228). The fractures associated with seizures commonly involve the hip, foot/ankle, humerus and wrist (232, 248, 249), although it is likely that the incidence of vertebral fractures is underestimated (223, 232, 249). It is generally held that vertebral fracture rates should be interpreted with caution, because unlike many other fractures there is almost never any clinically-apparent deformity. Given that diagnosis of vertebral fracture(s) relies on a clinical indication for a medical imaging procedure, it is probable that some fractures are never diagnosed. It has previously been shown that 50 – 75% of vertebral fractures may be asymptomatic, and thus only diagnosed by screening radiology, as opposed to that following a clinical indication (250, 251). However, the fracture rate among users of AEDs is approximately doubled even when only considering fractures not associated with seizures (224, 232). Users are at increased risk of falls (224), which may be due to incoordination, ataxia, visual changes, clumsiness or weakness resulting from coexisting neurological deficits or neurological side effects. Female AED users have been shown to be at increased risk of non-seizure falls compared to non-users (31% vs 17%, p = 0.027) and of multiple falls (18% vs 5%, p = 0.028) in a retrospective 12 month study (233). Balance performance is impaired in AED users compared to their matched non-user siblings (252). Increased risk of fracture has also been associated with the use of benzodiazepines, antidepressants and antipsychotics (224). These agents have not been linked with abnormal bone metabolism, adding weight to the concept that underlying brain disease or adverse events of medication can result in falls and fractures.

One obvious measure to prevent fractures in AED users is optimal control of seizures, especially the generalised variety, although this will not reduce non-seizure related fractures. Environmental modification to minimise risk is also important, especially where good seizure control proves elusive. This may include provision and correct use of walking aids, supply of hand rails and ramps, typically obtained with the assistance of an occupational therapist. Avoidance of over-treatment with AEDs should minimise side effects which could result in falls. It has been recommended that prophylactic vitamin D and possibly calcium supplementation should be considered whenever AED treatment is initiated (224, 253). In the general population aged over 50, a 2007 meta-analysis concluded that evidence supports the use of calcium, or calcium in combination with vitamin D supplementation, for the prevention of osteoporosis in people aged 50 and over (207). For best therapeutic effect, this meta-analysis recommended minimum doses of 1200 mg of calcium, and 800 IU of vitamin D. This may also be a reasonable approach for AED users, although the evidence base for the management of AED-
induced bone disease is weak. Higher doses, of the order of 2000-4000 IU/day have been recommended for individuals in whom osteopenia or osteoporosis have developed (253). It is also of interest that a small pilot study has suggested that normalisation of vitamin D levels in insufficient individuals with epilepsy reduced seizure frequency by 40%, although the mechanism for this effect is unclear (254).

In summary, whilst as a class AEDs are associated with reduced bone health, increased risk of falling and increased fracture risk, there is considerable variation between different agents. A more precise delineation of the pathophysiological mechanisms other than altered vitamin D metabolism would be helpful, as would well-designed, broad-based, comparative, and controlled longitudinal studies of this important chronic health issue in patients with epilepsy.
**Intellectual disability and bone health**

It is commonly accepted that people with intellectual disability (ID) have a higher prevalence of both mental health and physical disorders and disability compared with the general population (255, 256). There is a small but consistent body of literature showing that people with ID have poorer bone health than the general population, and suffer from a higher incidence of fractures. Published estimates suggest that fractures occur 1.7 – 3.5 times more frequently among people with ID (257-260). There are several reasons given in the literature for the poor bone health of people with ID, some of which are specific to people with ID, with others have been shown to be true of the general population, and may be reasonably extrapolated to people with ID.

Reduced mobility is a major risk factor, having been shown to cause profound bone loss. Loss of mechanical stress on bones results in both decreased bone formation and increased bone resorption (261), resulting in reduced BMD and thinning of cortical bone. These changes are known to increase fracture risk in the general population (262), a relationship which has also been shown in the population with ID (46).

Adequate vitamin D levels and calcium intake are essential for good bone health, as outlined previously. Low vitamin D levels have been found in people with ID (46, 263). The principal reason for the reduced vitamin D levels is probably lack of exposure to UV light (46, 264) associated with reduced mobility and thus less time spent outdoors. Other factors affecting both vitamin D and calcium may include inadequate dietary intake (46), particularly tube-feeding (260), and the use of AEDs, which as discussed previously interfere with vitamin D metabolism, may also affect calcium metabolism, and appear to have direct effects on bone.

Hypogonadism is more frequent in people with ID, either as a primary event or consequent on early menopause (265). The use of depot progesterone contraception is common amongst women with ID (266), and has also been linked to reduced BMD (267). Both male and female hypogonadism have been shown to be associated with osteoporosis and fractures (268, 269). Reduced BMD has been shown to be associated with ID consistently across all reported studies (260, 270-276).

Falling occurs frequently in people with ID (277), with one cohort study finding that 70% experienced a fall event over a 5 year period (278). In this same study, 79% of falls resulted in an injury, with 7% classified as a serious injury requiring significant medical intervention, and 3% resulting in fracture(s).
Higher injury rates were associated with male gender, mild to moderate levels of disability, group home residential settings, and age below 35 years. Other risk factors suggested by this study reflected a pattern of disability which is not unlike that of an aging population. These include the presence of a physical or sensory impairment, and the presence of comorbid illnesses. Other cohort studies have suggested that greater frequency of seizures is associated with a higher risk of fall events and injury (279).

Other medications have also been implicated in causing fractures in the elderly, including several antidepressants, major tranquilizers and hypnotics/anxiolytics, probably as a result of sedation and balance impairment (280). Given that such medications are very commonly used by people with ID, it is likely that they also contribute to falls and fractures in this group (277).

Down syndrome (DS) is a major cause of intellectual disability, with an incidence of approximately one in every 800 live births, and as such merits specific discussion. DS seems to be an independent risk factor for osteopenia and osteoporosis (281). DS is associated with multiple endocrine disorders that affect bone integrity. Growth retardation, hypogonadism, poor calcium and vitamin D intakes, and muscle hypotonia are recognized risk factors for low bone density in general, and in DS in particular. Compared with age-matched, healthy children, those with DS have lower BMD measurements at the spine, with mean decrements of 1 SD (281). As a result, low-or minimal-trauma spine and femur fractures are common, with the incidence of fracture in the adult DS population over 50 reported to be as high as 85% for long bones and vertebral bodies combined (282). Low BMD in adults with DS is correlated with a significant decrease in bone formation markers, compared to controls without DS, and is independent of gender. These data suggest that diminished osteoblastic bone formation and inadequate accrual of bone mass, with no significant differences in bone resorption, are responsible for the low bone mass in DS (McKelvey, #1267, 283).

