COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

First draft statement on adverse effects of high levels of vitamin D.

1. As COT members are aware, the Scientific Advisory Committee on Nutrition (SACN) are revising the Dietary Reference Values for vitamin D and the COT have been asked to consider the potential adverse effects of high intakes. This has been discussed in a number of COT papers to date, covering various aspects of the topic.

2. Some updating has taken place since the previous papers, for example new NDNS data on serum 25(OH)D levels have been included. However, members will wish to be aware that some data have been included from a paper by Markestad *et al*. (1987) in which 43 East German infants aged 1-20 months were monitored having been given doses of 15 mg vitamin D_2 every 3 -5 months as part of an existing prophylaxis program. Median serum 25(OH)D increased by up to 400 nmol/L two weeks after dosing compared to the level before each dose was given; individual increases were as high as 1000 nmol/L following the administration of the vitamin D dose. The infants were apparently healthy but hypercalcaemia was apparent in 34% of them, with levels of 2.81-3.32 mmol/L being measured (estimated from a figure in the paper) following dosing, the calcium levels appeared to be within normal levels by the time the next dose was given. It was stated that “repeated inquiries in GDR have failed to identify clinical vitamin D toxicity as a result of the prophylaxis program”.

3. The first draft of the COT statement is attached to this paper. However, since SACN have asked for a brief document to annex and refer to in their own report, it is envisaged that this will be based on the final section of the statement (paragraph 130 onwards), although the full statement will be provided to SACN and published in the usual way. Depending on the timelines and in particular SACN’s wish to publish the report for consultation as soon as possible, it may be necessary to finalise this section in advance of the rest of the statement.

4. Members are asked to comment on the overall structure and content of the statement and for more detailed comments on the final section and the conclusions.

Secretariat
August 2014

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

First draft statement on adverse effects of high levels of vitamin D.

Introduction

1. At the request of the Department of Health, the Scientific Advisory Committee on Nutrition (SACN) are revising the Dietary Reference Values (DRVs) for vitamin D and as part of this process the COT have been asked to consider the potential adverse effects of high vitamin D intakes. This would include the effects of both regular, long term intake and single or occasional doses as well as any effects on vulnerable groups.

Previous assessments

2. The safety of high intakes of vitamin D has been assessed by a number of expert bodies. In a 1991 report to the then Department of Health and Social Security, the Committee on the Medical Aspects of Food Policy (COMA) (the predecessor of SACN) established DRVs for a range of nutrients, including vitamin D. COMA briefly considered high intakes; they did not set a specific upper level, but noted that infants were most susceptible to hypervitaminosis D (COMA, 1991). It also cited a report by Markestad *et al*., (1987) that mild hypercalcaemia had occurred at intakes of 50 µg/day vitamin D per day or 15 mg every 3-5 months.

3. In 2002, the EU Scientific Committee on Food (SCF) established a Tolerable Upper Level (TUL)[1] of 50 µg[2] /day for adults (SCF, 2002). In 2003, the UK Expert Group on Vitamins and Minerals (EVM) recommended a maximum level of supplementary vitamin D intake of 25 µg/day for guidance purposes only, since it was considered that there was insufficient information to establish a Safe Upper Level (SUL) (EVM, 2003).

4. In 2011, an extensive review of vitamin D was undertaken by the US Institute of Medicine Food and Nutrition Board (IOM, 2011) which established an Upper Level of 100 µg/day vitamin D for adults; this was an increase from the upper level of 50

[1] SCF and EFSA have set TULs, IOM set ULs and EVM SULs. Although the terminology is different, the values represent a daily intake which, if consumed over a life time, would not be expected to result in adverse effects.

[2] The quantity of vitamin D can also be expressed as International Units (IU); 1 µg is equivalent to 40 IU

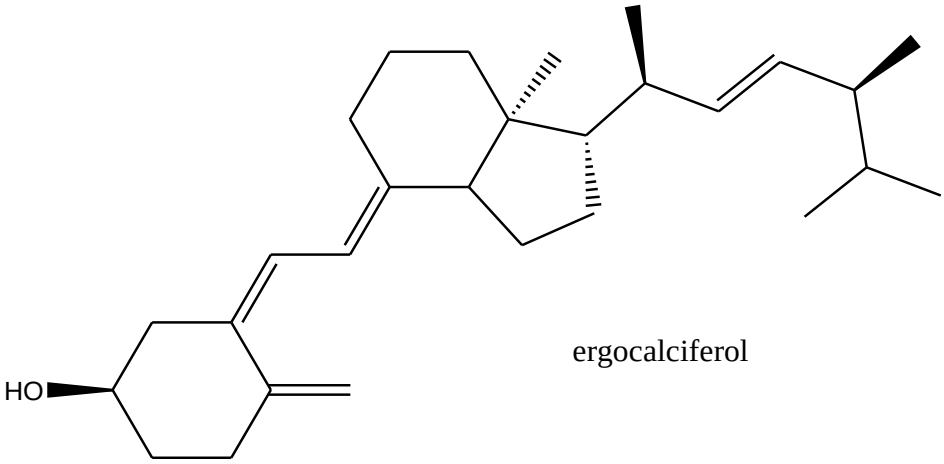
µg/day previously recommended (IOM,1997). The IOM noted the paucity of long term studies investigating the effects of vitamin D intakes above 250 µg per day or maintenance of serum 25-hydroxyvitamin D (25(OH)D) above 250 nmol/L. However, the IOM stated that it was unlikely that symptoms of toxicity would be observed at intakes below 250 µg/day, but that adverse effects would be observed at or above 1250 µg/day consumed for weeks or months. The IOM established ULs of 25, 38, 63, 75 and 100 µg/day vitamin D for infants and children aged 0-6 months, 6-12 months, 1-3 years, 4-9 years and 9-18 years respectively. The IOM document was used as the initial bibliographic source for the COT evaluation. The search strategy is attached at Annex A.

