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Review

The Problems of Vitamin D Insufficiency in Older People

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ABSTRACT: This report reviews evidence on disorders related to inadequate vitamin D repletion in older people. Vitamin D is as essential for bone health in adults as in children, preventing osteomalacia and muscle weakness and protecting against falls and low-impact fractures. Vitamin D is provided by skin synthesis by UVB-irradiation from summer sunshine and to a small extent by absorption from food. However, these processes become less efficient with age. Loss of mobility or residential care restricts solar exposure. Reduced appetite and financial problems often add to these problems. Thus, hypovitaminosis D is common world-wide, but is more common and more severe in older people. Non-classical effects of vitamin D, depending on serum circulating 25-hydroxyvitamin D concentrations, are present in most non-bony tissues; disorders associated with hypovitaminosis D include increased risks of sepsis [bacterial, mycobacterial and viral], cardiovascular and metabolic disorders [e.g. hyperlipidemia, type 2 diabetes mellitus, acute vascular events, dementia, stroke and heart failure]. Many cancer risks are associated with vitamin D inadequacy, though causality is accepted only for colo-rectal cancer. Maintenance of repletion in healthy older people requires intakes of ≥800IU/day [20μg], as advised by the Institute of Medicine [IOM], but achieving such intakes usually requires supplementation. Excessive intakes are dangerous, especially in undiagnosed primary hyperparathyroidism or sarcoidosis, but the IOM finds doses <4000 IU/day are safe. Many experts suggest that ≥1000-2000 IU [25-50μg] of vitamin D daily is necessary for older people, especially when independence is lost, or hypovitaminosis D could add to the clinical problem[s]. Much higher doses than these are needed for treatment of established deficiency or insufficiency.

Key words: vitamin D, elderly, deficiency, supplementation, aging, pathology

The aims of the review is to provide an overview of the evidence for hypovitaminosis D being associated with increased risks of many types of ill health in older people, especially those associated with loss of bone strength, and of some of the mechanisms by which hypovitaminosis D is thought to increase these risks; it is based on peer reviewed publications but necessarily reflects the author's interest and experience in vitamin D related matters, acquired during more than 50 years working in internal medicine, diabetes, metabolism & endocrinology as both clinician and clinical research worker.

Vitamin D metabolism and changes with advancing age

Vitamin D_3 [cholecalciferol or D3] is synthesised in mammalian skin under the influence of peak effective UVB, wavelength 295-297 nm from sunlight (eUVB) and absorbed into the circulation [1]. High local concentrations of D_3 suppress further D_3 formation, a valuable feedback system preventing vitamin D intoxication. Sub-erythemal doses of appropriate UVB on significant areas of skin can produce large amounts of D_3, enough in those regularly exposed to summer sunshine over much of their bodies, to maintain normal repletion during the months when sunlight provides no

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eUVB, as is seen in temperate zones for > 6 months of the year. However, adequate exposure to summer sunlight is unusual with current life styles, even in fit young adults, because most people live, work and exercise indoors, behind glass [which blocks UVB]. Furthermore, clothes also block UVB transmission; many immigrant females favour covered-up clothing and many people of all ages, but especially older women, use sunscreen regularly, and use it in make-up, often without knowing it. Most people world-wide avoid sunshine, to stay cool, to prevent sun-related skin aging, to reduce the risks of skin cancer and to avoid undue tanning. Thus, Westernised and urban communities world-wide, even in tropical countries, have a high prevalence of hypo-vitaminosis D, even in sunny climes [2, 3]. This is, however, no more a new problem in the old than it is at other ages [4,5]. In 1966 it was suggested that nutritional osteomalacia might 'contribute to the skeletal rarefaction found in old age', a suggestion now generally accepted [6]. These older findings make it especially regrettable that, >40 years later, hypovitaminosis D remains a public health problem at all ages, world-wide.This provides a bad start for those getting older, since ageing reduces outdoor activity, food intake and skin synthesis, and also gut absorption of vitamin D which can only worsen the problems relating to hypovitaminosis D in the elderly [7-9]. In Europe, representative residents from the SENECA study of older Europeans aged 75-76 years old showed 36% of men and 47% of women were vitamin D deficient, most markedly in southern Europe, in a survey of 19 towns in 11 countries in the winter of 1988-1989 [10]. Wearing short rather than long sleeves was associated with much better vitamin D status, especially in the sunnier southern countries, as was taking vitamin D supplements or using UVB lamps. Thus, baring the arms as well as the face usefully increases skin synthesis of vitamin D in sunny climates in older people. Furthermore, though a trial of increased skin exposure to sunlight in 602 female residents of 51 care facilities in Northern Sydney, Australia [being outdoors for 30-40 minutes early morning] failed to improve vitamin D status, possibly reflecting the 74% non-compliance with mornings outdoors or the lack of effective UVB in early morning sunshine, we have earlier shown serum 25(OH)D to be considerably increased by sub-erythemal doses of UVB in people in residential care [11,12]. Diet is a poor source of vitamin D, only wild oily fish, egg yolk, cod-liver oil and fortified foods [e.g. some breakfast cereals and margarine in the UK and some orange juices and milk in the USA]. Food fortification can improve vitamin D repletion at the population level, as recently achieved in much of the Finnish population, but may be less effective in elders as food intakes and vitamin D absorption fall. In counties like the UK, where there is no recommended daily intake for vitamin D for people aged 19-64 years old, the population remains unduly dependent on sun exposure, often impossible for elders to achieve, or on buying supplements.

Absorbed vitamin D2/3 is converted in the liver to 25(OH) vitamin D, the storage form of the vitamin that circulates bound to vitamin D binding proteins [DBPs], also made in the liver. This complex has a half life of 2-3 weeks and concentrations reflect availability of vitamin D over about 4-6 weeks in stable situations, which is why serum 25(OH)D concentration is generally accepted as suitable for assessment of vitamin D stores (repletion or availability) [13]. Treatment with 25(OH)D has been suggested , the half time of clearance of this metabolite is ~13.4 days, suggesting this metabolite may only bind partially to DBPs when given orally. Thus, oral D2, or D3, are likely to be better sources of circulating 25(OH)D than taking 25(OH)D, though data for 25(OH)D3 was not given [14]. Genetic variants of DBP influence serum concentrations of 25(OH)D, and thus the availability of 25(OH)D to the many tissues where activation takes place; a finding explaining the associations of serum 25(OH)D with so many health outcomes. Variants of the genes activating 25(OH)D and destroying 1,25(OH)2D also affect serum 25(OH)D concentrations but the overall effects of these 3 gene variants is small [15]. Well recognized causes of vitamin D deficiency should be treated routinely with vitamin D, e.g. fat malabsorption, and supplementation is needed for those taking drugs interfering with vitamin D metabolism, e.g. some anti-epileptic drugs [16].

How vitamin D works

The effects of vitamin D are largely produced by the activated hormonal metabolite produced by a second stage hydroxylation from 25(OH)D by a specific 1-alpha hydroxylase, making calcitriol [1,25-dihydroxyvitamin D], a process first identified in the kidneys and tightly regulated by parathyroid hormone [PTH], calcium and bone fibroblast growth factor-23 [FGF-23]. Serum PTH increases in response to hypovitaminosis D, but not consistently, making serum PTH measurements unhelpful for assessing vitamin D status. Circulating calcitriol [1,25(OH)2D] concentrations are tightly regulated in health and are maintained, or may rise somewhat, in vitamin D deficiency - only with extreme deficiency does serum calcitriol fall so that they too are most unhelpful for assessment of vitamin D status which depends on serum 25(OH)D concentration. Vitamin D activation was first thought to be limited to the kidney but also takes place locally in vitamin D target tissues.

Tissues able to activate vitamin D locally, as well as respond to it, include pancreatic islets, gut, arterial walls, myocardium, skin and placenta [17]. This 'local' tissue calcitriol production has autocrine and paracrine effects and only in the case of calcitriol produced in the placenta and pathological accumulations of macrophages [e.g. in sarcoidosis], does locally produced calcitriol spill into the circulation [17]. Local activation of vitamin D is not, however, dependent on PTH but probably on availability of 25(OH)D in the circulation and activity of the catabolic 24(OH)ase enzyme, locally, explaining why the associations of health outcomes with serum 25(OH)D or other measures of available vitamin D (eUVB exposure or vitamin D intakes) are so common. Calcitriol, and some of the 25(OH)D, are inactivated locally by catabolism to 24-(OH) metabolites by a specific 24-hydroxylase (CYP24); splice variants of this enzyme, associated with over or under activity, likely contribute to modulation of local calcitriol production [18]. Biological effects of vitamin D are induced through two main pathways. Firstly, calcitriol binds to nuclear vitamin D receptors [VDR], often complexed with retinol X receptors to form heterodimers, inducing genomic signaling through target genes after binding to their promoter regions. Over 3000 genes contain vitamin D response elements in their promoter regions. Only ~158 human genes were reported to alter their expression in response to calcitriol in 2005, mostly showing increased expression [19]. Rapid non-genomic responses to calcitriol follow ligand-binding to non-nuclear VDRs, as in cytoplasmic membrane caveolar pits, enhancing gut calcium absorption, calcium enhancement of insulin release from islet beta cells, opening of calcium channels in osteoblasts and increased endothelial cell migration [20]. In addition, excess vitamin A, though necessary for many metabolic processes induced by vitamin D, antagonizes vitamin D. Rickets is induced in vitamin D-replete animals by large doses of retinol, probably due to RXR complexation with the excess of ligand-bound retinoic acid receptor [RAR], produced in vitamin A excess. Literature review suggests that prevention of death from toxic doses of vitamin D and induction of rickets in normally vitamin D replete animals by large amounts of vitamin A are likely explained by such mechanisms, suggesting important interactions between these vitamins [21]. Thus, vitamin A status is a likely confounder in studies on vitamin D and should be assessed in all future trials of supplementation.