In summary, there are multiple factors, some associated with the cause(s) of ID or also part of the syndromes which include ID in their definition, and some as a consequence of treatments often used in people with ID, which result in poor bone health and an increased incidence of fractures.
**Bone Structure & Function**

The skeletal system consists of bone, cartilage and specialised connective tissues such as ligaments and intervertebral discs. Its functions may be grouped into 3 categories:

1. **Mechanical**: Allowing locomotion by providing rigid support and sites for muscle action;
2. **Protective**: As a shield interposed between external forces and/or objects and internal organs and structures, and as a housing for haemopoietic tissues;
3. **Metabolic**: As a dynamic reservoir of ions (especially calcium and phosphate) to allow serum mineral homeostasis, which is in turn vital for the normal operation of numerous metabolic processes.

**Anatomy & Structural Organisation**

There are two major types of bones, long bones and flat bones. Examples of long bones include the radius, femur and tibia. Examples of flat bones include the skull, scapula and ilium. In general, long bones are found in the appendicular skeleton, also referred to as the peripheral skeleton. Flat bones are generally found in the axial skeleton, also referred to as the central skeleton. In order to perform its mechanical role bone must possess two apparently contradictory properties, stiffness and flexibility, as well as the ability to absorb & dissipate energy (284). This is because bones must to varying degrees act as both levers and as springs. Long bones, such as the femur, tend to function primarily as levers. Such bones require greater structural stiffness so that they will bend less and tolerate greater loads (284, 285). Bones such as the vertebrae have a sponge-like structure, allowing them to act as spring-like ‘shock absorbers’ (286).

Macroscopic inspection of a long bone shows a relatively cylindrical, hollow tube in the central region (the diaphysis), wider regions at each end (the epiphysis) and a transitional zone in between (the metaphysis). The diaphysis contains the large, central medullary cavity. In adults, this is filled with yellow (inactive) marrow, which is mostly fatty tissue. Red (active) marrow is confined to the proximal epiphyses of larger adult long bones (287). The outer surface of the epiphysis forms the joint surface and is covered with a layer of articular cartilage. These features are displayed in Figure 3.
Features of long bone (adapted from Knight, 2003 (287))

The external surface of bone is a dense layer of calcified tissue, the cortex. Resistance to bending is a function of the square of the distance between the outer surface of the cortex and the long axis (288, 289). Towards the metaphysis and diaphysis the cortex becomes thinner as it surrounds a sponge-like network of calcified rods and spaces termed trabecular bone, also known as cancellous bone. The vertebral bodies also consist mainly of trabecular bone. Although cortical and trabecular bone are composed of the same cells and matrix elements, they are distinguished by both their structure and function. The mineralised honeycomb of interconnected plates that makes up trabecular bone functions
like a spring, enabling it to absorb energy by deforming reversibly during loading (269). The trabecular structure also results in a larger area of bone surface per unit of volume than is found in cortical bone. Because bone resorption and formation occur at bone surfaces, this means that trabecular bone is substantially more metabolically active than cortical bone. As a result, any changes in bone which result from age, diet, disease or drugs occur more rapidly and are much more pronounced in trabecular bone than in cortical bone (290).

Bone obtains material stiffness by the impregnation of collagen with mineral, predominantly calcium hydroxyapatite, \( \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 \). Increasing tissue mineralisation will increase stiffness at the expense of flexibility (269). Bones which need high levels of stiffness, such as the ossicles of the ear, have a very high mineral content, but lack flexibility. Bones that require greater flexibility have a lower mineral content (269). Structural stiffness is produced in long bones by the hollow, tubular diaphysis, which achieves stiffness without excessive weight.

The cellular component of bone consists of osteoblasts, osteoclasts and osteocytes, the latter embedded in an extracellular matrix. Osteoblasts lie on the inner periosteum and endosteum. They secrete an organic bone matrix in which they become entrapped, forming osteocytes. Osteocytes are located in small cavities called lacunae. Each cell has many protruding cell processes that interconnect with other osteocytes. Osteoclasts are large, multinucleated cells with many branched processes. They resorb bone, as outlined below. Bone matrix has an organic component, osteoid, composed of collagen and proteoglycans, and an inorganic component, predominantly calcium hydroxyapatite. The structural unit of cortical bone is the Haversian system, or osteon. These units effectively form a series of parallel, stacked columns parallel to the long axis of the bone. In the centre of the osteon is a central canal, called the Haversian canal. The central canal is surrounded by concentric layers of matrix called lamellae. These lamellae are laid down one after the other over time, each successive one inside the preceding one. Collagen fibres in a lamella run parallel to each other but the orientation of collagen fibres across separate lamellae is oblique. The fibre density is also lower at the border between adjacent lamellae, which accounts for the distinctive appearance of an osteon (see Figure 4).
Osteoblasts and osteoclasts are derived from precursors originating in the bone marrow. Osteoblasts derive from multipotent mesenchymal stem cells (292). These same stem cells also give rise to bone marrow stromal cells, chondrocytes, muscle cells and adipocytes. Osteoclasts derive from haematopoietic cells of the monocyte/macrophage lineage (293). The development and differentiation of osteoblasts and osteoclasts are controlled by growth factors, cytokines and adhesion molecules. Several systemic hormones as well as mechanical signals also exert significant effects on osteoclast and osteoblast development and differentiation (293). Although many details remain to be established.
concerning the precise operation of this system, a few themes have emerged (293). First, several of the growth factors and cytokines control each other’s production in a cascade fashion, in some cases forming positive and negative feedback loops. Second, the same factor(s) will sometimes be capable of influencing both osteoblast and osteoclast differentiation. Third, systemic hormones exert their effects on osteoblast and osteoclast formation via their ability to control the production and/or action of local mediators.