5. In 2012, the dietetic products, nutrition and allergies (NDA) panel of the European Food Safety Authority (EFSA) also published a review of vitamin D drawing on the IOM document (EFSA, 2012). The EFSA panel established a TUL for adults of 100 µg vitamin D per day which is an increase from the previous TUL of 50 µg/day (SCF, 2003). The EFSA panel established TULs of 25, 50 and 100 µg/day vitamin D for infants and children aged up to 12 months, 1-10 years and 11-17 years respectively. A TUL applies to all groups of the general population (excluding those receiving the nutrient under medical supervision), including sensitive individuals, throughout life stages such as pregnancy - except in some cases discrete, identifiable sub-populations (e.g. those with genetic predisposition or certain disease states) that may be especially vulnerable to one or more adverse effects (EFSA, 2006).

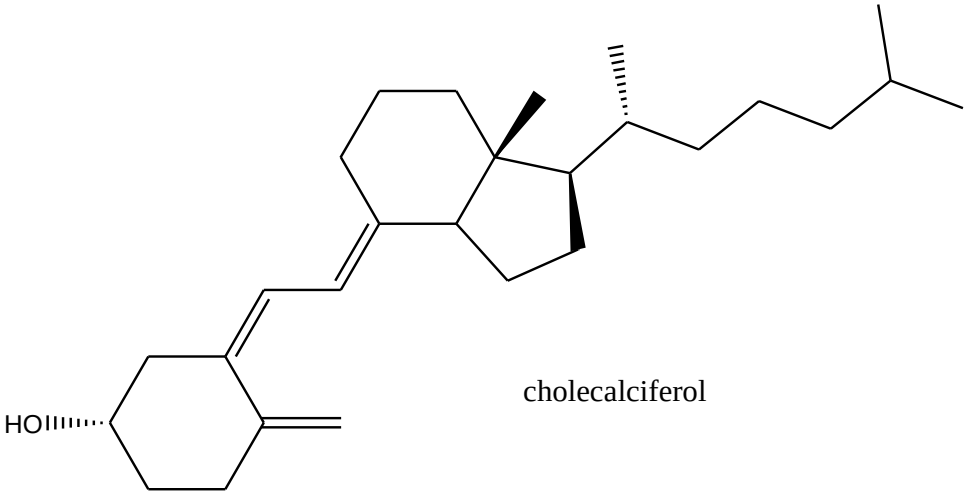
Background

6. Vitamin D is a pro-hormone also known as calciferol. It comprises a group of fat soluble seco-sterols. The two major forms are vitamin D_2 (ergocalciferol) which is produced by UV irradiation of plant and fungal materials (Lips, 2006; Jäpelt and Jakobsen, 2013) and vitamin D_3 (cholecalciferol) which is synthesised in the skin in response to UV irradiation. The two forms of vitamin D vary only in their side chain and, while some differences have been reported, this does not substantially affect their metabolism or biological efficacy.

Fig 1 Structure of vitamin D



ergocalciferol



cholecalciferol

7. Vitamin D undergoes sequential hydroxylation to form 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) also known as calcitriol, which is the active form of vitamin D.

Function of vitamin D

8. The main function of vitamin D is to elevate the levels of plasma calcium and phosphate which are required for the mineralisation of bone, the functioning of the neuromuscular junction, vasodilation, nerve transmission and hormonal secretion. This elevation is achieved by the stimulation of intestinal calcium absorption, mobilisation of calcium from bone via parathyroid hormone (PTH) and stimulation of the renal distal tubule reabsorption of calcium by the kidney. The molecular mechanisms for these processes have not yet been fully elucidated.

9. As serum calcium rises, PTH secretion drops. If serum calcium levels become too high, the parafollicular cells of the thyroid secrete calcitonin which blocks calcium resorption from bone and helps to keep calcium levels within the normal range. 1,25(OH)D2 suppresses parathyroid gene expression and parathyroid cell proliferation via the vitamin D receptor (VDR), providing important feedback loops.

10. VDRs are widespread throughout the body including in tissues not involved with calcium or phosphate homeostasis, the classical role of vitamin D. The

presence of VDRs in these tissues implies that vitamin D may play a more general role, or that ligands other than calcitriol can activate VDRs. The non-classic tissues also contain cytochrome P450 (CYP) 27B1 which is able to produce the active form of the vitamin (Bikle, 2009). Vitamin D-responsive elements (VDREs) are present in a large number of genes associated with both the classical and non-classical roles of the vitamin, such as the regulation of cell proliferation, cell differentiation and apoptosis. It has been suggested that calcitriol exerts immunomodulatory and anti-proliferative effects through autocrine and paracrine pathways (Adams and Hewison, 2008).

Vitamin D status

11. The best indicator of vitamin D status of both cutaneous synthesis and dietary intake is the serum 25(OH)D concentration, since the active form, 1,25 (OH)D2, has a short half-life and its formation is not directly regulated by vitamin D exposure but may be influenced by factors such as PTH (IOM, 2011). In addition, even in severe vitamin D deficiency, 1,25(OH)D2 levels may be normal or elevated through the up regulation of the 1-α hydroxylase enzyme.]. The normal circulating level of 25(OH)D in the blood is in the range 25-200 nmol/L[3] (Jones, 2009). Hollis (2005) stated that in sun-rich environments, where cultural practices permit sun exposure, circulating levels are 135 to 225 nmol/L, suggesting that current circulating levels may be too low. It has also been stated that "sun worshippers and life guards" (Holick, 2006) could have serum 25(OH)D levels of up to 312 nmol/L.