Leukocyte telomere length (the length of the protective DNA 'tips' of chromosomes) falls with age; unduly short telomeres for one's age predict reduced lifespan due to increased risks from ageing-related illness. Better vitamin D status is associated with longer telomeres even after adjustment for age, menopausal status, obesity, the use of hormone replacement therapy and physical activity; reduction in telomere length in the lowest vs. the highest tertile of serum 25(OH)D after adjustment was 107 base pairs [p=0.0009], the equivalent of 5 years of telomeric ageing, a difference enhanced by adjustment for the presence of inflammation using C-reactive protein (CRP) concentrations [22]. Genomic instability contributes to ageing-related diseases and telomere dysfunction and defective repair of DNA breaks aggravate the problems. The enzyme telomerase re-forms telomere tips and in peripheral blood mononuclear cells (PBMC) its activity increases with vitamin D supplementation in overweight African Americans, suggesting how vitamin D might protect against changes of ageing [23]. In addition, experimental evidence suggests that vitamin D protects against DNA damage by reducing oxidative nuclear damage and reducing loss with age [24]. Vitamin D also promotes DNA stability by inhibiting undue expression of the cysteine protease, cathepsin, which regulates stability of an essential factor in DNA repair, 53BP1 [25]. There is, however, little known as yet on how vitamin D may contribute to human DNA stability, or what vitamin D status may be optimal for such effects.

The Klotho [KL] gene product, a β-glucoronidase-like type-1 membrane protein, appears to protect mice against ageing since KL knock out (KO) mice age rapidly, and KL-loss accelerates degenerative processes with changes suggestive of excessive effects of vitamin D, are ameliorated by restriction of vitamin D intake and by genetic knock-out of vitamin D receptors. KL gene activity modulates calcium ion channels and potassium and phosphate transporters in mice and influences fibroblast growth factor-23 (FGF-23) action, important in regulating PTH production and, thereby, the renal activation of vitamin D. These data have led to fears that better vitamin D repletion may have adverse effects on human health, especially since prostate cancer risks are optimally reduced at moderate serum 25(OH)D concentrations of 20-40 nmol/l and calcitriol induces ageing in FGF-23 and in KL-KO mice [26-29]. However, human mesenchymal stem cells exposed to calcitriol show evidence of delayed senescence and reduced apoptosis and it may be that the changes seen in mice were due to adverse effects of species-specific increases in systemic phosphate concentrations [30]. If these findings are supported by further work in human tissues, it would make it unlikely that the upper limits of vitamin D status seen in humans with high sunshine exposure world-wide increase ageing processes; it would also imply that such values could safely be used as

targets for normalization of vitamin D status and that avoidance of hypovitaminosis D could be included in the range of measures for use in reducing ageing in the skin and other tissues, as has already been suggested [31].

The 'classical' role of vitamin D in musculoskeletal health in the elderly

Vitamin D is well known to be essential for older people [32]. Osteomalacia in adults, like childhood rickets, develops in vitamin D deficiency, commonly presenting with severe aching in bone and muscles, marked proximal muscle weakness making standing up and walking difficult and painful and a marked 'waddling' gait. This condition is common in older people, especially women and especially Asian women whose life style puts them at high-risk for hypovitaminosis D, even in sunny countries. Routine blood tests are often unhelpful and X rays were used for diagnosis for decades. Pseudofractures develop [Looser's zones], are visible early in isotope bone scans, sometimes incidentally and often before being detectable on X-rays. These zones become painful and may even fracture. Biochemical tests used for diagnosis before serum 25(OH)D assays became routine, sometimes showing normal serum calcium, reduced serum phosphate [secondary to the increased serum PTH] and often a raised serum alkaline phosphatase. Treatment with vitamin D, (D2 or D3) is curative. As bone heals, serum alkaline phosphatase rises, often for 2-3 months, before returning to normal. Early work trialling vitamin D supplementation for muscle weakness in deficiency found no improvement [33]. However, with larger doses, muscle weakness and balance are improved by vitamin D treatment [though not gait], though mainly in deficient subjects [34,35]. Supporting this view, amongst 368 community-dwelling adults aged 70-89 years old, improved physical performance was found when natural increases in 25(OH)D took subjects out of baseline-deficiency after a year's lifestyle intervention without supplementation, after adjustment for relevant factors including physical activity [36]. However, in long-standing weakness, with muscle wasting, muscle regeneration can take months.

Poor vitamin D repletion is associated with increased risk, and severity, of osteoporosis and supplementation [800 IU daily or more in older people] reduces rates of bone loss over time [37]. Osteoporotic fragility fracture rates in the UK average ~ 200,000/year with a 20% one-year mortality, recent costs to the NHS being ~£1.7 billion/year, 50% of sufferers losing their independence and 27% needing admission to residential care as a result of their fracture and the surgery for these fractures, mainly hip fractures, fills ~20% of UK orthopedic beds [38]. Supplementation, often given with calcium for osteoporosis, but which reduces compliance, can improve bone mineral density and reduce fall and fracture risks, benefits appearing on doses of 700-1000 IU/day [39-41b]. Giving 6500 IU daily did no better than 800 IU daily, the current Institute of Medicine of North America (IOM) recommendation for healthy over 70 year olds] in a recent one year randomized controlled trial (RCT) in 50-80 year old women [42]. Some RCTs showed no changes in fracture risk with supplementation with vitamin D, calcium or both, e.g. the RECORD trial in 5292 people over 70 years old in the UK [43]. However, many RCTs have shown fracture risk reductions, possibly because of better compliance, higher doses, starting in younger people or longer duration. An early RCT using 100,000 IU of cholecalciferol orally, 4 monthly, for 5 years in community dwellers aged 65-85 years in rural England reduced 'first incident fracture' rates [OR = 0.78 (95% CI 0.61-0.99)] and for first fractures of hip, wrist, forearm or vertebrae [OR = 0.67 (95%CI 0.48-0.93)] [39]. Recent meta-analyses suggest benefits from supplementation to those who were, or were likely to be, vitamin D deficient and that fracture risk reduction with supplementation is less in community free-living older adults than in those in residential care [44]. These data support the need for ageing populations as a whole to avoid hypovitaminosis D to protect their independence with intakes at least as high as current IOM recommendations [800IU/day], to reduce the burden of osteoporotic fragility fractures on individuals and on health-care funding. If vitamin D repletion were ensured throughout the lifespan it seems likely that ageing hypovitaminosis D related problems would be less common, reducing costs to individuals and heath providers alike, since childhood, especially adolescent, bone mineral density (BMD) is well known to predict later life BMD. Even In pregnancy, better maternal repletion leads to better BMD in children aged 9 years old [45].

Non-classic roles of vitamin D in health

Immune system and infection

Vitamin D has protective effects against bacterial and viral infection risks, including tuberculosis; supplementation can reduce some infection rates and the severity or mortality of some infections. Innate immune defence is enhanced by vitamin D which boosts early inflammatory processes including Toll-like receptor 2 formation, increasing production of bactericidal cathelicidin [46, 47], enhances capillary increases in

permeability in response to both PAMPS and DAMPS (pathogen associated and tissue damage associated 'molecular patterns'), allowing defence factors, (e.g. white cells and bactericides) into affected tissues. White cells produce vitamin D receptors (VDRs) and the vitamin D-activating hydroxylase and thus vitamin D stimulates white cell cathelicidin production [46, 48], enhancing bactericidal killing of pathogens and clearance of tissue breakdown products [49]. Calcitriol stimulates monocyte maturation into macrophages, enhancing defence against chronic infections [e.g. tuberculosis]. Macrophages also invade active atheromatous plaque, containing matrix metalloproteinase enzymes [MMPs, especially MMP9], digesting interstitial tissue. This causes tuberculous caseation and atheromatous plaque breakdown, triggering thrombus formation and resultant arterial occlusion. Longer-term, paradoxically, vitamin D reduces inflammation, allowing healing and reducing inappropriate prolongation of inflammation and the risks of acquired immune tissue damage but enhancing anti-inflammatory cytokine secretion [e.g. IL-10] and suppression of inflammatory cytokine secretion(e.g. of IL-6 , tumour necrosis factor) [46, 49]. Such effects contribute to reducing risks and severity of acute inflammatory conditions. The incidence of confirmed influenza A fell with supplementation [at 1200IU/day] in children, though a further superiority trial showed 3 monthly boluses were ineffective in infants [50, 51]. This data led to the suggestion that vitamin D supplementation should be mandatory in influenza A (HINI) outbreaks [52]. In 6789 British Birth Cohort subjects across the UK, better vitamin D status at 45 years old was associated with better lung function cross-sectionally after adjustment for co-variates; each 10 nmol/l increase in baseline 25(OH)D independently predicted 7% [95% CI 3,11%] reductions in subsequent respiratory infections [53]. Similarly, better long-term vitamin D status [serum 25(OH)D > 38 ng/ml [~90 nmol/l] vs. <38 nmol/l] was associated with two-fold reductions in acute respiratory viral infections in winter, and reductions in viral illness duration in 198 healthy adults [54]. These data suggest that better repletion is beneficial; however, supplementation to reduce influenza-like illness among 569 adults in an RCT of ~1000-<7000 IU vitamin D daily vs. placebo was ineffective, though disease duration may have shortened [55]. Adequate RCTs are needed to determine whether supplementation can reduce incidence, or severity, of respiratory tract infections in older people.