Growth factors that have been shown to influence cell development and differentiation include bone morphogenetic proteins (BMPs), transforming growth factor β (TGF β), platelet-derived growth factor (PDGF), insulin like growth factors (IGFs) and fibroblast growth factors (FGFs) (292). Bone cells also produce proteins that modulate the activity of growth factors either by binding to them, competing for the same receptors or by promoting the activity of a particular factor. A large group of cytokines and colony stimulating factors that are involved in haematopoiesis also affect osteoclast development. Given the derivation of osteoclasts outlined above, this is not surprising. This group includes several interleukins, leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotropic factor (CNTF), tumour necrosis factor (TNF), granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte colony stimulating factor (M-CSF) and c-kit ligand (292). The two principal hormones of the calcium homeostatic system, parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D₃ (1,25D) are potent stimulators of osteoclast formation, whereas calcitonin inhibits osteoclast development and promotes osteoclast apoptosis.

Several other hormones regulate the production and/or action of several cytokines (292). Oestrogen acts on both osteoblasts and osteoclasts to regulate bone mass (294), with the decline in oestrogen levels after menopause leading to increased bone resorption (269). Androgens have been shown to act directly on osteoclasts, although their effects on osteoblasts are less well understood (295, 296). Testosterone also exerts indirect effects on bone through its aromatisation to oestrogen (297). Glucocorticoids impair the replication, differentiation and function of osteoblasts and induce the apoptosis of mature osteoblasts and osteocytes, suppressing bone formation (298). Glucocorticoids also favour osteoclastogenesis and as a consequence increase bone resorption (298). Thyroxine directly stimulates both osteoclastic and osteoblastic activity, with a predominance of bone resorption during hyperthyroidism, resulting in decreased bone mass (299).

Most adult skeletal disease are due to excessive osteoclastic activity, leading to an imbalance of bone remodelling which favours resorption (300). Such diseases include osteoporosis, periodontal disease,
rheumatoid arthritis, multiple myeloma and metastatic cancers (301). Peri-prosthetic osteolysis (i.e. immediately surrounding joint prostheses) also falls into this category (302). It has been shown that two haematopoietic factors are both necessary and sufficient for osteoclastogenesis, the TNF-related cytokine RANK ligand (Receptor Activator of Nuclear factor Kappa b ligand) and the polypeptide growth factor CSF – 1 (Colony Stimulating Factor 1) (301). Together, these factors induce expression of genes that typify the osteoclast lineage, including those encoding tartrate-resistant acid phosphatase (TRAP), cathepsin K (CATK), the calcitonin receptor and β3 – integrin, which leads to the development of mature osteoclasts. Osteoprotegerin (OPG) has been shown to act as a natural antagonist of RANK ligand, and thus suppresses osteoclast differentiation and activity (303). Loss of oestrogen after the menopause may result in the removal of an important control on RANK ligand action, and decrease the synthesis of OPG (304).

In addition to autocrine, paracrine and endocrine signals, cell-cell and cell-matrix interactions are also integral to osteoclast and osteoblast development. These interactions are mediated by proteins expressed on the surface of these cells, known as adhesion molecules. These are responsible for contact between osteoclast precursors and stromal/osteoblastic cells. They are also involved in the migration of osteoblast and osteoclast progenitors from the bone marrow to sites of bone remodelling and the control of osteoclastic bone resorption. The list of adhesion molecules that influence bone cell development and function includes the integrins, selectins and cadherins, as well as a family of transmembrane proteins containing a disintegrin and metalloprotease domain (292). Each of these proteins recognises distinct ligands.
Bone modelling and remodelling

Bone modelling is defined as the process by which bone formation occurs alone, without prior bone resorption (290). This occurs during skeletal growth and during fracture repair. Bone remodelling is the continual destruction and reformation of bone, a process which is lifelong. These processes are coupled, with formation preceded by resorption. Remodelling has a structural function, preserving skeletal integrity by allowing bone to respond to altered mechanical loading and to repair accumulated damage. It also has a metabolic function, primarily concerned with the maintenance of serum calcium homeostasis. It is estimated that 5-10% of the skeleton is renewed by remodelling each year (290). Remodelling activity is 5-10 times higher in trabecular than cortical bone (269).

Bone remodelling follows a set sequence of events. Osteoclasts resorb a volume of bone, leaving a small defect. After a period of delay known as the reversal phase osteoblasts fill the cavity with new bone matrix. The matrix undergoes primary mineralisation (70-80% of full mineralisation) quite rapidly, followed by a slower process of secondary mineralisation. The time period from commencement of absorption to completion of primary mineralisation is typically 3-6 months. During this time a resorption pit of approximately 60-100 μm in depth will have been excavated and completely refilled with new bone matrix (305).

In normal circumstances the tight coupling between resorption and formation results in no net bone loss or structural damage. As described above, in a number of disease states resorption exceeds formation, resulting in a net deficit in bone mass. This also occurs as a result of falling oestrogen levels after the menopause.
Growth and Ageing

From a macroscopic, structural perspective, long bones may be regarded as hollow tubes that grow in length (306). As they do so, the central cavity also enlarges such that the cortical ‘shell’ moves further away from the central axis of the bone. Hormonal changes at puberty result in the fusion of the epiphysis to the diaphysis, leading to growth arrest (307). The effects of oestrogens and androgens differ, causing males to develop longer and wider bones with a slightly thicker cortex than females (308). The greater strength of long bones in males than in females is thus the result of differences in bone size and geometry, not density. However, oestrogen is still important for bone health in males. Experiments using mice with inactivated sex steroid receptors demonstrated that both activation of the oestrogen receptor (ER)α and activation of the androgen receptor result in a stimulatory effect on both the cortical and trabecular bone mass in males (309). There is also clinical evidence suggesting that a threshold exists for oestrogen effects on bone in men, with rates of bone loss and fracture risk being highest in men with oestradiol levels below 40 pmol/L (310, 311). Growth builds a bone that is bigger, not more dense (269). Vertebral bodies in adult males are also wider and slightly taller. The larger male skeleton is able to tolerate a larger load than the female skeleton (269). The average absolute load imposed on the skeleton is greater in men than women because men tend to be taller and heavier than women. However, the load per unit area is identical because of the bigger skeleton (289).

Peak bone mass is achieved during the third decade of life, although 90% of this peak is typically attained by adolescence (308, 312). It has been estimated that genetic factors account for 80% of peak bone mass (308). Factors known to adversely affect peak bone mass include lack of weight-bearing exercise, inadequate calcium intake and smoking. In women, bone density generally remains stable for the 10-15 years following attainment of peak bone mass (313). Bone loss in the proximal femur usually starts premenopausally, typically in the fourth or fifth decade. Vertebral bone loss before the menopause is less well established. In men, bone loss probably starts in the fifth decade (314), and accelerates after the age of 70 (315).