12. In the UK, data from the NDNS rolling programme up to 2012 reported that mean 25(OH)D levels were 44.9 and 41.1 nmol/L in boys and girls aged 11-18 years respectively and 43.5 and 47.3 nmol/L in men and women respectively (Bates, 2014). Levels at the upper 2.5 centile were 100.0 and 87.5 nmol/L in boys and girls aged 11-18 years respectively and 92.4 and 106.0 nmol/L in men and women respectively.

Sources of dietary vitamin D

13. Vitamin D_2 is found in non-animal foods, particularly fungi, and vitamin D_3 in animal-based foods such as fatty fish, fish liver oil and egg yolk. Foods such as milk, margarine and breakfast cereals may be fortified with vitamin D. Both forms of vitamin D are found in food supplements. Single supplements are available containing up to 500 µg vitamin D per daily dose, with most multi-vitamin supplements containing 5 µg vitamin D per daily dose; the latter amount being the EU Recommended Daily Amount, a harmonised value used for labelling purposes (EC, 2008).

14. Human milk contains low levels of vitamin D, but infant formula is fortified with 1-2.5 µg vitamin D/100 Kcal (EC,2006).

15. The Department of Health currently recommends that most individuals can obtain all the vitamin D they need by eating a healthy balanced diet, but that some

[3] Serum 25(OH)D is sometimes reported in ng/ml; 1 ng/ml = 2.49 nmol/L

population groups should take supplements. These groups are pregnant and breast-feeding women, children aged 6 months to 5 years, infants consuming small quantities of infant formula or breast fed infants, whose mothers did not take supplements during pregnancy or lactation, and adults aged > 65 years who are not exposed to much sunshine (NHS Choices, 2014).

Absorption, distribution, metabolism and excretion

Absorption, distribution and metabolism

16. Vitamin D is fat soluble and is absorbed in the small intestine with dietary fats. Efficient absorption of vitamin D depends on the presence of fat in the lumen which triggers the release of bile acids and pancreatic lipase.

17. Within the intestinal wall, vitamin D is incorporated into chylomicrons with cholesterol, triglycerides, lipoproteins and other lipids, the chylomicrons reach the systemic circulation via the lymphatic system. Chylomicron lipids are metabolised in peripheral tissues that express lipoprotein lipase, particularly adipose tissue and skeletal muscle. During hydrolysis of the chylomicron triglycerides, a fraction of the vitamin D can be taken up by the tissues. After this hydrolysis, a chylomicron remnant remains; this is a particle which contains a fraction of the original vitamin D content.

18. Vitamin D is cleared from the lymph or skin within a few hours. A fraction of newly absorbed intestinal vitamin D is also transported directly to the liver via the portal system along with amino acids and carbohydrates. Vitamin D is also sequestered in adipose tissue as a result of its hydrophobic nature, but these are probably non-specific stores; the mechanisms of accumulation or mobilisation are uncertain (IOM, 2011).

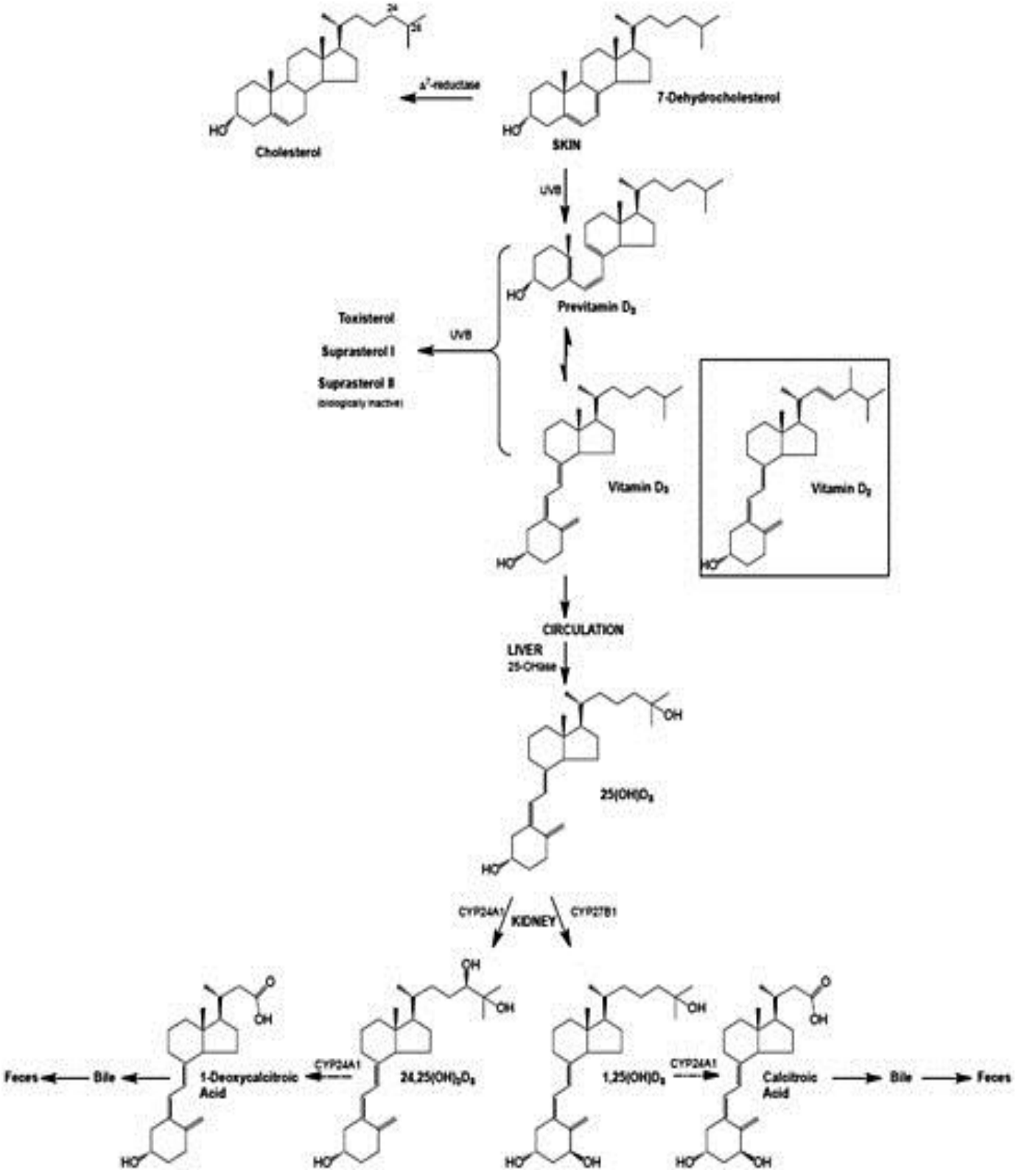
19. Vitamin D is hydroxylated in the liver to 25(OH)D by the 25 hydroxylase enzyme, most likely CYP 2R1 (IOM, 2011); there appears to be little, if any, feedback regulation of this enzyme with 25(OH)D then circulating in the blood bound to albumin and to vitamin D Binding Protein (DBP), a protein which has a high homology to albumin.