Better vitamin D repletion may contribute to reducing chronic inflammatory problems such as periodontitis [56-58] and atheromatous disease, and may reduce acute vascular events due to arterial plaque disruption, inflammation being a major factor in atheromatous disease progression [59]. Tuberculosis infections were treated with sunshine and/or vitamin D before anti-tuberculous drugs emerged. Sputum conversion rates fall with supplementation, but only in specific VDR genotypes [60]. Vitamin D status relates inversely to circulating MMP9, a marker of atheromatous damage, which falls after supplementation [61]. MMP9 formation in tuberculous lesions is suppressed by vitamin D [62]. Further RCT data on whether supplementation improves responses to anti-tuberculous treatment is needed. Though mechanisms exist for vitamin D to reduce common autoimmune disease risks in older people, [hypothyroidism, insulin-dependence in T2DM], there is no actual evidence to this effect in humans. Better vitamin D status enhances response to vaccination and immunization, possibly improving protection of older people from influenzal illness after flu immunization [63]. Wound healing, often slow in older people, can be enhanced experimentally by vitamin D [64, 65]. Hypovitaminosis D was associated with increased pressure sore risks in community dwellers in their 70s to 90s but not as an independent determinant since hypovitaminosis D was determined by existing co-morbidities [66]. Clearly, adequate RCTs of supplementation are required to see whether causality can be demonstrated. Low baseline vitamin D status predicted reduced healing in the year after surgery for periodontitis, a common problem in older people, and giving vitamin D to subjects with severe periodontal problems improved post-operative bone defect resolution, supporting suggestions that hypovitaminosis D may worsen periodontitis [67, 68]. Organ rejection risks after transplantation are reduced by vitamin D supplementation [69]. If causality can be demonstrated for any or all of these suggestions by adequate RCTS of vitamin D supplementation, then provision of adequate vitamin D could be used in management of these situations.

Treating rickets with vitamin D caused respiratory infection rates to fall, but bolus supplementation does not necessarily improve respiratory infection rates or outcomes in children. In older people findings have also been mixed. Community-acquired pneumonia mortality increased in severe vitamin D deficiency (25(OH)D < 30 nmol/l) vs. those without deficiency, OR = 12.7 [95%CI; 2.2-73.3, p = 0.004] in New Zealand, though baseline status was not related to circulating cathelicidin [70]. The RECORD RCT of vitamin D and/or calcium for secondary prevention of osteoporotic fractures in the UK analyzed 3444 community-dwelling older subjects responses to questionnaires ~18 (11-25) months post-

randomization; 45% of subjects reported discontinuation of trial medication and the remaining subjects showed doubtful reductions in reported infection rates and antibiotic use during the RCT amongst those vitamin D vs. placebo [71]; greater reductions were found on analysis for data on subjects continuing their medication] OR [95%CI] =0.80[0.64-1.01]and 0.74[0.52-1.06] respectively. Adverse effects were equally rare in treatment and control groups but these findings may have been compromised by dependency on recall rather than diary keeping.

Metabolic syndrome

The physical variables associated with increased risks of type 2 diabetes (T2DM) and atheromatous cardio-vascular disease [obesity, usually defined by waist size, adjusted for gender and ethnicity, hypertension, hyperglycemia and dyslipidemia (raised triacylglyceride (TAG), raised total or LDL-cholesterol and low HDL-cholesterol [see Table 1]]. Three or more such abnormalities in an individual are taken to define risks sufficient to warrant preventative measures. Grouped together, this situation is called Reaven's Syndrome, Metabolic Syndrome or Syndrome 'X' (see Table 1).

Table 1. Range of criteria used for diagnosis of 'Metabolic Syndrome' in North American and European adults [72]

Criterion	European men	European women	South and East Asian Men
Waist size	≥ 94-103	≥ 80-88	≥ 90 cm in men ≥ 80 cm in women
Triglycerides (Triacylglycerol)	≥ 1.7 mmol/l	≥ 1.7 mmol/l	≥ 1.7 mmol/l
HDLCholesterol	≤ 40 mg/dl	≤ 50 mg/dl	as for Europeans
Blood Pressure	≥ 130/85-140/90 (or on medication for hypertension)	≥ 130/85-140/90 (or on medication for hypertension)	≥ 130/85-140/90 (or on medication for hypertension)
Fasting Glucose	≥ 5.1, 5.6 or 6.1 mmol/l	≥ 5.1, 5.6 or 6.1 mmol/l	≥ 5.1, 5.6 or 6.1 mmol/l

Non-alcoholic fatty liver disease is another important component of 'metabolic syndrome' as is increased insulin resistance. Common environmental and life style risk factors [lack of physical activity, stress, cigarette smoking, excess alcohol usage and inappropriate diet] are well- known but not used for defining ' metabolic syndrome', even though regular exercise and weight loss reduces T2DM risk by 58%, and reductions in insulin resistance or improvements in beta cell function (whether induced by life-style changes, metformin, renin-angiotensin system (RAS) blockade or other medication), produces similar benefits [73, 74].

Review of earlier literature shows hypovitaminosis D is associated dose-dependently, with increased abnormality of each of the variables used to define metabolic syndrome [75]. Many more recent cross-sectional studies show dose-effects of lower vitamin D status for metabolic syndrome prevalence and for risks of T2DM and overt CVD. Such risks are also reported prospectively [72, 76]. Furthermore, correcting vitamin D deficiency reduces insulin resistance and improves insulin secretion experimentally and can have similar benefits in humans, though available evidence suggests this only happens with early metabolic syndrome abnormalities [72, 77, 78].

Many problems affect associative, prospective, and randomized controlled trial findings for vitamin D. Obesity is associated with reductions in serum 25(OH)D concentration, probably due to increased sequestration of vitamin D, even at concentrations normal in fat, by the increased fat mass; thus this association may be due to reverse confounding (the increased fat mass lowering serum 25(OH)D so that associations of 25(OH)D with abnormalities are due to obesity rather than by hypovitaminosis D). This problem is aggravated by the fact that many obese people sunbathe less than slim people. However, many studies show independent associations of vitamin D status with metabolic

syndrome, T2DM and CVD outcomes cross-sectionally and this has also been found prospectively after adjustment for obesity and all other baseline findings [e.g. 72, 76], though 2 of the 40 or so cross-sectional studies reviewed recently either show these associations in men but not women or not in older or very obese people [79]. Abnormalities in lipid profiles are commonly associated with reduced vitamin D status findings, in particular for TAGs which can be reduced by supplementation, but ApoA1 and HDL-C are directly, and independently, associated with vitamin D status in several studies [80, 81], as is blood pressure, though this has been reduced by supplementation only in younger people. Vitamin D blocks renal renin secretion which reduces blood pressure, unless secondary renal damage is irreversible which is increasingly likely in older people [46, 82]. Pancreatic islet renin-angiotensin-system [RAS] activity increases in hyperglycaemia but can be blocked and prevented by vitamin D in islets [83]. Interestingly, RAS blockade reverses the increases in islet RAS activity seen with hypovitaminosis D. These effects may account for the reductions in T2DM risk seen in people treated with RAS blockade in RCTs [72, 84]. However, whether adequate vitamin D provision may reduce risks of T2DM or CVD, especially in older people, requires testing in adequate RCTs of vitamin D supplementation. In addition, the possibility that supplementation given together with RAS blockade could have additive protective effects for T2DM and CVD, as consequences of the metabolic syndrome, remains to be tested in adequately powered RCTs vitamin D for both younger and older people (using IOM recommended intakes [600 IU/day and 800 IU/day for those < and > 70 years old respectively)). RCTs also need to be done at the higher doses recommended by most vitamin D experts [between 1000 and 4000 IU and 2000-4000 IU/day for people under or over 70 years old, respectively, 4000 IU being the maximum dose categorically regarded as safe in adults by the IOM [40].

Type 2 diabetes mellitus

Though there are strong associations of reductions in vitamin D status, however assessed, with increased risks of type 2 diabetes in many reported cross-sectional studies and in the few prospective studies that have been reported, very few randomised controlled trials have been carried out to see whether correction of insufficiency can prevent T2DM diabetes. There is a wealth of evidence showing that vitamin D is necessary for insulin secretion and can reduce abnormal increases in insulin resistance in humans as well as experimentally [21, 72, 78]. Improving vitamin D status before irreversible defects develop in islet cells, insulin resistance and related defects also appears to be important. For example, in deficient humans, supplementation improved reduced insulin responses to oral glucose more in those with higher baseline responses than in those with markedly reduced initial baseline responses to oral glucose [77]. In line with this, though low 25(OH)D predicted progression to T2DM in those with prediabetes, this was not the case in those with normal baseline glucose tolerance in a group of 2378 people aged 35-56 years old [85]. Similarly, supplementation with vitamin D and calcium appears, on meta-analysis, to lead to better glucose tolerance in those with impaired, but not normal, glucose tolerance at baseline [86, 87]. A study from a nationally representative population survey (the Health Survey for England 2005) in 2038 community dwelling people aged >65 years old showed independent and reducing dose effects, cross-sectionally, for the risk of hyperglycaemia with increasing 25(OH)D concentrations up to 50-79 nmol/l; OR for hyperglycemia = 2.3 [95%CI, 1.2-4.4], for 25(OH)D < 25 nmol/l; 2.09 [1.22-3.58] for 25(OH)D between 25 and 49.9 nmol/l but for increases of 50-74.9 nmol/l the increase OR was not significant at 1.49 [95%CI, 0.85-2.62] [88].