Bone size varies little during adult life, apart from a continuous and slight expansion of bone outer dimensions. This is found mainly in men, and affects both the axial and appendicular skeleton (316). However, endosteal absorption continues in long bones, resulting in a gradual expansion of the marrow space (316). The rate of this endosteal absorption exceeds the external periosteal expansion, resulting in a thinning of the bone cortex. With age, the efficiency with which osteoblasts refill resorption cavities is reduced (314). Age-dependent bone loss is thought to be a result of this phenomenon, together with
an increase in cortical porosity and destruction of cancellous bone through trabecular thinning and perforation (67). Sex hormone deficiency, most notably oestrogen deficiency in postmenopausal women, results in increased bone turnover, with an imbalance between bone formation and resorption (314). Increased bone remodelling or bone turnover has been shown to be a predictor of osteoporotic fractures independent of absolute bone mineral density (317). It is believed that this increase in skeletal fragility results from both decreasing bone mass and deteriorating microarchitecture due to trabecular plate perforations (67). The initial bone loss following the menopause is mainly trabecular, probably because of the higher metabolic activity of trabecular bone, but with the passage of time bone loss is approximately equal in both trabecular and cortical bone, resulting in increased porosity (316). Although men do not have the equivalent of the menopause and hence lack the rapid early phase of bone loss present in postmenopausal women, rates of age-related bone loss in elderly men are comparable to those in older women (318-320). This translates into a significant increase in the risk of osteoporotic fractures in elderly men (321). Incidence rates of vertebral fractures among men decline at older ages, in contrast to women where they continue to rise (322, 323). Factors other than BMD are likely to be important in men, given that the majority of fractures occur in men who are not osteoporotic by BMD criteria (324).

In summary, bone is a largely rigid, yet dynamic, organ that is continuously moulded, shaped and repaired. Bone microstructure is patterned to provide maximal strength with minimal mass, as determined by the physiological needs of the body. Imbalances in the control of remodelling result in abnormal skeletal structure and function, thus contributing to morbidity and in some cases early mortality.
Genetic determinants of bone health

In the past decade there has been considerable interest in the genetic variations which predispose individuals to a wide range of diseases, with several genetic variations having been identified in the VDR, expression and nuclear activation of which are necessary for the effects of vitamin D. DNA sequence variations, which occur frequently in the population, are referred to as “polymorphisms” and can have biological effects. To test whether there is a linkage between VDR polymorphisms and diseases, epidemiological association studies are performed. Other examples which have been studied include the oestrogen receptor and the collagen type I A gene (325). In these studies, the presence of a variation of the gene is studied in a population of patients, and then compared to a control group. These association studies are aim to establish a link between gene polymorphisms and diseases. Since the discovery of VDR polymorphisms a number of papers have been published studying its role in bone biology and also non-skeletal diseases. Linkage analysis in monogenic bone disorders such as osteoporosis-pseudoglioma syndrome, sclerosteosis, high-bone-mass syndrome and Paget's disease have yielded important advances in recent years and highlighted the importance of the WNT signalling (a network of proteins that propagates signals from the cell surface to the nucleus) (326), and RANK-RANKL-OPG pathways in the regulation of bone mass and bone turnover (327). However, linkage studies for the common form of osteoporosis have had limited success (328). As with other complex diseases, most of the candidate gene association studies in osteoporosis have produced conflicting results with limited replication (328), mostly owing to small sample sizes (329). However, large studies of major candidate gene polymorphisms have been successful in obtaining consistent evidence of association between some genetic variants, BMD and fracture risk (330-333).

A different approach is taken in a genome-wide association study (GWAS), in which the density of genetic markers and the extent of linkage disequilibrium are sufficient to capture a large proportion of the common variation in the human genome in the population under study, and the number of specimens genotyped provides sufficient power to detect variants of modest effect. GWAS is able to analyse rapidly and cost-effectively the genetic differences between people with specific illnesses and healthy individuals. The purpose of the studies is to explore the connection between specific genes, i.e. genotype information, and their outward expression, i.e. phenotype information, and to facilitate the identification of genetic risk factors for the development or progression of disease. Unlike linkage studies, which aim to test an hypothesis regarding the association between a specific variant gene and a disease state, GWAS effectively performs an hypothesis-free search for genetic determinants (334). Several loci have already been identified that are associated at a GWAS level with BMD (335, 336),
some of which encode members of the Wnt and RANK-RANKL-OPG signalling pathways, highlighting their role in monogenic forms of osteoporosis as well as in the common form.

In summary, this is a rapidly expanding area which shows considerable promise in terms of clarifying the role of genetic factors in bone health and emphasising the importance of signalling pathways which have already been identified. In the future it is likely that genetic approaches will assist in identifying individuals at greater risk of poor bone health and fractures, and also in the design of novel therapeutic agents.
Polyunsaturated fatty acids and bone

Polyunsaturated fatty acids (PUFAs) are essential fatty acids necessary for normal cellular function at all stages from conception to adulthood (22). They have been divided into 2 main classes, n–3 and n–6 fatty acids, according to the placement of a carbon – carbon double bond at their methyl end, which determines their saturation. The n–3 fatty acids eicosapentanoic acid (EPA) and docosohexanoic acid (DHA) are obtained mainly from fish in the human diet, and to a lesser degree from plants (21). The principal n–6 fatty acid, linoleic acid (LA) is derived from vegetable oils (21). The n–3 and n–6 fatty acids are incorporated into the phospholipid membranes of several cell types. In turn, the enzymes cyclooxygenase, lipooxygenase and epoxygenase convert arachidonic acid (AA) into prostaglandins, leukotrienes, and thromboxanes. Ingestion of dietary n–3 fatty acids leads to these compounds partially displacing the n–6 fatty acids, especially AA, from cell membranes, altering their n–6/n–3 ratio and thus altering their function. Increasing proportions of n–3 fatty acids can decrease the production of substances such as IL-1, IL-6 and TNF-α, as well as prostaglandin E₂, thromboxane A₂ and leukotriene B₄ (337). These cytokines are important mediators of platelet aggregation, immune response and vasoreactivity. A diet rich in omega –3 fatty acids may have beneficial effects in a wide range of normal developmental processes and disease states. This list includes cardiovascular disease, infant brain & retinal development, autoimmune disorders, cancer (breast, colon, prostate) and arthritis, and is likely to continue to expand as further research is conducted (22). Human studies suggest that omega –3 fatty acids and the omega –6/omega –3 ratio alter calcium absorption, bone turnover, peak bone mass and postmenopausal bone loss (23, 338). However, few clinical studies have been performed and the current data are inconclusive.