20. In response to changes in PTH stimulated by low calcium or phosphate, a second hydroxylation reaction takes place in the kidney where 25(OH)D is hydroxylated by 1α-hydroxylase (CYP27B1) to $1,25(OH)_2D$. This active metabolite enters the cells and binds to the VDR. This complex then forms a heterodimer with the retinoid receptor and binds to a vitamin D responsive element on a responsive gene such as that of osteocalcin, calcium binding protein or 24-hydroxylase. This is followed by transcription and translation of proteins.

21. When $1,25(OH)_2D$ is sufficiently available, 24,25 dihydroxyvitamin D ($24,25(OH)_2D$) is formed in the kidney which is then further catabolised (Lips, 2006). The 24-hydroxylase enzyme (CYP24A1) is found in all target tissues and is induced in response to calcitriol interacting with the VDR. CYP24A1 is also responsible for the metabolic degradation of 25(OH)D and ultimately produces calcitroic acid from

calcitriol and 1- desoxycalcitroic acid from (24,25(OH)2D. Vitamin D metabolism is described in Fig 2 below:

Fig 2. The metabolism of vitamin D_3 from synthesis/intake to formation of metabolites. (taken from IOM, 2011)


Cholesterol
Δ7-reductase
7-Dehydrocholesterol
SKIN
UVB
Previtamin D3
Toxisterol
Suprasterol I
Suprasterol II
(biologically inactive)
UVB
Vitamin D3
Vitamin D2
CIRCULATION
LIVER
25-OHase
25(OH)D3
CYP24A1
KIDNEY
CYP27B1
Feces
Bile
1-Deoxycalcitroic Acid
CYP24A1
24,25(OH)2D3
1,25(OH)2D3
CYP24A1
Calcitroic Acid
Bile
Feces

22. The vitamin D metabolites in the circulation are also bound to DBP which has a high affinity for 25(OH)D, 1,25$(OH)_2$D and 24,25$(OH)_2$D. The dihydroxy metabolites have a range of affinities, both higher and lower than that of 25(OH)D. At any one time, only 1–2% of the DBP sterol-binding sites are bound, this excess of sites indicates that the primary biological role of DBP extends beyond acting solely as a transport molecule for vitamin D (Gomme and Bertolini, 2004).

Excretion

23. Vitamin D metabolites are largely excreted through the bile and into the faeces, with very little being eliminated in the urine, partly due to renal reuptake of vitamin D metabolites bound to DBP. The mono, di and tri-hydroxylated metabolites show progressively increasing polarity, culminating with the water-soluble biliary form calcitroic acid. The whole body half-lives of vitamin D, 25(OH)D and 1,25(OH)2D are 2 months, 15 days and 15 hours respectively (Jones, 2008).

Formation of vitamin D by UV exposure

24. The synthesis of vitamin D_3 is a two stage process which begins with the irradiation of 7 dehydrocholesterol by UV light resulting in the formation of pre-vitamin D (Webb, 2006). Pre-vitamin D then undergoes thermal isomerisation to form vitamin D_3. Irradiation of pre-vitamin D also results in the formation of lumisterol and tachysterol which are inactive (Bikle, 2011) or it can revert back to 7 dehydrocholesterol. The formation of pre-vitamin D_3 is rapid and reaches a maximum within hours. Continued irradiation results in the formation of the biologically inactive lumisterol (but not tachysterol or further pre-vitamin D_3). Lumisterol can form pre-vitamin D if levels of the latter fall.