Cardiovascular system disorders

Ageing is associated with reduced peripheral arterial endothelium-dependent dilatation, probably due to increases in oxidative stress and reductions in locally synthesized nitric oxide. Whilst vascular endothelial function is adversely affected by lack of exercise, obesity, the menopause and other factors, it is also reduced in hypovitaminosis D [89], and is improved by supplementation [90, 91]. Compliance [elasticity] of blood vessel walls falls with age and is associated with increases in blood pressure, as also seen with age. Reducing blood pressure reduces risks of an acute vascular event such as stroke or coronary occlusion. Therapeutic options for hypertension include renin-angiotensin blockers since hypertension can be associated with increased renin production, especially in people of Afro-Caribbean descent. Vitamin D status is commonly related inversely to blood pressure and supplementation has reduced blood pressure in humans, but only it appears, in those few RCTs those with reasonably normal baseline blood pressure. This effect may be due to suppression of the renin gene, and hence of production, by vitamin D as discussed above. However, giving vitamin D can improve peripheral vascular function and improves vasodilator responses and endothelial function in vivo in humans in RCTs

(though results are variable, probably due to variation in RCT design) [92]; these effects may also contribute to the beneficial effects of supplementation in deficiency, at least in those with early hypertension (probably before established hypertension has led to irreversible renal damage). It may contribute to the more long term beneficial effects reported for supplementation in adults on all-cause and, also, specifically, on cardiovascular mortality in adults. Considerable amounts of data showing inverse associations of vitamin D status (serum 25(OH)D concentration) with mortality have accumulated over recent years. A recent meta-analysis of mortality risks in the general population from 14 prospective cohort studies (with 5562 deaths amongst 62,548 adults [93]) found dose effects for increases in baseline serum 25(OH)D; overall RR for highest vs. lowest category of 25(OH)D was 0.71[95% CI: 0.5, 0.91]. However, there were dose-effects – for increases in serum 25(OH)D above the basal category of 27.5 nmol/l of 12.5, 25 and 50 nmol/l the RRs were 0.86, 0.77 and 0.69 [95% CI: 0.82, 0.91; 0.7, 0.84 and 0.6, 0.78] respectively. For baseline values of >87.5 nmol/l above the reference category there was no decrease in mortality (perhaps because there was no benefit with such higher status, or there were too few subjects in this category for reliable analysis, or, because higher status has an adverse effect). Thus, this analysis suggests optimal serum 25(OH)D concentrations for adult survival are between 75-87.5 nmol/l [93]. Heart failure outcomes are also reported to be improved in those with higher baseline status but also by supplementation, in a study of 3009 adults over ~1 ½ years (Hazard Ratio for mortality on supplementation = 0.68 [95% CI 0.54-0.85, P < 0.0001) [94]. Furthermore, Cochrane review of data for supplementation with vitamin D_3 (but not with calcitriol or its analogues) found that supplementation decreased overall mortality, [RR 0.94, 95% CI 0.91-0.98] in adults as a whole but that this benefit was most present mainly in older women, but especially for those in residential care [95]. Similarly, deficiency was common in a recent report on 10,899 patients in the USA, 70.3 % having inadequate vitamin D status [defined by serum 25(OH)D < 30 ng/ml] and follow-up over more than 5 years showed associations of poor vitamin D status with hypertension, coronary artery disease, cardiomyopathy and diabetes risks [p<0.05]. Deficiency was also an independent predictor of mortality [all-cause], OR 2.64[95%CI; 1.9-3.66, p<0.0001], and in addition, supplementation reduced mortality [OR for death 0.39; 95%CI: 0.277-0.534, p<0.0001 [96]. An 8% reduction in mortality for each 20nmol/l increase in baseline serum 25(OH)D [RR 0.92, 95% CI: 0.89-0.95]was reported in a review and meta-analysis of data from 12 studies on 32,142 mainly elderly subjects of whom 6921 died during follow up [97], reductions close to those reported with supplementation. Supplementation has also been reported to improve outcomes in heart failure but unduly high circulating serum 25(OH)D (but not deficiency) is associated with increased risk of atrial fibrillation [98]. Much of the population is taking a statin for secondary risk reduction after having a cardiovascular event and, increasingly, these drugs are being given for primary prevention. Current concerns are a possible increase in risk of T2DM, but the benefits appear to outweigh these risks [99]; indeed there are proposals in the UK for a statin to be given to everyone over 50 years old. Myalgia, with muscle aches, fatigue and post-exertional muscle pain, often with myopathic weakness, are such common symptoms in people taking statins that they are classified as 'class-effects'. Treatment of these symptoms has proved difficult and many people cannot continue taking statins. Supplemental co-enzyme Q10 eases these symptoms in some people but is not available on prescription on the UK NHS and is very expensive. More recently, correcting vitamin D deficiency has been found to be remarkably effective in relieving these symptoms, allowing statin use to continue, in 92% of sufferers, possibly because the myopathy of hypovitaminosis D, which has very similar symptoms, is worsened by statins. However, while statins have been suggested to mimic vitamin D effects, recent work suggests that hypovitaminosis D may, by reducing the activity of certain cytochrome P450 (CYP) enzymes, increase the toxicity of certain statins whilst also reducing their efficacy as treatment for hyperlipidemia (since vitamin D is known to induce certain CYP enzymes important for metabolizing statins to their active metabolites). Vitamin D metabolites also have some statin-like HMG-CoA reductase activity of their own - thus, ensuring vitamin D repletion may not both relieve myalgic side effects of statins and increase their activity in treatment of hyperlipidemias, but may also lead to reductions in the doses of statins needed for adequate control of lipid abnormalities [99-101].

Central and peripheral nervous system

Dementia is increasingly common with ageing and most commonly results, in older people, from atherosclerotic small vessel disease (multi-infarct dementia). Thus, any vascular benefits of improvement of vitamin D status in the elderly may be expected to reduce, or delay, this risk. A group of tests for cognitive function were found to be related inversely to vitamin D status in 377 black and 703 mainly Caucasian non-black elders requiring support

to live at home, after adjustment for relevant factors (including lipids, homocysteine, B vitamin status and multivitamin use, as well as age, sex, ethnicity, BMI, education, center, renal function, season, physical activity and alcohol usage) [102]. Associations of vitamin D status with risks of Alzheimer's disease or with degrees of impairment in this condition suggest that the role of vitamin D should be examined in this condition. One trial of vitamin D for loss of cognition whilst the disease was mild to moderate used intakes of 1000 IU/day for 8 weeks before randomisation to higher vs. 1000 IU/day for another 8 weeks, followed by intranasal insulin challenge (known to improve cognition temporarily in this disorder) but showed no benefit from the higher dosage, despite significant increases in serum 25(OH)D [103].

Respiratory system

Better vitamin D status has been associated with better lung function, independent of other factors including allergic status and in chronic pulmonary disease (CPD) though not in all cohorts [104-106]. However, there could be an element of reverse causation in these associations since reduced exercise capacity may well reduce outdoor activity. Poor status was also associated with increased risks of CPD, i.e., chronic bronchitis, in a recent study of 6872 adults and persisted after adjustment for age, itself a major predictor for increased prevalence of CPD in this cohort [107]. However, poor vitamin D status was not a predictor of rates of deterioration in smokers with mild to moderate CPD [108]. Thus, whether supplementation can improve functional capacity in chronic pulmonary disease (CPD) is unknown, and this question awaits further data from adequate trials of supplementation. Lung surfactant is important for reducing alveolar surface tension and its production is well known to be enhanced by corticosteroids and is enhanced by vitamin D; this may help explain the benefits of improving vitamin D status in acute lung infections in general and in chronic renal failure [109, 110]. The increased secretion of the bactericide cathelecidin induced by vitamin D would also be helpful [46]. The available evidence has also led to suggestions that preparedness for influenzal pandemics would be improved if all health workers were vitamin D replete when immunised so as to optimise immune responses to flu vaccines [63], a proposition that, if proven, would warrant extension to susceptible populations. The role of vitamin D in defence against infections [innate immunity] is discussed further under infections above.

Chronic Renal disease

Renal replacement therapy [haemo- or peritoneal dialysis] prolongs life in renal failure. Renal function falls with age and renal failure in older people is increasingly managed actively. Since renal activation of vitamin D is lost with extensive renal damage, renal osteodystrophy is a common and potentially severe bone disease for which activated vitamin D or its analogues are usually given as prophylaxis, together with measures for controlling serum calcium and phosphate concentrations. Respiratory infection risk is high in renal failure but reduced in those on calcitriol [109]. New onset diabetes, hypertension and early cardiovascular death in patients on dialysis are major problems but replacement of vitamin D activity can reduce the risk of, or even correct, glucose intolerance, reduce blood pressure and greatly reduce CVD mortality [110, 111]. However, this risk can increase if unduly high replacement doses are used, narrowing the therapeutically acceptable range of dosage of activated vitamin D. Increases in albuminuria are associated with increased risks of renal failure and also of CVD and cardiovascular deaths but albuminuria is reduced by supplementation with vitamin D, probably due to beneficial effects on function of small blood vessel walls [92]. CVD mortality is also reduced by improving vitamin D status (higher serum 25(OH)D) with supplemental vitamin D3, probably because this allows local tissue activation to proceed normally. If this observation is confirmed it may prove possible to protect against CVD in renal failure or on chronic dialysis by using vitamin D3 together with lower doses of calcitriol so as to reduce the risks of both hypercalcaemia, and of non-optimal calcitriol dosage, since local activation appears to be self regulating [112, 113].

Nervous system, central and peripheral, cognition and dementia, MS, eyes

Vitamin D is postulated to protect eyes from some common changes of ageing, including inflammation. In ageing mice, 6 weeks supplementation reduced retinal inflammation and amyloid-beta accumulation and showed significant improvements in visual function whilst, in a cohort of 481sibling pairs discordant for early AMD and in prospective nested case-control studies of those with age-related macular degeneration [AMD] vs. controls, variants of the CYP24A1gene (coding for the 24-hydroxylase enzyme catabolic for vitamin D) influenced AMD risks amongst 2528 subjects [114, 115]. Thus further studies of supplementation for reduction in human AMD risks are warranted.