As described above, human bone is subject to a continuous process of remodelling. Remodelling has a structural function, preserving skeletal integrity by allowing bone to respond to altered mechanical loading and to repair accumulated damage and a metabolic function, primarily concerned with the maintenance of serum calcium homeostasis. Remodelling is the result of the balance of bone resorption, performed by osteoclasts, and bone formation, performed by osteoblasts. In normal circumstances the tight coupling between resorption and formation results in no net bone loss or structural damage. However, if resorption exceeds formation, then a net deficit in bone mass and alteration of microarchitecture will ensue, which is termed osteoporosis.

In animal models, n–3 fatty acids have been shown to inhibit osteoclast activity and to promote osteoblast activity, thus favouring bone formation over bone resorption (339). N–3 fatty acids have
also been shown in animals to potentiate the effects of oestrogen on bone (340), to reduce bone loss during oestrogen deficiency (341), and to moderate peroxisome proliferator-activated receptor γ (PPAR-γ) (337), which influences the marrow adiposity which accompanies osteoporosis. It appears that the n – 6/n – 3 ratio may be important, in addition to the absolute quantities of n – 3 fatty acids ingested (341). It is known that lipid metabolism differs between animals and humans, so data cannot always be easily extrapolated.

Human studies have suggested that n – 3 fatty acids and the n – 6/n – 3 ratio alter a variety of markers of bone health. Griel et al (342) performed a controlled-feeding study on 23 healthy volunteers, in order to investigate the association between markers of bone turnover and dietary plant-sourced n – 3 PUFA intake. Markers of bone resorption were suppressed, suggesting that the incorporation of plant sources of n – 3 PUFAs may be beneficial to bone health. Hogstrom et al (343) examined the relationship between serum concentrations of n – 3 PUFAs and bone accrual in 78 healthy young men with a mean age at study entry of 16.7 years. N – 3 PUFA levels were measured at age 22, with DEXA BMD measurement at entry, age 22 and age 24. They found a positive correlation between n – 3 PUFA concentrations and changes in BMD (total and spinal) between the ages of 16 and 22, an effect which was most pronounced for DHA. Weiss et al (344) used self-administered food frequency questionnaires to investigate the association between the ratio of dietary n – 6 to n – 3 PUFAs in 1532 community-dwelling men and women aged 45 – 90 years. They found that a higher n – 6/n – 3 ratio was associated with lower hip BMD in both genders, and with BMD at the spine in women who were not using hormone replacement therapy. Collectively, these studies suggest that PUFA intake and type/ratio may play a role in bone health, both in achieving optimal peak bone mass and in maintaining skeletal integrity in old age. However, they represent a very modest number of participants and have very diverse protocols, and so cannot be regarded as providing a coherent narrative.
Vitamin D and Aboriginal Australians

Little is known about the vitamin D status of Aboriginal Australians. They represent only about 2.5% of the total Australian population (6), and for a variety of reasons have not always been a population group in which conducting research has been seen as straightforward (345).

One small study found that one of the potential sequelae of vitamin D deficiency, muscle pain, is highly prevalent in Aboriginal people, and that it is often associated with low 25D levels (112). Hip fracture rates may provide an indirect indication of population vitamin D status, given that there has been an association shown between femoral neck fractures and vitamin D levels (45). A study from North Queensland found that the age-standardised rates for femoral neck fracture were significantly higher for indigenous males. However, the rates for females were identical in the indigenous and non-indigenous groups, and the female indigenous mean age was significantly greater than the non-indigenous mean age (346). Aboriginal Australians have reduced life expectancy when compared with the total population (20), which could reduce the impact of diseases which are usually associated with advanced age, including osteoporosis. However, the higher incidence and earlier age of onset of a number of other diseases associated with ageing in Aboriginal Australians suggests that bone health may still be important. These diseases include cardiovascular disease, type 2 diabetes and some cancers (20).

There is a larger body of literature regarding the vitamin D status of other dark-skinned groups, including black/African Americans (8, 16, 347, 348), Pacific Islander and Maori people (9, 349) and Canadian Aboriginal women (166), which consistently shows that they have lower 25D levels than those seen in comparable non-indigenous Caucasian populations. It is likely that this is also true of Australian Aboriginals. However, the consequences of these lower 25D levels have yet to be fully defined. From an evolutionary perspective, if it is assumed that darker-skinned people groups have experienced lower 25D levels than light-skinned people groups over many generations, one would expect that their physiology will have adapted to these lower levels. Research in North America has shown differences in vitamin D levels between different racial groups (16), with the suggestion that both 25D and 1,25D levels are closely linked to levels of vitamin D binding protein, which is in turn strongly genetically determined (350). Variations in vitamin D binding protein have also been associated with fasting plasma insulin levels in several ethnic groups (351-353).

A number of studies have investigated differences in calcium, vitamin D and PTH and bone health between American blacks and whites. The incidence of hip fractures (354, 355) and non-vertebral
fractures as a group (356) is lower in black than in white Americans, and BMD values have been reported to be higher in blacks than in whites (357, 358). Some investigators have found that serum 25D was significantly lower and serum 1,25D was significantly higher in both black adults (347, 356, 359, 360) and black adolescents (361) when compared to whites, although other studies did not confirm these differences (362, 363). Similarly, serum PTH was significantly higher in black individuals in some studies (347, 360, 362) but not all (363, 364). The 25D level threshold at which PTH levels begin to rise has been used to infer the optimal 25D level for bone health, with some data suggesting that black American women tolerate lower 25D levels before PTH begins to rise (365). However, there is a wide variation in the reported threshold levels in different regions, which may be at least partially due to significant heterogeneity between 25D levels as measured by different laboratories (366). Bone turnover markers suggest that bone turnover is either higher in blacks than in whites (347) or at least similar (360). Urinary calcium excretion has consistently been lower in black than in white people (347, 360, 361, 363). A detailed study of calcium absorption in response to calcitriol (1,25D) showed a lower response in blacks than in whites, suggesting a gut resistance to 1,25D (360). It has also been estimated that black American women require 300 mg/day less calcium than do white women (367). Bone strength is also a function of geometry, with black American women having been shown to have different bone geometry from white American women, particularly in their cross-sectional areas at the neck of femur, which increases bone strength (368).
The first paper to be presented in this thesis, “Vitamin D and fractures in people with intellectual disability”, was published in 2006 in the Journal of Intellectual Disability Research. It is the result of a short period working as a locum general practitioner at Strathmont Centre, a residential facility for intellectually disabled adults located in suburban Adelaide. During my period working at Strathmont I was quite surprised to see several patients who had sustained fragility fractures at a young age, when such fractures would normally be associated with much older individuals in the general population. These fractures included sites such as the pelvis, neck of femur, femoral shaft and humerus. I also noticed that the practitioner whose absence I was covering for had measured vitamin D levels in some patients, and that many of the levels were very low. A short time later I commenced my position as a Lecturer at the University of Adelaide and took the opportunity to perform a literature search to try to learn more about what I had observed whilst at Strathmont. I was struck by the lack of literature in this area, and approached my now-returned colleague, Dr Michael Nugent, to see if he would be interested in researching the matter. He agreed, and this paper is the means by which we have reported our findings.