25. Prolonged exposure to sunlight does not result in the formation of toxic levels of vitamin D_3 due to the formation of tachysterol and lumisterol and because vitamin D_3 itself can be photoconverted to suprasterols I and II and 5,6 trans-vitamin D_3 which are also inactive. Prolonged irradiation results in a quasi-equilibrium mixture of isomers. The relative amount of each isomer depends on the irradiation spectrum and length of irradiation but in sunlight there is a limit to the amount of pre-vitamin D_3 which forms within the mixture, this being less than 12-15% (Webb, 2006). In contrast to oral vitamin D, vitamin D_3 then enters the circulation attached to DBP where hydroxylation to 25(OH)D and subsequently to 1,25$(OH)_2$D occurs. In the absence of supplementation, the majority of vitamin D_3 in the circulation occurs from exposure to sunlight.

26. Hollis (2005) reported that a 10-15 minute whole body exposure to summer sun could generate and release up to 500 µg vitamin D_3 into the circulation.

27. Vitamin D_2 is found in yeast and fungi through the irradiation of ergosterol. Vitamin D2 in plant material may result from the presence of fungal contamination (Jäpelt and Jakobsen, 2013).

D_2 and D3

28. Whether there is a difference in potency between the two forms of vitamin D is uncertain. Qualitatively, vitamins D_2 and D_3 exhibit virtually identical biological responses throughout the body (i.e., through gene expression) that are mediated by the VDR (IOM, 2011).There is some suggestion from animal data that vitamin D_2 is less toxic than vitamin D_3 and data from human trials suggest that it is also less effective at increasing serum levels. This issue is discussed in more detail by IOM (2011), who concluded that firm conclusions could not be drawn but that at low doses the two forms appeared to be equivalent but that at high doses vitamin D_2 was less effective, and might also be less toxic. The differences, if any, may be due to minor differences in metabolism, as below.

29. *In vitro* evidence suggests that the rates of inactivation are virtually identical for the two forms of vitamin D. However, the routes of catabolism of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ differ following the initial deactivating 24-hydroxylation step. As a result of the actions of non-specific enzyme systems as well as phase II enzymes, the overall half-life of $1,25(OH)_2D_2$ is slightly reduced compared to $1,25(OH)_2D_3$. The biliary metabolites of vitamin D_2, are comparable to those of vitamin D_3.

30. Where known, the form of vitamin D used has been noted in this statement.

Vitamin D toxicity

Hypercalcaemia

31. Excess levels of vitamin D are associated with the occurrence of hypercalcaemia and hypercalcuria. Vitamin D promotes the absorption of calcium from the gut as well as calcium resorption from bone, therefore sustained high levels of vitamin D exposure and the ensuing hypercalcaemia can result in calcium deposition in soft tissues, diffuse demineralisation of bones and irreversible renal and cardiovascular toxicity. Resorption of calcium from bone may be the most important component of the hypercalcaemia (Selby *et al*., 1995).

32. Clinical signs and symptoms may include anorexia, nausea, vomiting, weakness, lethargy, constipation and non-specific aches and pains (Barrueto *et al*., 2005) as well as weight loss, polyuria and heart arrhythmia. These effects have been observed in a number of case reports of vitamin D intoxication. Hypercalcaemia has also been reported in a few individuals taking part in vitamin D supplementation intervention studies.

33. Because of the lipophilicity of vitamin D and its storage in fat tissue, the effects of vitamin D toxicity can persist for more than 2 months after the exposure has ceased (Barrueto *et al*., 2005).

Calcium status and the definition of hypercalcaemia

34. Free (ionised) calcium is the biologically active form of calcium, but calcium is also present in serum bound to protein or complexed with anions. In the blood and extracellular fluid, total calcium is maintained at a concentration of 2.5 mmol/L (range

2.25-2.6 mmol/L) and ionised calcium is maintained at 1.1-1.4 mmol/L (EFSA, 2012). Hypercalcaemia is generally considered to be a total calcium concentration greater than 2.75 mmol/L. However, hypercalcaemia also occurs when ionised calcium alone becomes elevated.

35. Since total calcium might not accurately reflect calcium status, it is sometimes adjusted for protein or albumin concentration to improve its reliability. The majority of studies where serum calcium is measured report total or total corrected calcium. The COT noted that the correlation between total serum calcium and ionised serum calcium/ calcium status is not perfect and whilst this might have clinical implications for the individual, it would still be sufficient to detect treatment related changes in a population or group of individuals.

36. When serum calcium increases above 3 mmol/L, the ability of the kidney to reabsorb calcium becomes limited and hypercalcuria can occur. This is considered to be where urinary excretion of calcium exceeds 250 mg/day in women and 275-300 mg/day in men. The COT noted that urinary calcium concentration is unlikely to provide useful information, since this has high individual variability and is not often measured.

Case reports

37. As noted above, a number of cases of vitamin D intoxication have been reported in the literature. These have been summarised in Table 1 below. The case reports have not occurred through normal dietary exposure, but have often occurred through consumption of high levels of vitamin D, often over a sustained period of time. The high level of exposure has frequently arisen from errors in formulation or dosing.

38. Intoxication cases are generally associated with serum 25(OH)D levels of at least 300 nmol/L but more usually in excess of 600 nmol/L and frequently reaching levels >1000 nmol/L. Where lower serum 25(OH)D levels have been reported, this may be due to a delay in diagnosis or analysis.

Table 1- Case reports of vitamin D intoxication, where serum 25(OH)D was measured.