Cancer

Many cancers are thought to be more common in vitamin D deficiency and there is much convincing epidemiological and especially ecological evidence, for this [116]. However, to date [May 2012] only in colo-rectal cancer has the available evidence been accepted as demonstrating causality by bodies such as the International Agency for Research against Cancer [117]. Other cancers await the outcomes of further trials, though there is good mechanistic evidence to suggest that this association is likely to be confirmed for several other cancers, such as breast, lung and pancreas [118]. Interestingly, the evidence for good vitamin D status over time being protective against melanoma, a skin tumour precipitated by sunburn, is currently strengthening [119]. Remarkable reductions in overall cancer rates over time were seen with vitamin D supplementation in 1179 women aged >55 years enrolled in a randomised controlled trial, with no effects of additional calcium supplementation. However since this reduction did not appear during the first year of the trial but only over the last 3 years of the 4 year trial, when the risk ratio for any cancer with supplementation was 0.231[95% CI, 0.09-0.6] [120], many workers have not accepted the findings as meaningful. This non-acceptance can be considered perverse since in other studies for cancer prevention no reductions in risk have been expected to appear within as short a period as one year [121]. For prostate cancer the debate is clouded by whether there may be a U-shaped curve for cancer risk with vitamin D status [122], though this may prove spurious for various reasons, including the small numbers of people studied in some trials, the small proportions with high vitamin D repletion in most studies and the lack of data for many likely confounders, including relevant genetic polymorphisms [123].

Vitamin D requirements

Recent position statements on vitamin D requirements include that from the IOM in 2010, discussed above [40]. One specific position statement on vitamin D and postmenopausal health [124], recognizes that diet is an inadequate source of vitamin D, when recommending daily intakes of 600 IU/day in adults, increasing to 800 IU/day from the age of 71 years; it also states that regular summer sunshine exposure, without sunscreen, for 15 min 3-4 times weekly in the middle of the day in summer can generate adequate amounts of vitamin D and that supplementation with D2 or D3 can be used, with monitoring if concomitant medical problems require it or there is doubt about dosages being used. In 2012 joint guidance from the Chief Medical Officers of all 4 countries of the UK on the advice to be given on vitamin D intakes to people over 65 years old was circulated to all general practitioners, practice nurses, health visitors and community pharmacists [125]. This advice recommended at-risk groups, such as those over 65 years old, to take supplements of 10 μg/day, [400 IU] which is less than is recommended in North America for adults in general [600 IU/day] and only half of the daily intake of 800 IU/day recommended for the healthy population aged over 70 year by the IOM, with the aim of achieving 25(OH)D values of ~ 20ng/ml [40]. For those presenting to clinicians, including those with osteoporosis, falls and fragility fractures, the Endocrine Society Clinician Vitamin D Guideline of 2011 recommends achieving a serum 25(OH)D of at least 30 ng/ml, the level required to achieve a plateau in the reduction of serum PTH with increasing 25(OH)D amongst large numbers of healthy adults: in addition, no trial of vitamin D has ever achieved reductions in falls or fracture rates in older people without achieving this level of serum 25(OH)D or with supplemental doses of vitamin D of less than 800 IU daily [126]. Whether adequate supplementation can reduce the risks of cancer generally and not just those of colo-rectal cancer, as is suggested by the data for years 2-4 in the Lappe trial [120], and whether it can reduce the risk of developing a first cardiovascular event (stroke or an acute coronary event) is currently being tested in a RCT of supplementation with 2000 IU of vitamin D3 daily amongst a group of 20,000 healthy adults over 5 years, recruiting from 2010[http://clinical/ct2/show/NCT01169259]. Hopefully this trial may also produce data on outcomes for metabolic syndrome abnormalities and the development of impaired glucose tolerance and T2DM since these are pertinent to CVD risk.

When and when not to measure serum 25(OH)D?

Where whole groups of the population, such as in older people, are at high risk of deficiency, adequate intakes of vitamin D should be ensured as a matter of public health. This author agrees that there should be no need to measure serum 25(OH)D concentrations on healthy people [127], other than for research or audit purposes. A single 25(OH)D assay in the UK costs as much as a year of supplementation for 3 people at IOM recommended amounts. Furthermore, serum 25(OH)D measurements have not been as well standardized as most routine biochemical test measurements and have varied widely. Thus, only assays from laboratories regularly performing well on international quality control schemes (such as DEQUAS [128]) should be

used, even with the newer automated Liquid Chromatography-Tandem mass Spectrographic methods that are now being widely introduced. In addition, serum 25(OH)D, though generally accepted as reflecting vitamin D repletion, may not be comparable across all population groups since at least 3 genetic factors affect 25(OH)D findings and there may be other unrecognised variations due to factors affecting measurements or the activation or catabolism of 25(OH)D; one possible factor increasing catabolism may be betel chewing in Asian populations [21]. However, assessment of vitamin D status may well be judged clinically necessary in people presenting for medical advice when there is a need to confirm or exclude vitamin D deficiency as a cause of the problems presented. Follow-up monitoring may also be warranted if the clinical response to initial supplementation is poor and whenever ongoing health problems or medication require routine vitamin D supplementation or it is judged necessary to ensure that the vitamin D dosages used in treatment are adequate.

Means of improving vitamin D provision

For treatment of proven deficiency in people aged over 65 years a cumulative dose of ~600,000 IU, given in daily doses of 5000-10,000 IU over 2-4 months is often needed. Though interval doses of 50,000 to 100,000 IU have proved beneficial in deficiency much of a single large dose is simply excreted. Doses of 300,000 -500,000 IU are not advisable since some studies have reported adverse effects on bone health from such doses and also because, should the patient have undiagnosed primary hyperparathyroidism, or be unduly sensitive to vitamin D due to an unknown condition such as sarcoidosis, such large doses might well lead to severe hypercalcaemia and, given yearly, these doses have failed to improve bone health [129,130]. For prevention of hypovitaminosis D in the general population, current IOM recommendations suggest daily doses of 600 IU/day for adults and 800 IU/day for those at risk or over 70 years old [40]. It is important that ways be found to get advice out to those 'at risk' of hypovitaminosis D and to give them advice on supplementation appropriate to their age. For prevention of hypovitaminosis D at the population level provision of supplementation for all individuals would be cumbersome and expensive; food supplementation is used in many countries to improve daily intakes since food is a poor source of vitamin D, only wild oily fish and egg yolks providing useful amounts of the vitamin. Food fortification, e.g. of milk and orange juice, is used in some countries, e.g. the USA. Finland has recently introduced food fortification and this has virtually abolished deficiency in most parts of the population. The problem is that the elderly need larger intakes than young adults whilst babies and the very young need less. Thus, intakes suiting the elderly can be too high for babies and infants and have caused hypercalcaemia, in the UK at least, when several fortified infant foods were given as well as fortified milk feeds [131]. Thus food fortification, which would help reduce the risk of being vitamin D insufficient even before age-related changes develop, probably needs to be combined with the use of modest supplementation by older people.

Adverse effects of improved vitamin D provision

Supplementation of people with deficiency, who also happen to have undiagnosed primary hyper-parathyroidism, is well recognised to increase the risk of hypercalcaemia, and, since this condition may not cause hypercalcaemia when vitamin D deficiency is present, but gets more common with age, especially in women, this risk is significant. Thus if people started on supplementation become in unwell, especially if they get nausea, vomiting, gut upsets or thirst, they should have an urgent blood test including measurement of serum calcium, urea, creatinine and electrolytes. If serum calcium is high, and especially if renal function has been compromised, urgent admission for rehydration may well be needed [16]. Supplementation of adults, especially older adults, with calcitriol or its analogues rather than vitamin D_3, specifically increases the risks of nephrolithiasis [RR 1.17, 95% CI 1.02-1.34] [95], though this also a feature of hyperparathyroidism and is seen with excessive vitamin $D_{2/3}$ supplementation, even without concomitant hypercalcaemia, due the development of hypercalcuria. However, consideration of evidence already available suggests that aiming to achieve serum 25(OH)D concentrations of 75 nmol/l rather than 50 nmol/l is likely to have health benefits [132]. However, policy makers such as the IOM are unlikely to change their recommendations without further evidence of long-term safety.

Conclusions

Inadequate amounts of vitamin D in older people reduces well being, aggravates the ageing process, in particular reducing mobility and adds to the severity of osteoporosis and the risks of falls and fragility fractures with all of their severe consequences. It also reduces longevity, increasing the risk of cardiovascular deaths in particular, but may also increase the risks of type 2 diabetes and certain common cancers, notably colo-rectal cancer. Some describe vitamin D, feeding into genetic

mechanisms, as acting as a 'Fountain of Youth' [133]. At the most prosaic level, it is at least certain that reasonable intakes are protective against many common disorders that worsen with age. Since adequate exposure to summer sunshine becomes increasingly difficult for everyone with modern lifestyles, and more so with age, maintaining good intakes of vitamin D throughout life and increasing the amount taken in those aged 60-70 years or more would ensure that the problems that hypovitaminosis D aggravates with ageing are minimized. Current Institute of Medicine recommendations [40] for healthy adult intakes [600 IU/day] and for those over 70 [< 800 IU/day], together with the recommendations emerging from other bodies for those recognized as being at increased risk of deficiency (e.g. the dark skinned, those wearing veils or otherwise heavily clothed and those with little sun exposure, on poor diets or over 65 years old) of intakes of 1000-2000 IU/day would be protective for most people [32, 125]. These intakes cannot be achieved from a normal diet. Unless food fortification is in place, healthy people need to take these doses as supplements. Vitamin A supplementation should be avoided unless insufficiency is a problem locally. Supplements used should provide pure vitamin D3, without other nutrients. Supplemental calcium or other nutrients should only be taken if medically indicated. These recommendations provide advice based on available evidence but do not allow for the possibility that much higher doses may be more beneficial. However, adequate RCT data is not yet available for the potential benefits and risks of doses larger than 4000 IU/day over the long-term. Health care providers at all levels would be wise, therefore, to ensure that healthy people do achieve intakes of vitamin D in accordance with current advice. If higher intakes in the long-term prove to have added benefits, especially in older people, then dose recommendations will need to be increased as a public health priority.