ID is a collective diagnostic label which has a wide range of possible aetiologies. Deinstitutionalisation of care of people with ID has led to most of their primary health care being delivered in community general practice (369). Among the more common underlying causes are cerebral palsy, Down syndrome, neurofibromatosis, Fragile X syndrome, Noonan syndrome, Prader-Willi syndrome, Angelman syndrome, Rett syndrome, tuberous sclerosis, phenylketonuria and Williams syndrome (370). As would be expected, a wide range of possible underlying causes results in an even wider range of possible adverse health outcomes. Although some such outcomes are specific to particular underlying diagnoses, several issues have been shown to occur frequently in people with ID as a group.

People with ID have high levels of impaired vision, hearing or both (371, 372), especially people with Down syndrome (282, 373). Up to 50% of people with moderate to severe ID have gastro-oesophageal reflux (374), with *H. pylori* infection being common, especially in the institutionalised (375, 376). Oesophageal stricture, peptic ulcer disease and Barrett’s oesophagus are all more common than in the general community (376). Predisposing factors include impaired mobility, scoliosis and drugs, particularly AEDs and psychotropics (377). Common dental problems include poor oral hygiene,
gingival disease, untreated caries and heavy tooth wear due to grinding of the teeth (378). As discussed elsewhere in this thesis, epilepsy is common in people with ID, especially in those affected by cerebral palsy. This epilepsy is often severe and difficult to treat, with high rates of clusters of seizures, prolonged seizures and status epilepticus (379). The rates of psychiatric disorders including schizophrenia, depression and anxiety are much higher in people with ID than in the general community (380). Underlying brain damage, social isolation and lack of autonomy may all be contributing factors. Behavioural disorders such as aggression, inappropriate sexual expression, destructive behaviour and self-injurious behaviour are common in adults with intellectual disability (380). Such behaviours may cause injury and/or lead to exclusion from activities. Behavioural problems are more likely in the more severely disabled, particularly those with limited communication skills, and are believed to often reflect frustration (381). Premature Alzheimer’s disease is common, particularly in people with Down syndrome (282). With regard to health screening and preventive medicine, it has been shown that women with ID have low rates of cervical cancer screening, breast screening and screening for risk factors for cardiovascular disease, including blood pressure and lipid levels (382).

In summary, people with ID are at increased risk of developing a wide range of chronic diseases, compounded by problems with communication and behavioural issues. This has been recognised by the academic community in Australia, resulting in the development of a comprehensive health assessment tool for people with ID, intended for use in general practice (383). This has been shown to produce substantial increases in health-promotion and disease-prevention activity, when compared with usual care (384). Funding for an annual assessment of this type is provided by Medicare (385).

The research which led to this paper was conducted without any grant funding, and I was greatly assisted by Dr Nugent and also by medical students on placement at Strathmont Centre, who volunteered to access the participants’ medical records and extract the necessary information. At that time the medical records were partially electronic and partially paper-based. The medical records program was used principally for prescribing of medications and as such was not structured or coded in a manner which allowed for computerised searches or data extraction. The process of going through each paper file manually was labour-intensive and relied on volunteers. Some items of information, for example the menopausal status of female participants, were not routinely collected and/or included in the patient summary section of each file, which meant that they could not be included in the retrospective audit component of the research. Detailed dietary information for each participant was also not available, as the staffing levels at Strathmont Centre did not permit routine recording of individual dietary habits and consumption for all residents. However, I was advised that the meals
provided were under the supervision of a dietician, and that the intended minimum calcium intake for each resident was 1200 mg daily. There would undoubtedly have been a number of participants who did not always consume all food and drink offered to them, and so some must have had a lower calcium intake than that which was targeted, but I had no means of quantifying this more precisely.

Scopus states that this paper has been cited 8 times. It also led to our being invited to take part in a working party under the auspices of the Australian Association for Developmental Disability Medicine. This Working Party produced a guide for clinicians, of which I was the first-named author. “A guide for the assessment and management of vitamin D status in people with intellectual disability (developed as an AADDM Working Party initiative)” was published in the Journal of Intellectual & Developmental Disability in 2008. A sub-group of the working party also produced a paper designed for general practitioners, rather than those with a special interest in intellectual disability, and this was published in Australian Family Physician in 2008. I was also the first-named author of this paper. At conferences I have been an invited presenter on this topic twice (Intellectually Disabled Service Council Annual Meeting, 2006; Hanson Centre Bone & Joint Symposium, 2007), and have had submissions accepted for presentation 4 times (Australia & New Zealand Bone & Mineral Society ASM 2005; Australian Association for Developmental Disability Medicine ASM, 2005 & 2007; The Osteoporosis Society (UK) ASM, 2007).

NOTE:
This publication is included on pages 63-69 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1111/j.1365-2788.2006.00841.x
**Paper 2 : “Vitamin D insufficiency in Aboriginal Australians”**

The second paper presented in this thesis, “Vitamin D insufficiency in Aboriginal Australians”, was published in the Medical Journal of Australia in 2011. It also sprang from questions arising from my day-to-day clinical practice. In 2005 I began working part-time at Nunkuwarrin Yunti, an Aboriginal community-controlled health service located in central Adelaide. Conversations with other medical staff at the service indicated that we believed that vitamin D insufficiency and deficiency were more prevalent in our Aboriginal patients than was the case for the general community. Once again, a literature search revealed only one published paper which addressed the issue of vitamin D levels in Aboriginal Australians. This paper had a sample size of 8, and examined 25D levels in patients presenting with muscle pain rather than attempting to describe the range of 25D levels seen in healthy individuals, so I was encouraged by my colleagues to design and conduct research into this area. This was a logical extension of my existing interest in vitamin D, bone health and distinctive population sub-groups. Colleagues from Tullawon Health Service in the far west of South Australia heard that the research was being conducted, and asked if they could also participate, which added a very small number of rural/remote participants.