Population	Dose/ Exposure µg	Duration	Serum 25(OH)D nmol/L	Serum/plasma Ca mmol/L	Symptoms	Reference
Boy 2y	Liquid supplement. 15,000/day	4 days	1175 (peak)	3.6	Colic constipation, vomiting lethargy	Barrueto *et al*., 2005
2 infants aged 3 and 4 mo	750-1500/day	1-3 months		4.4-4.9		Beşbaş *et al*., 1989
1 child (anephric)			1588			Counts *et al*., 1975*
Boy 6 y	Calciferol tablets 1250	9 months			Thirst, bed wetting (diabetes insipidus)	Dent, 1964.
Boy 5 ½ y	2500 vitamin D + cod liver oil and multivitamins	2-3 months for all, vitamin D carried on for 1 y afterward.		4.25	Irritability, restlessness, nausea, lumps on tibia. Patterns of increased and decreased bone density in X rays. Loss of bone density and tissue calcification persisted after vitamin D stopped. Osteosclerosis, severe calcinosis, fatal renal failure.	De Wind., 1961
Female 66y	5 (2 x day). Symptoms started with new pack 200 µg/pill	3 years	696	4.04	"severe constitutional symptoms" anaemia	Puig *et al*., 1998
Male 32y	Lab technician working with vitamin D_3 dust	65 days in a 3 y period	1240 (at 1 mo)	3.5	Polydipsia, anorexia nausea, general malaise	Jibani and Hodges, 1985
Female 58y	Supplements containing 4674 µg per serving	2 months	1171	3.75	Fatigue, forgetfulness, constipation, back pain, nausea, vomiting.	Klontz and Acheson, 2007
Male 42y	Supplement powder – variable D_3 content. 3900-65100 µg/day consumed	2 years	1218	3.75	Hypercalcaemia	Koutkia *et al*., 2001
Female 70 y	15000/day	3 weeks	1474		Hypercalcaemia not apparent when	Lilienfeld Toal *et al*.,

Population	Dose/ Exposure µg	Duration	Serum 25(OH)D nmol/L	Serum/plasma Ca mmol/L	Symptoms	Reference
					25(OH)D declined to 711 nmol/L 9 wks after vitamin D withdrawn.	1978
Female 49 y	2500	6 years	706, 666		Weight loss, anorexia, pruritis, back pain and bone pain. Hypercalcaemia	Streck *et al*, 1979
Male 69y	2500	3 weeks		5	Abnormal ECG. Patient also had a tumour which may have contributed to the hypercalcaemia.	Sterling and Rupp, 1967
7 cases (3 adults, 4 children)	Unknown	Unknown (1 week for 2 adults)	832-1287	2.72-4.08	Vomiting, anorexia, constipation, polydipsia	Thomson and Johnson, 1986
Family, 2 adults + 11 mo infant	Food cooked in nut oil – Vit D_3 125000 µg /ml	Not stated, (IOM say single exposure)	600- 3750	4-4.3	Nausea, vomiting, abdominal pain, weakness and sensory loss. Suffused conjunctivae. Negative calcium balance suggesting Ca mobilisation from bone. Miscarriage at 10 weeks. 11 years later, all well but persistent nephrocalcinosis in adult male.	Down *et al.,* 1979
8 patients (7 aged 39-82y, 1 aged 15 mo)	Milk – 118-710 ml/day consumed. D_3 levels ND to 5814 µg /L)	Sporadic excess in milk.	Mean = 293± 435	3.15 ± 0.5	Weight loss, anorexia, fatigue, weakness, vomiting, constipation	Jacobus *et al*., 1992
2 children				“Elevated”	Anorexia, diarrhoea, vomiting (IOM) Hypercalcaemia	Jacqz *et al*., 1985*
3 mo	-		321			
7 mo	300		314			
4 infants						Ross, 1952
14 mo	250-750/day	12 mo		4.45	Fever, anorexia, weight loss, skeletal changes. Latter 2 cases fatal.	
8 mo	250-750/day	5 mo		4.68		
10 mo	600-1000/day	8 mo		-		
10 mo	600-1000/day	8 mo		-		
Males, 63 and 29y	Approximately 1.3 g/ month in table sugar	7 months	3700 and 1555	4.39 and 3.82	Right sides flank pain. Conjunctivitis, anorexia, fever, chills, weight loss, thirst, vomiting	Vieth *et al*., 2002

Population	Dose/ Exposure µg	Duration	Serum 25(OH)D nmol/L	Serum/plasma Ca mmol/L	Symptoms	Reference
Male, 62 **Female, 55**	Slow release i.m. preparation 3x 25,000 µg day for 20 days/month	3 months 1.5 months	375 >375	3.83 and 2.83	Renal failure, calcification of ileac artery and skeletal muscle	Chiricone *et al*., 2003
11 patients, 8-69 y	Vitamin D concentrate 50,000 µg /g used as cooking oil	Single exposure	847-1652	Mean = 3.99± 0.3	Abdominal cramps, vomiting, neurological symptoms	Pettifor *et al*., 1995
33 cases (93 controls)	Over-fortified milk-dose unknown	Unknown – could be over 5y in some cases	Mean= 896	3.28	Anorexia, weight loss, weakness, fatigue, disorientation, vomiting dehydration, polyuria, constipation. Renal impairment, 2 deaths due to hypercalcaemia.	Blank *et al*., 1995
234 survey respondents	Over-fortified milk-dose unknown	Unclear – assessed by daily intake	32.8, 39.5, 41.3, 44.7 in Qs in increasing intake	6	Symptom scores and individual symptoms not associated with intakes.	Scanlon *et al*., 1995
6 cases (14-57y) –variety of indications **UK**	2500/day 5000/day 2500/day 2500/day ? 5000/day	10y 2 y 10y 13 y ? 2y	866 802 1005 533 643 1203 Mean = 842	3.19 3.00 3.24 3.31 3.07 3.77 Mean = 3.22		Selby et al., 1995
7 cases (50-84y) Treated with vitamin D for osteoporosis, osteomalacia or hypoparathyroidism	250-750	Various- 3 wks to 7.2y	710 ± 179 mean (range 221-1692)	Mean = 3.3 ± 0.25	Asthenia, weight loss, nausea, polydipsia, polyuria, bradypsychism, sleepiness, pruritus, dizziness, episcleritis Urinary calcium 0.192 ± 0.067 mmol/L	Rizzoli *et al*., 1994
4 cases (all female) 42-	1250 µg /? 1250 µg/week	Long term 6 weeks	354 -	3.35 2.88	Lethargy, tenderness in some joints, pain, nausea, weakness,	Schwartzman and Franck, 1987