References

[1] Holick MF (2011). Photobiology of vitamin D. Chapter 2:113-22. In Vitamin D, Vol 1. Eds Feldman D, Pike JW, Adams JS. Academic Press, Elsevier. ISBN: 978-0-12-387035-3

[2] Calvo MS, Whiting SJ, Barton CN (2005). Vitamin D intake: a global perspective of current status. J Nutr, 135:310-6

[3] Prentice A (2008). Vitamin D deficiency: a global perspective. Nutr Rev 66,(10 Suppl 2): S153-64

[4] Editorial (1968). Nutrition of the elderly. Lancet, 5619:629-30

[5] Corless D, Gupta SP, Sattar DA, Switala S, Boucher BJ.(1979). Vitamin D status of residents of an old people's home and long-stay patients. Gerontology, 25:350-5

[6] Exton-Smith AN, Hodkinson HM, Stanton BR (1966). Nutrition and metabolic bone disease in the elderly. Lancet, 2:999

[7] MacLaughlin J, Holick MF (1985). Ageing decreases the capacity of human skin to produce vitamin D3. J Clin Invest, 76: 1536-8

[8] Matsuoka LY, Wortsmann J, Haddad JG, Hollis B (1989). In vivo threshold for cutaneous synthesis of vitamin D3. J Lab Clin Med, 114:301-5

[9] Barragry JM, France MW, Corless D, Gupta SP, Switala S, Boucher BJ, Cohen RD (1978). Intestinal cholecalciferol absorption in the elderly and in younger adults. Clin Sci Mol Med, 55: 213-20

[10] Van der Wielen RP, Lowik MR, van den Berg H, de Groot LC, Haller J, Moreiras O, van Staveren WA (1995). Serum vitamin D concentrations among elderly people in Europe. Lancet, 346:207-10

[11] Sambrook PN, Cameron ID, Chen JS, Cumming RG, Durvasula S, Herrmann M, Kok C, Lord SR, Macara M, March LM, Mason LM, Seibel MJ, Wilson N, Simpson JM (2012). Does increased sunlight exposure work as a strategy to improve vitamin D status in the elderly: a cluster randomised controlled trial. Osteoporosis, 23:615-24

[12] Corless D, Gupta SP, Switala S, Barragry JM, Boucher BJ, Cohen RD, Diffey BL (1978). Response of plasma 25-hydroxyvitamin D to ultraviolet irradiation in long-stay geriatric patients. Lancet, 23:649-51

[13] Whyte MP (2011). Approach to the patient with metabolic bone disease. Chapter 46:807-822. In Vitamin D, Vol 1. Eds Feldman D, Pike JW, Adams JS. Academic Press , Elsevier. ISBN: 978-0-12-387035-3

[14] Jones KS, Schoenmaker I, Bluck LJC, Ding S, Prentice A (2012). Plasma appearance and disappearance of an oral dose of 25-hydroxyvitamin D2 in healthy adults. Brit J Nutr, 107:1128-37

[15] Berry D, Hyppönen E (2011). Determinants of vitamin D status: focus on genetic variations. Curr Opin Nephrol Hypertens, 20: 331-6. Review

[16] Anonymous. (2006). Primary vitamin D deficiency in adults. Drug Ther Bull, 44:25-9

[17] Hewison M, Burke F, Evans KN, Lammas DA, Sansom DM, Liu P, Modlin RL, Adams JS (2007). Extra-renal 25-hydroxyvitamin D3-1alpha-hydroxylase in human health and disease. J Steroid Biochem Mol Biol, 103:316-21

[18] Ren S, Nouyen L, Wu S, Encinas C, Adams JS, Hewison (2005). Alternative splicing of vitamin D-24-hydroxylase: a novel mechanisms for the regulation of extra renal 1.25-dihydroxyvitamin D synthesis. J Biol Chem, 280:20604-11

[19] Wang TT, Tavera-Mendoza LE, Laperriere D, Libby E, MacLead NB, Nagai Y, Bourdeau V, Konstorum A, Lallemant B, Zhang R, Mader S, White JH (2005). Large-scale in silico and micro-array-based identification of direct 1,25-dihydroxyvitamin D3 target genes. Mol Endocrinol, 19:2685-95

[20] Norman AW (2006). Mini review: new assignments for an already busy receptor. Endocrinology, 147: 5542-48

[21] Boucher BJ (2011). Vitamin D insufficiency and diabetes risks. Curr Drug Targets, 12:61-87.

[22] Richards JB, Valdes AM, Gardner JP, Paximadas D, Kimura M, Nessa A, Lu X, Surdulescu GL, Swaminathan R, Spector TD, Aviv A (2007). Higher serum 25-hydrocyvitamin D concentrations are associated with longer telomere length in women. Am J Clin Nutr, 86:1420-5

[23] Zhu H, Guo D. Li K, Pedersen-White J, Stallman-Jorgensen IS, Huang Y, Parikh S, Liu K, Dong Y (2011). Increased telomerase activity and vitamin D supplementation in overweight African Americans. Int J Obes [Lond], doi: 10.1038/ijo.2011.197. [Epub ahead of print]

[24] Nair-Shalliker V, Armstrong BK, Fenech (2012). Does vitamin D protect against DNA damage? Mutat Res, Feb 24 [Epub ahead of print]

[25] Gonzalez-Suarez I, Redwood AB, Grotsky DA, Neumann MA, Cheng EH, Stewart CL, Dusso A, Gonzalo S (2011). A new pathway that regulates 53BP1 stability implicates Cathepsin L and vitamin D in DNA repair. EMBO J, 30:3383-96

[26] Lanske B, Razzaque MS (2007). Vitamin D and ageing: old concepts and new insights. J Nutr Biochem, 18:771-7

[27] Tuohimaa P (2012). Vitamin D and ageing. J Steroid Biochem Mol Biol, 114:78-84

[28] Keisala T, Minasyan A, Lou YR, Zou J, Kalueff AV, Pyykkö I, Tuohimaa P (2009). Premature aging in vitamin D receptor mutant mice. J Steroid Biochem Mol Biol, 115:91-7

[29] Kuro-O M (2011). Klothos and the ageing process. The Korean J Int Med, 26:113-22

[30] Klotz B, Mentrup B, Regensburger M, Zeck S, Schneidereit J, Schupp N, Linden C, Merz C, Ebert R, Jakob F (2012). 1,25-dihydroxyvitamin D3 treatnment delays cellular ageing in human mesenchymal stem cells while maintaining their multipotent capacity. PLoS One, 7:e29959: 1-11

[31] Zouboulis CC, Makrantonaki E (2012). Hormonal therapy of intrinsic ageing. Rejuvenation Res, Apr 25. [Epub ahead of print].

[32] Jansen BC (1950). The necessity of vitamin D in old age. Rev Med Liege. 5:667-9. Perez-Lopez FR, Brinat M, Erel CT, Tremollieres F, Gambacciani M, Lambrinoudaki I, Moen MH, Schenk-Gustafsson K, Vujovic S, Rozenberg S, Rees M (2012). EMAS position statement: vitamin D and postmenopausal health. Maturitas, 71:83-8

[33] Corless D, Dawson E, Fraser F, Ellis M, Evans SJ, Perry JD, Reisner C, Silver CP, Beer M, Boucher BJ (1985). Do vitamin D supplements improve the physical capabilities of elderly hospital patients? Age Ageing, 14 76-84

[34] Muir SW, Montero-Odasso M (20011). Effect of vitamin D supplementation on muscle strength, gait and balance in older adults: a systematic review and meta-analysis. J Am Geriatr Soc, 59:2291-3000

[35] Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL (2011). Effect of vitamin D on muscle strength: a systematic review and meta-analysis. Osteoporosis In, 22:859-71

[36] Houston DK, Tooze JA, Hausman DB, Johnson MA, Micklas BJ, Miller ME, Neiberg RH, Marsh AP, Newman AB, Blair SN, Kritchevsky SB (2011). Change in 25-hydroxyvitamin D and physical performance in older adults. J Gerontol A Biol Sci Med Sci, 66:430-6

[37] Mosekilde L (2005). Vitamin D and the elderly. Clin Endocrinol, 62:265-81

[38] Kanis JA, Brazier JE, Stevensen M, Calvert NW, Lloyd-Jones M (2002). Treatment of established osteoporosis: a systematic review and cost-analysis. Health Assessment technology. Vol 6: No 29 *Executive summary*. NHS R&D HT

[39] Trivedi DP, Doll R, Khaw KT (2003). Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomized double blind controlled trial. BMJ, 326:469.

[40] Ross AC, Manson JE, Abrams SA, Aloia JF, Clinton SK, Duraxo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Mayne ST, Rosen CJ, Shapses SA (2011). The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab, 96:53-8.

[41] (a). Bischoff-Ferrari HA, Kiel DO, Dawson-Hughes B, Oray JE, Priegelman D, Dietrich T, Willett WC (2009). Dietary calcium and serum 250hydroxyvitamin D status in relation to BMD among U.S. adults. J Bone Min Res, 24:935-42. (b) Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Oray JE, Stuck AE, Theiler R, Wong JB, Egli A, Kiel DP, Henschkowski J (2009). Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomized controlled trials. BMJ, 339:b3692

[42] Grimnes G, Joakimsen R, Figenschau Y, Torjese PA, Almås B, Jorde R (2012). The effect of high-dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass--a randomized controlled 1-year trial.Osteoporos Int, 23:201-11.

[43] Grant AM, Avenell A, Cambell MK, McDonald AM, MacLennan GS, McPherson GC, Anderson FH (2005). Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. Lancet, 365:1621-8

[44] Chung M, Lee J, Terasawa T, lau J, Trikalinos TA (2011). Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventative Services Task Force. Ann Int Med, 155:827-38

[45] Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, Arden NK, Godfrey KM, Cooper C; Princess Anne Hospital Study Group (2006). Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet, 367:36-43

[46] Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL (2006). Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science, 311: 1770-73.