The conduct of research involving Aboriginal Australians brings with it additional requirements. Approval from an Aboriginal Ethics Committee is required, in this case the Aboriginal Health Council of South Australia’s Aboriginal Health Research Ethics Committee (AHREC). When I first began to discuss the possibility of conducting a research project at Nunkuwarrin Yunti it was made clear to me that the local Aboriginal community was concerned to ensure that any such research had a clear likelihood of being beneficial to the community, and that it would not impose any undue burdens on participants or on the health service staff. Some elements of research may have complicated connotations for Aboriginal people. An example of this sort of area of particular sensitivity was my wish to attempt to test for an association between depth of skin pigmentation and vitamin D blood level. I
was informed that many Aboriginal people find the issue of skin colour to be uncomfortably reminiscent of attempts made in earlier times to quantify an individual’s ‘degree’ of Aboriginality (e.g., half-caste, quarter caste, octoroon etc). Apart from appearing nonsensical to many Aboriginal people (“Which quarter of me is the black bit?”, as one community elder asked me), this classification is perceived as having been an integral part of attempts to discriminate against Aboriginal people, and to justify enforced removal of children from their birth families. Once the rationale was explained, together with the efforts taken to standardise/objectify the classification (participants’ skin colour on the inner forearm was compared with a reference coloured guide), this issue was rapidly resolved, however. It should be noted that the method used for assessing skin colour was not formally validated. Rockell et al (9) describe using reflectance colorimetry, but in the absence of a colorimeter we attempted to standardise assessment by providing photographic examples of each category (pale, intermediate, dark) and conducting training attended by staff members who would be classifying skin colour.

The literature regarding the conduct of research in an Aboriginal community-controlled health service is very limited—I could only find one paper which dealt with this specific issue. However, this paper, by Sibthorpe et al, published in 2002, was catalysed by the authors’ experiences in attempting to conduct research at Nunkuwarrin Yunti, the same organisation that hosted my research project (345), and as such had a significant effect on the way in which I planned and developed my research proposal. Drawing on their own experiences and also referring to a limited literature set which was not specific to indigenous research, the authors emphasised the following key features for a successful research project. The research processes must fit with existing clinic processes, and must not be perceived by staff as being a “hassle”; the project should attempt to avoid areas which might cause resentment or be regarded as embarrassing by participants; any questionnaires or other instruments must be capable of easy administration by Aboriginal health workers, and must avoid perceptions of excessive length or intrusiveness; perception of pressure on Aboriginal health workers to recruit participants should be
avoided, as they are likely to result in reduced recruitment rates; participants need to perceive some benefit or incentive for their participation in the project.

With this in mind, a single-page questionnaire was designed in consultation with a senior Aboriginal health worker and presented for discussion and endorsement to the clinic team as a whole. Whilst this was important in enabling the project to proceed, it did result in some limitations being placed on the project methodology. The most significant of these was the exclusion of a detailed dietary questionnaire with particular reference to calcium intake. Whilst this would have substantially enhanced the value of the study, the clinic team was unwilling to add to the length and complexity of the study questionnaire, and so this aspect was omitted.

Participant recruitment proved slower and more difficult than expected, despite the lengthy and extensive consultative process involving community members and the clinic team. Reasons given for this by clinic team members included the length of time and administrative burden involved in explaining the project and obtaining informed consent, which was felt to be difficult to fit into the busy clinical routine, and the significant number of individuals who were already under investigation for possible vitamin D deficiency or taking vitamin D supplementation. The inclusion of individuals who were already under investigation for suspected vitamin D deficiency would have increased participant numbers with very little additional effort, but would have biased the study sample population, and would have led to an overestimation of the prevalence of deficiency. Inclusion of participants from the Tullawon Health Service was very welcome, as I hoped that in addition to boosting overall participant numbers this would also allow comparison between the metropolitan Nunkuwarrin Yunti population and their more remote Tullawon counterparts. Unfortunately, despite the enthusiasm of the Tullawon staff, numbers were small (7 participants). Tullawon staff suggested that this was partly because of the high frequency with which individuals were being investigated for vitamin D deficiency (thus excluding them
from the project), and also because of the high rate of non-attendance by participants for their fasting blood tests. The small number of participants from Tullawon precluded statistical comparisons between the two groups. The small overall sample size also precluded a proper investigation of the relationship between serum 25D levels D status and fasting glucose. Assuming an effect size similar to that reported by Von Hurst and Sabharwal, a sample size of 202 would have been required for adequate statistical power, using an $\alpha$ value of 0.05. Despite the difficulties in both design and conduct, this remains the first published study of its type and can be used to inform future research.

Scopus lists this paper as having been cited 3 times. Material from this study has also been accepted for presentation at 3 conferences (The Vitamin D Conference, 2009; Australia & New Zealand Bone & Mineral Society ASM, 2010; International Osteoporosis Foundation Asia-Pacific Regional Meeting, 2010).
*Medical Journal of Australia, v. 194(3), pp. 131-134*

NOTE: This publication is included on pages 74-77 in the print copy of the thesis held in the University of Adelaide Library.
Paper 3: “Efficacy and tolerability of calcium, vitamin D and a plant-based omega-3 oil for osteopenia: A pilot RCT”

The final paper presented in this thesis, “Efficacy and tolerability of calcium, vitamin D and a plant-based omega-3 oil for osteopenia: A pilot RCT”, was accepted for publication in Maturitas in October 2011. It also arose from questions relating to my clinical interests, in addition to continuing my ongoing interest in vitamin D & bone health. Because of this interest in bone health I had acted as a consultant to Osteoscan, a community-based bone mineral density testing service. I observed that patients with established osteoporosis had ready access to effective medications via the Pharmaceutical Benefits Scheme (PBS) which could reduce the risk of future fractures. However, options available to patients with osteopenia (i.e. whose bone density was reduced, but not yet in the osteoporotic range) were much more limited. International (386) and Australian (387) studies have shown that most fragility fractures occur in these osteopenic individuals, primarily because they are so much more numerous than those with osteoporosis. Therefore, any strategy which aims to reduce fragility fractures must also take into account people with osteopenia. My reading of the literature indicated that the omega-3 fatty acids might have a beneficial effect, with the added bonus that this nutritional approach may be attractive to those individuals who have a reluctance to use prescription medications.