Population	Dose/ Exposure µg	Duration	Serum 25(OH)D nmol/L	Serum/plasma Ca mmol/L	Symptoms	Reference
77y Patients with osteoporosis or osteomalacia	1250 µg /day 1250 µg /2 x week	6 weeks 5 years	319 586	3.75 -	confusion, hypertension.	
8 cases (15-60y)	1250 – 5000 µg /day	4 months-10y		3.1-4.4	Back ache, sore eyes, nausea, vomiting, anorexia, pruritus,	Davies and Adams, 1978
Female 71y (only case with 25(OH)D	3750	2y	1123		polydipsia. Polyuria. No symptoms in 1 patient.	
10 cases (48-75y)	525,000 µg 270,000 µg ? ? 300,000 ? 1,350,000 75000 ? 1,500,000	1-4 months	302 172 200 165 164 283 100 236 176 306	3.38 3.0 3.48 3.1 3.2 3.1 3.5 3.4 3.2 3.55	Lassitude, vomiting, polyuria, polydipsia, altered sensorium, anorexia, oliguria. NB. The patients were from an area of India with endemic hypovitaminosis D. 25(OH)D levels seem low, normal range stated to be up to 144 nmol/L	Koul *et al*., 2012

*Abstract only

Hypercalcaemia in studies in human volunteers.

39. The case reports of vitamin D intoxication provide limited information for risk assessment purposes since exposure, where known, is of varying doses and durations.

40. Numerous supplementation studies have been conducted using vitamin D as a means of improving vitamin D status and/or improving bone health endpoints. These end points have frequently been investigated in population groups vulnerable to low vitamin D status and its consequences, such as older adults.

41. The most relevant studies have been tabulated in Annex B.

42. In view of the extensive database, the studies have been grouped into randomised controlled trials (RCTs) identified in the systematic review included in the IOM report, other studies cited by IOM and additional studies identified by EFSA. The studies vary widely in design, though there are few which use daily doses greater than 100 µg and where this has happened this is rarely for more than a few months.

43. The available studies have reported only isolated instances of hypercalcaemia. In some instances mean calcium levels have increased, but not above the normal range. These studies are considered further in paragraphs which consider dose response relationships and the establishment of a TUL.

44. Adverse effects arising from vitamin D exposure unrelated to hypercalcaemia have not been reliably documented. However, a potential increase in fracture risk arising from high annual doses of vitamin D and a non-monotonic relationship between vitamin D and all-cause mortality, and the risk of certain cancers have been reported. The mechanism(s) underpinning these relationships are unclear. These are considered elsewhere in the statement.

Animal data

45. The animal data generally support the findings from case reports and studies in humans and have not been considered in detail in this statement. In the majority of studies, hypercalcaemia and calcification of soft tissues was observed with clinical signs such as anorexia, weight loss, weakness, lethargy, polyuria and polydipsia are also being reported in animals treated with excess vitamin D. In reproductive studies, adverse effects such as fetal loss, reduced fetal growth and bone lesions occurred at doses where maternal toxicity was apparent or where significant disruption of calcium and phosphate homeostasis was observed in the mothers. The data suggest that rodents seem to be able to tolerate higher levels of vitamin D than other species, including humans.

Summary and conclusions –toxicity and hypercalcaemia

46. Vitamin D toxicity occurs following high and/or sustained vitamin D intake. Toxicity in humans does not occur as a result of UV exposure due to thermal

deactivation and has not been reported from exposure to vitamin D present in the normal diet but has often arisen through errors in dosing or fortification. Serum 25(OH)D levels associated with toxicity are at least 300 nmol/L but more usually in excess of 600 nmol/L and frequently reach levels >1000 nmol/L.

47. Vitamin D promotes the absorption of calcium from the gut as well as calcium resorption from bone, therefore sustained high levels of vitamin D exposure and thus hypercalcaemia can result in calcium deposition in soft tissues, diffuse demineralisation of bones and irreversible renal and cardiovascular toxicity. Hypercalcaemia is the appropriate endpoint to assess vitamin D toxicity since adverse effects not mediated by hypercalcaemia have not been reliably demonstrated. Hypercalcaemia with accompanying clinical symptoms are observed in vitamin D poisoning cases but have not been reported in supplementation studies, where only isolated instances of hypercalcaemia have been observed.

48. Free (ionised) calcium is the biologically active form of calcium, but total calcium is more usually measured. The correlation between total serum calcium and ionised serum calcium/calcium status is not perfect and whilst this might have clinical implications for the individual, it would still be sufficient to detect treatment related changes in a population or group of individuals.

49. At high serum calcium concentrations, the ability of the kidney to reabsorb calcium becomes limited and hypercalcuria can occur. However, urinary calcium concentration is unlikely to provide useful information, since this has high individual variability and is not often measured.