[47] Martineau AR, Wilkinson KA, Newton SM, Floto RA, Norman AW, Skolimowska K, Davidson RN, Sorensen OE, Kampmann B, Griffiths CJ. Wilkinson RJ (2007). IFN-gamma and TNF-independent vitamin D-inducible human suppression of mycobacterial: the role of cathelicidin LL-37. J Immunol, 178:190-8

[48] Borges MC, Martini LAM, Rogero MM (2011). Current perspectives on vitamin D, immune system, and chronic diseases. Nutr, 27: 399-404

[49] Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier MC (2010). Vitamin D and inflammation. Joint Bone Spine, 77: 552-7.

[50] Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H (2010). Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr, 91:1255-60

[51] Manaseki-Holland S, Maroof Z, Bruce J, Mughal MZ, Bhutta ZA, Walraven G, Chandranohan D (2012). Effect on the incidence of pneumonia of vitamin D supplementation by quarterly bolus doses to infants in Kabul: a randomized controlled superiority trial. Lancet, 379:1419-27

[52] Goldstein MR, Mascitelli L, Pezzetta F (2010). Pandemic influenza A (HINI): mandatory vitamin D supplementation? Med Hypotheses, 74:756.

[53] Berry DJ, Hesketh K, Power CM, Hyppönen E (2011). Vitamin D status has a linear association with seasonal infections and lung function in British adults. Br J Nutr, 106:1433-40

[54] JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA, Landry ML (2010). Serum 25-hydroxyvitamin D and the incidence of acute viral respiratory infections in healthy adults. PLos One, 5: e11088.

[55] Jorde R, Witham M, Janssens W, Rolighed L, Borchard K, de Boer IH, Grimnes G, Hutchinson MS (2012). Vitamin D supplementation did not prevent influenza-like illness as diagnosed retrospectively by questionnaires in subjects participating in randomized clinical trials. Scand J Infect Dis, 44:126-32

[56] Grant WB (2008). Vitamin D, periodontal disease, tooth loss and cancer risk. Lancet Oncol, 9:612-3

[57] Jabbar S, Drury J, Fordham J, Datta HK, Francis RM, Tuck SP (2011). Plasma vitamin D and cytokines in periodontal disease and postmenopausal osteoporosis. J Periodontal Res, 46: 97-104.

[58] Amano Y, Komiyama K, Makishima M (2009). Vitamin D and periodontal disease. J Oral Sci, 51: 11-20.

[59] Zhou J, Chew M, Ravn HB, Falk E (1999). Plaque pathology and coronary thrombosis in the pathogenesis of acute coronary syndromes. Scand J Clin Lab Invest, 230 (Suppl 1): 3-11. Review.

[60] Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, Packe GE, Moore-Gillon JC, Darmalingam M, Davidson RN, Milburn HJ, Baker LV, Barker RD, Woodward NJ, Venton TR, Barnes KE, Mullett CJ, Coussens AK, Rutterford CM, Mein CA, Davies GR, Wilkinson RJ, Nikolayevskyy V, Drobniewski FA, Eldridge SM, Griffiths CJ (2011). High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet, 377:242-50

[61] Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D, Aganna E, Price CP, Boucher BJ (2002). Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? QJM, 95:787-96.

[62] Coussens A, Timms PM, Boucher BJ, Venton TR, Ashcroft AT, Skolimowska KH, Newton SM, Wilkinson KA, Davidson RN, Griffiths CJ, Wilkinson RJ, Martineau AR (2009). 1alpha, 25-dihyrdroxyvitamin D3 inhibits matrix metalloproteinases induced by Mycobacterium tuberculosis infection. Immunology, 127:539-48

[63] Edlich RF, Mason SS, Dahlstrom JJ, Swainston E, Long WB 3rd, Gubler K (2009). Pandemic preparedness for swine flu influenza in the United States. J Environ Pathol Toxicol Oncol, 28:261-4

[64] Ramesh KV, Mahindraker MB, Bhat EP (1993). A new role for vitamin D: cholecalciferol promotes dermal wound strength and re-epithelization. Indian J Exp Biol, 31: 778-9

[65] Tian XQ, Chen TC, Holick MF (1995). 1,25-dihydroxyvitamin D3: a novel agent for enhancing wound healing. J Cell Biochem, 59: 53-56.

[66] Kalav UR, Cha SS, Takahashi P (2011). Association between vitamin D and pressure ulcers in older ambulatory adults: results of a matched case-control study. Clin Interv Ageing, 6:213-9

[67] Grant WB, Boucher BJ (2010). Are Hill's criteria for causality satisfied for vitamin D and periodontal disease? Dermatoendocrinol, 2:30-6

[68] Bashutski JD, Eber RM, Kinney JC, Benavides E, Matra S, Braun TM, Giannobile WW, McCauley LK (2011). The impact of vitamin D status on periodontal surgery. J Dent Res, 90: 1007-12

[69] Bitetto D, Fabris C, Falleti E, Fornasiere E, Fumolo E, Fontanini E, Cussigh A, Occhino G, Baccarani U, Pirisi M, Toniutto (2010). Vitamin D and the risk of acute allograft rejection following human liver transplantation. Liver Int, 30: 417-44.

[70] Leow L, Simpson T, Cursons R, Karalus N, Hancox RJ (2011). Vitamin D, innate immunity and outcomes in community acquired pneumonia. Respirolog, 16:611-6

[71] Avenell A, Cook J, MacLennan GS, MacPherson GC (2007). Vitamin D supplementation to prevent infections: a sub-study of a randomised placebo-controlled trial in older people. (RECORD trial, ISRCTN 5164738). Research Letter. Age and Ageing, 36:574-92

[72] Boucher BJ (2012). Is vitamin D status relevant to metabolic syndrome? Dermatoendocrinol, 4: 1-12.(In Press).

[73] Kujala UM, Jokelainen J, Oksa H, Saaristo T, Rautio N Moilanen L (2011). Increase in physical activity and cardiometabolic risk profile change during lifestyle intervention in primary healthcare: 1-year follow-up study among individuals at high risk for type 2 diabetes. BMJ Open. 19;e000292

[74] Athyros G, Tziomalos K, Karagiannis A, Mikhailidis DP (2010). Preventing diabetes mellitus: room for residual risk reduction after lifestyle changes? Curr Pharm Des, 16:3939-847

[75] Boucher BJ (1998). Inadequate vitamin D status: does it contribute to the disorders comprising 'Syndrome 'X'? Br J Nutr, 79: 315-27

[76] Forouhi NG, Luan JJ, Cooper A, Boucher BJ, Wareham NJ (2008). Baseline serum 25-hydroxyvitamin D is predictive of future glycaemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. Diabetes, 57:2619-25

[77] Boucher BJ, Mannan N, Noonan K, Hales CN, Evans SJW (1995). Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. Diabetologia, 38:1239-45

[78] Von Hurst PR, Stonehouse W, Coad J (2010). Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient – a randomised, placebo-controlled trial. Br J Nutr, 103:549-55

[79] Rueda S, Fernandez-Fernandez C, Romero F, Martinez de Osaba J, Vidal J (2008). Vitamin D, PTH, and the metabolic syndrome in severely obese subjects. Obes Surg, 18:151-4

[80] Auwerx J, Bouillon R, Kesteloot H (1992). Relation between 25-hydroxyvitamin D3, apolipoprotein A-1 and high density lipoprotein cholesterol. Arterioscler Thromb, 12:671-4

[81] John WG, Noonan K, Mannan N, Boucher BJ (2005). Hypovitaminosis D is associated with reductions in serum apolipoprotein A-I but not with fasting lipids in British Bangladeshis. Am J Clin Nutr, 82:517-22.

[82] Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J (2004). Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. J Steroid Biochem Mol Biol, 89-90:387-92.

[83] Cheng Q, Li YC, Boucher BJ, Leung PS (2011). A novel role for vitamin D: modulation of expression and function of the local renin-angiotensin system in mouse pancreatic islets. Diabetologia, 54:2077-81

[84] Cheng Q, Boucher BJ, Leung PS (2012). Modulation of hypovitaminosis D-induced glucose homeostasis impairment and islet dysfunction through direct suppression of the pancreatic islet renin-angiotensin system. *(Submitted)*

[85] Deleskog A, Hilding A, Brismar K, Hamsten A, Afendic S, Ostenson CG (2012). Low serum 25-hydroxyvitamin D level predicts progression to type 2 diabetes in individuals with prediabetes but not with normal glucose tolerance. Diabetologia, Mar 17 [ahead of print].

[86] Pittas AG, Dawson-Hughes B, Li T, Van Damn RM, Willett WC, Manson JE, Hu FB (2006). Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care, 29:650-6

[87] Pittas AG, Lau J, Hu FB, Dawson-Hughes B (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J Clin Endocrinol Metab, 92:20127-9

[88] Hirani V (2011). Relationship between vitamin D and hyperglycemia in older people from a nationally representative population survey. J Am Geriatr Soc, 59: 1786-9

[89] Seals DR, Jablonski KL, Donato AJ (2011). Ageing and vascular endothelial function in humans. Clin Sci (Lond), 120:357-75

[90] Ertek S, Akgül E, Cicero AF, Kütük U, Demirtaş S, Cehreli S, Erdoğan G (2012). 25-Hydroxy vitamin D levels and endothelial vasodilator function in normotensive women. Arch Med Sci, 29:47-52.

[91] Harris RA Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, Shah Y, Zhu H, Ding Y (2011). Vitamin D3 supplementation for 16 weeks improves flow-mediated dilatation in overwieght African-American adults. Am J Hypertens, 24:557-62

[92] Vaidya A, Forman JP (2012). Vitamin D and vascular disease: the current and future status of vitamin D therapy in hypertension and kidney disease. Hypertens Rep, 14:111-9.

[93] Zitterman A, Iodice S, Pilz S, Grant WB, Bagnardi V, Gandini S (2012). Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. Am J Clin Nutr, 95:91-100

[94] Gotsman I, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C, Admon D (2012). Vitamin D deficiency is a predictor of reduced survival in patients with heart failure; vitamin D supplementation improves outcome. Eur J Heart Fail, 14: 357-66.