The project was conducted with the aid of a small grant ($5000) from the Royal Australian College of General Practitioners. This precluded a full-scale RCT, which would also have been premature given the very limited evidence-base in existence at the time that this project was being planned. Our project had elements that were novel and distinctive. It investigated the use of a plant-origin omega-3 supplement, in contrast to the majority of published studies where marine animal (i.e. fish) sources were used. It also incorporated assessment of both BMD and bone turnover, which had not previously been documented in studies of the effects of omega-3 fatty acids. We also recognised that the
requirement for participants to take 4 tablets daily might prove too onerous, and wished to assess how good adherence to such a regime would be.

As expected, the small sample size of this pilot project did mean that statistical power was low and firm conclusions could not be drawn regarding the effects of the study agent (an omega-3 oil). However, the project did show that the regime used was feasible, with good tolerability and acceptability combined with high adherence. It also gave an indication of the effect size of the study agent on bone turnover, which allows sample size calculations to be performed to aid the design of future projects.

Specifically, assuming an effect size of a 10–15% suppression of c-terminal telopeptide by docosahexanoic acid and a power value of 80%, 360 participants would be required to properly examine this relationship. Finally, it also confirmed a relationship between bone turnover and changes in BMD, which has been sparingly documented elsewhere in the literature.

This paper deals specifically with the issues of bone density and bone turnover. As discussed earlier, these are independent predictors of future fracture risk. However, there are other important risk factors, including age, gender, history of having fallen, history of previous fractures and medication use. Although these were not examined in this study, it is worth noting that Osteoscan, the service at which the DEXA testing was conducted, routinely provides estimates of future fracture risk using validated tools such as the FRAX tool (388) and the Garvan Institute calculator (389). These tools integrate a range of clinical, historical and biometric data in order to provide a more wholistic estimate of risk. These tools allow a more accurate stratification of risk than that provided by use of bone densitometry alone.
This paper has yet to be cited. However, I did have a submission for presentation accepted for an oral presentation at the Royal Australian College of General Practitioners’ ASM, in October 2011.
Maturitas, v. 71(1), pp. 44-48

NOTE:
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**Conclusion**

Vitamin D is essential for a wide range of physiological processes involving numerous cell types and organ systems. Although attention has historically been focused upon bone health and fractures, a growing body of evidence has associated vitamin D deficiency with a wide range of disease states and adverse health outcomes. Although the depth and quality of the available evidence from intervention studies is highly variable, it appears that rendering individuals vitamin D replete, usually via oral supplementation, has significant positive health effects. Further investigation via well-designed, adequately powered prospective studies is urgently required in order to give define more clearly the precise benefits of vitamin D repletion, especially in areas other than bone health.

These three community-based, quantitative studies, using different methodologies, have shown that there are sub-populations which are at particularly high risk of vitamin D deficiency. The first study showed that fractures and vitamin D deficiency are common among intellectually disabled people, and provided carers and GPs with a list of readily observable patient characteristics which may highlight those individuals at highest risk of deficiency. It also suggested that both daily and 4-monthly oral supplementation may help achieve vitamin D sufficiency in this population, and that such supplementation should be routinely considered in those at highest risk. As judged by invitations to present, requests for advisory panel participation and direct inquiries to the author, there was a strong response to the publication of the study's results, and it is likely to have influenced the care provided to this vulnerable group. In order to properly establish the benefits of vitamin D repletion in this group, as judged by an effect on falls and fractures, a substantially larger interventional study, or studies, of longer duration will be required. This project will aid in the design of such studies, but given the barriers to obtaining funding for research into the effects of a non-proprietary agent in a ‘hidden’ sub-population, it is difficult to be optimistic that these studies will be conducted and reported on in the near future.
The second study, conducted in the challenging environment of an Aboriginal community-controlled health service, was the first to attempt to describe the vitamin D status of Aboriginal Australians. It confirmed the hypothesis that adult Aboriginal people are at high risk of vitamin D deficiency, and suggested that sun exposure is the major means by which vitamin D is derived in this group. It adds to the broader literature concerning the vitamin D status of darker-skinned populations globally, and will aid the design of future studies. The current interest in genetic determinants of risk factors for diseases and disease states, and in epigenetic influences on disease development suggests that Aboriginal Australians have the potential to be a group of special interest who may add to the broader literature in these areas. However, because of the clear requirement for community engagement in and endorsement of research projects involving Aboriginal and Torres Strait Islander people, and the sensitivities which have arisen as a result of past practices and perceptions, the decision as to whether further research is undertaken and what the nature of this research will be must be shared between the scientific/clinical community and the indigenous community.

The third study showed that a novel regime of calcium, vitamin D and DHA was tolerable and acceptable to osteopenic individuals, who demonstrated good adherence. In fracture risk and prevention terms, osteopenic individuals could be regarded as the ‘forgotten’ population, as they have very limited eligibility for subsidised treatments, despite the fact that the largest absolute number of fractures occurs amongst them as a group. It also gave an indication of the effect size of the study agent on bone turnover, which allows sample size calculations to be performed to aid the design of future projects. Finally, it confirmed a relationship between bone turnover and changes in BMD, which has been sparingly documented elsewhere in the literature and may be of use in assessing response to therapy. Since this paper was published there have been other publications suggesting that bone health omega-3 PUFA intake (390, 391). Further research is needed to define the optimal type and
dose of PUFA, and to quantify more precisely any beneficial effect(s). It is also possible that timing of intake (i.e. during youth versus senescence) may also be important (392).

Vitamin D insufficiency and deficiency are common in Australia, with reported general population prevalence varying from 31 – 67%, depending on which region is being discussed (393, 394). In many cases, appropriate sun exposure, preferably whilst undertaking weight-bearing exercise, is likely to be helpful. A combination of targeted testing of vitamin D levels, and where appropriate simple, inexpensive and safe supplementation with vitamin D is effective at producing adequate vitamin D levels, with the probable outcomes of a reduction in falls and fractures. Additional beneficial effects on other disease states are widely speculated upon, but await the outcome of large scale clinical trials in order to be fully described.
Bibliography

92


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