[95] Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wettersley J, Simonetti RG, Bjelakovic M, Gluud C (2011). Vitamin D supplementation for reduction of mortality in adults. Cochrane Database Syst Rev, 6:CD007470

[96] Vacek JL, Vanga SR, Good M, Lai SM, Lakkireddy D, Howard PA (2012). Vitamin D deficiency and supplementation and relation to cardiovascular health. Am J Cardiol, 109:359-63

[97] Schöttker B, Ball D, Gelfert C, Brenner H (2012). Serum 25-hydroxyvitamin D levels and overall

mortality. A systematic review and meta-analysis of prospective cohort studies. Ageing Res Rev, Feb 17 [ahead of print]

[98] Rienstra M, Cheng S, Larson MG, McCabe EL, Booth SL, Jaques PF, Lubitz SA, Yin X, Levy D, Magnani JW, Elinor PT, Benjamin EJ, Wang TJ (2011). Vitamin D status is not related to development of atrial fibrillation in the community. Am Heart J, 162: 538-41

[99] Goldfine AB (2012). Statins: Is it really time to reassess benefits and risks? N Eng J Med, 366:1752-5

[100] Grimes DS (2009). Statins and vitamin D. Cardiovasc Drugs Ther, 23:261-2

[101] Bhattacharyya S, Bhattacharyya K, Maitra A (2012). Possible mechanisms of interaction between statins and vitamin D. Commentary. QJM, 105:487-91

[102] Buell JS, Scott TM, Dawson-Hughes B, Dallal GE, Rosenberg IH, Folstein MF, Tucker KL (2009). Vitamin D is associated with cognitive function in elders receiving home health services. J Gerontol A Biol Sci Med Sci, 64:888-95

[103] Stein MS, Scherer SC, Ladd KS, Harrison LC (2011). A randomised controlled trial of high-dose vitamin D2 followed by intranasal insulin in Alzheimer's disease. J Alzheimers Dis, 26: 477-84

[104] Topannen AM, Williams D, Hendersen J, Lawlor DA (2011). Serum 25-hydroxyvitamin D and ionised calcium in relation to lung function and allergen skin tests. Eur J Clin Nutr, 65:493-500

[105] Romme EA, Rutten EP, Smeenk FW, Spruit MA, Menheere PP, Wouters EF (2012). Vitamin D status is associated with bone mineral density and functional exercise capacity in patients with chronic obstructive pulmonary disease. Ann Med, Apr 2. [Epub ahead of print]

[106] Shaheen SO, Jameson KA, Robinson SM, Boucher BJ, Syddall HE, Sayer AA, Cooper C, Holloway JW, Dennison EM (2011). Relationship of vitamin D status to adult lung function and COPD. Thorax, 66:692-8

[107] Zhao G, Ford ES, Tsai J, Li C, Croft JB (2012). low concentrations of serum 25-hydroxyvitamin D associated with increased risk for chronic bronchitis among US adults. Brit J Nutr, 107: 1386-92

[108] Kunisaki KM, Niewoehner DE, Singh RJ, Connett JE (2010). Vitamin D status and longitudinal lung function decline in the Lung Heath Study. Eur J Resp J, 37:238-43

[109] Tsujimoto Y, Tahara H, Shoji T, Emoto M, Koyama H, Ishimura E, Tabata T, Nishizawa Y, Inaba M (2011). Active vitamin D and acute respiratory infections in dialysis patients. Clin J Am Soc Nephrol, 6:1361-7.

[110] Tillis CC, Huang HW, Bi W, Pan S. Bruce SR, Alcorn J (2011). Glucocorticoid regulation of human pulmonary surfactant protein-B (SP-B) mRNA stability is independent of activated glucocorticoid receptor. Am J Physiol Lung Cell Mol Physiol, 300. L940-50

[111a] Mak RH (1998). 1,25-dihydroxyvitamin D3 corrects insulin and lipid abnormalities in uremia. Kidney Int, 53: 1353-7

[111b] Shoji T, Shinohara K, Kimoto E, Emoto M, Tahara H, Koyama H, Inaba M, Fukumoto S, Ishimura E, Miki T, Tabata T, Nishizawa Y (2004). Lower risk for cardiovascular mortality in oral 1alpha-hydroxy vitamin D3 users in a hemodialysis population. Nephrol Dial Transplant, 19:179-84.

[112] Querfeld U, Mak RH (2010). Vitamin D deficiency and toxicity in chronic kidney disease: in search of the therapeutic window. Pediatr Nephrol, 25: 2413-30. Review.

[113] Jones G (2010). Why dialysis patients need combination therapy with both cholecalciferol and a calcitriol analog. Semin Dial, *23:* 239-43.

[114] Lee V, Rekhi E, Kam JH, Jeffrey G (2012). Vitamin D rejuvenates ageing eyes by reducing inflammation, clearing amyloid beta and improving visual function. Neurobiol Ageing, Jan 2 [ahead of print]

[115] Morrison MA, Silveira AC, Huynh N, Jun G, Smith SE, Zacharaki F, Sato H, Loomis S, Andreoli MT, Adams SM, Radeke MJ, Jelcick AS, Yuan Y, Tsiloulis AN, Chatzoulis DZ, Silvestri G, Kotoula MG, Tsironi EE, Hollis BW, Chen R, Haider NB, Miller JW, Farrer LA, Hageman GS, Kim IK, Schaumberg DA, DeAngelis MM (2011). Systems biology-based analysis implicates a novel role for vitamin D metabolism in the pathogenesis of age-related macular degeneration. Hum Genomics, 5:538-68

[116] Grant WB (2012). Ecological studies of the UVB-vitamin D-cancer hypothesis. Anticancer Res, 32:223-36

[117] IARC Working Group Report 5: Vitamin D and cancer (Nov 25 2008). http://www.iarc.fr/en/Mediia-Centre/IARC-News?Vitamin D and Cancer

[118] Wolpin BM, Ng K, Bao Y, Kraft P, Stampfer MJ, Michaud DS, Ma J, Buring JE, Sesso HD, Lee IM, Rifai N, Cichrane BB, Wactawski-Wende J, Chlebowski RT, Willett WC, Manson JE, Giovannucci EL, Fuchs CS (2012). Plasma 25-hydroxyvitamin D and risk of pancreatic cancer. Cancer Epidemiol Biomarkers Perv, 21:82-91

[119] Newton-Bishop JA, Chang YM, Elliott F, Chan M, Leake S, Karpavicius B, Haynes S, Fitzgibbon E, Kukalizch K, Randerson-Moor J, Elder DE, Bishop DT, Barrett JH (2011). Relationship between sun exposure and melanoma risk for tumours in different body sites in a large case-control study in a temperate climate. Eur J Cancer, 47: 732-41.

[120] Lappe JM, Travers-Gustavson D, Davies KM, Recker RR, Heaney RP (2007). Vitamin D and calcium supplementation reduce cancer risk: results of a randomized trial. Am J Clin Nutr, 85: 1586-91

[121] Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, Lee R, Belch JF, Wilson M, Mehta Z, Meade TW (2012). Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet, 379:1602-12.

[122] Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, Stattin P, Harvei S, Hakulinen T, Luostarinen T, Dillner J, Lehtinen M, Hakama M (2004). Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer, 108:104-8

[123] Vieth R (2004). Enzyme kinetics hypothesis to explain the U-shaped risk curve for prostate cancer vs. 25-hydroxyvitamin D in Nordic countries. Int J Cancer,111:468

[124] Perez-Lopez FR, Brincat M, Erel CT, Temollieres F, Gambacciani M, Lambrnoudaki I, Moen MH, Schenk-Gustafsson K, Vujovic S, Rozenberg S, Rees M (2012). EMAS position statement: Vitamin D and postmenopausal health. Maturitas, 71:83-8

[125] Chief Medical Officers for England, Wales, Northern Ireland and Scotland (2012). Vitamin D – Advice on supplements for at risk groups. Circular to General Practitioners, Practice Nurses, Health visitors, Community Pharmacists. Ref CEM/CMO/2012/04. Gateway ref 17193. Department of Health (UK). Dangers of vitamin D deficiency highlighted. http://www.dh.gov.uk/health/2012/02/vitamin-d/accessed 07/02/2012

[126] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM (2011). Evaluation, treatment , prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab, 96:1911-30

[127] Sattar N, Welsh P, Panarelli M, Forouhi NG (2012). Increasing requests for vitamin D measurement: costly, confusing, and without credibility. Lancet, 379:95-6

[128] Carter GD, Carter R, Jones J, Berry J (2004). How accurate are assays for 25-hydroxyvitamin D? Data from the International Vitamin D External Quality Assessment Scheme. Clin Chem, 50: 2195-97

[129] Haines ST, Park SK (2012). Vitamin D supplementation: What's known, what to do, and what's needed. Pharmacotherapy, 32:354-82

[130] Stephens WP, Limiuk PS, Berry JL, Mawer EB (1981). Annual high-dose vitamin D prophylaxis in Asian immigrants. Lancet,28:1199-202

[131] Food and Agriculture Organization [United Nations] (2002). Corporate document report. Human Vitamin and Mineral Requirements. Chapter 8: Vitamin D.http://www.fao.org/docrep/004/Y2809E/y2809/ee00.htm

[132] Vieth R (2012). Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/l (30 ng/ml). Best Pract Res Clin Endocrinol Metab, 25:681-91

[133] Haussler MR, Haussler CA, Whitfield GK, Hsieh J-C, Thompson PD, Barthel TK, Bartik L, Egan JB, Wu Y, Kubicek JL, Lowmiller CL, Moffet EW, Forster RE, Jurutka PW (2010). The nuclear vitamin D receptor controls the expression of genes encoding factors which feed the "Fountain of Youth" to mediate healthy ageing. J Steroid Biochem Mol Biol, 121:88-97