The U shaped curve

50. In the 2011 IOM review, it was noted that the dose-response relationship between serum 25(OH)D concentration and the risk of certain endpoints had a reverse J or U shape, suggesting that both low and high serum 25(OH)D levels were associated with increased health risk. The IOM considered the relationships between serum 25(OH)D and all-cause mortality and the risk of certain cancers, notably pancreatic and prostate cancer in detail. From this (and taking into account data on African-Americans[4]), IOM considered that serum 25(OH)D levels should not exceed 125 nmol/L. The dose-response relationships for all-cause mortality, pancreatic cancer and prostate cancer are considered below.

51. The IOM also considered data on a potential U shaped curve were for breast cancer, cardiovascular disease and falls and fractures. The latter endpoint will be addressed in paragraphs 122-124.

[4] The IOM noted that emerging data suggested that there was a positive association between serum 25(OH)D levels and calcified atherosclerotic plaque in the aorta and carotid arteries of African Americans and that the risk for all-cause mortality among non-Hispanic blacks compared to whites, occurred at lower levels of 25(OH)D. The IOM stated the data were limited and might eventually be explained by factors other than serum 25(OH)D but they were concerning and increased uncertainty.

All-cause mortality

52. Five cohort studies were identified by IOM (2011) which focussed on the association between serum 25(OH)D levels and all-cause mortality. In general these indicated that serum 25(OH)D levels <30 nmol/L were associated with an increased risk of mortality, the risk of mortality then decreased as serum 25(OH)D increased. However, three of these studies suggested a U or reverse J shaped dose response, with a flattening of the dose-response curve and a slight increase in all-cause mortality being observed at the highest serum 25(OH)D levels.

53. Jia *et al*., (2007) reported a statistically significant trend between increasing serum 25(OH)D levels and lowered odds ratios (ORs) for all-cause mortality (*p*= 0.03) in a population of community dwelling men (n= 208) and women (n=191) aged over 75 years living in Scotland. Blood samples were taken at baseline with median follow up being 69.2 months. Hazard Ratios (HRs) were estimated using Cox proportional hazard models for quintiles 1-4 referent to quintile 5. Although not discussed by the authors, the IOM considered that a U or reverse J shaped relationship was apparent with the lowest mortality at serum levels below 50 nmol/L but an apparent increase in risk in the highest quintile (47.1-82 nmol/L in men and 39.1- 82 nmol/L in women) compared to the fourth quintile (30.3-39 nmol/L in women and 37.0– 47 nmol/L in men). The HRs for the different quintiles are given in Table 2 below:

Table 2. Hazard Ratios of death referent to the highest quintile of serum 25(OH)D from Jia *et al*., (2007)

Quintile	**HR***	**HR‡**	**HR†**	**HR§**
25(OH)D nmol/L				
6-23 (men) **7-19 (women)**	2.22 (1.22-4.06)	2.02 (1.10-3.72)	1.97 (1.03-3.75)	1.74 (0.91-3.34)
23.1-30.0 (men) **19.1-24.0 (women)**	1.75 (0.95-3.22)	1.64 (0.89-3.02)	1.54 (0.80-2.97)	1.40 (0.73-2.70)
30.1-37.0 (men) **24.1-30.2 (women)**	1.03 (0.53-2.00)	1.01 (0.52-1.96)	0.95 (0.48-1.88)	0.90 (0.45-1.79)
37.1-47.0 (men) **30.3-39.0 (women)**	0.92 (0.46-1.84)	0.87 (0.43-1.75)	0.86 (0.43-1.74)	0.80 (0.39-1.62)
47.1- 82.0 (men) **39.1-82.0 (women)**	1	1	1	1
P for trend	0.001	0.008	0.01	0.03

*Model 3, adjusted for age, sex, taking ≥5 medicines, self-perceived health status and pre-existing heart disease and/or diabetes, ‡Model 4. Model 3 + sunlight exposure, †Model 5, Model 3 + use of supplements containing vitamin D, §Model 6. Model 3 + variables in models 4 and 5.

54. A similar pattern was reported by Visser *et al*., (2006) in a sample of 1260 independent community-dwelling persons aged ≥ 65 years who were participating in the Longitudinal Aging Study Amsterdam. The time between blood sampling and the end of the study was 5-6 years. Mortality was reduced at higher than deficiency levels of 25(OH)D (≥ 25 nmol/L) but was slightly increased at the highest quartile of

blood levels (≥75 nmol/L) compared to the third quartile (50-74.9 nmol/L). The HRs are given in Table 3 below:

Table 3.Hazard Ratios of death referent to the highest quartile of serum 25(OH)D from Visser *et al*., (2004)

Quartile	Model 1*	Model 2§	Model 3†	Model 4‡
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
25 OHD nmol/L				
<25	1.61 (1.09, 2.37)	1.54 (1.04, 2.29)	1.47 (0.99, 2.19)	1.28 (0.85, 1.92)
25-49.9	1.17 (0.85, 1.62)	1.11 (0.80, 1.54)	1.08 (0.78, 1.51)	1.00 (0.72,1.40)
50-74.9	0.93 (0.67, 1.29)	0.95 (0.68, 1.32)	0.95 (0.68, 1.32)	0.91 (0.65, 1.26)
≥ 75	1.0	1.0	1.0	1.0
P for trend	0.0058	0.021	0.046	0.19

*Model 1 is adjusted for gender, age, and education; **§**model 2 is adjusted for model 1 + chronic disease, serum creatinine concentration, cognitive status, and depressive symptoms; **†**model 3 is adjusted for model 2 + lifestyle variables including BMI, smoking status, alcohol consumption, and physical activity; **‡** model 4 is adjusted for model 3 + frailty indicators: mobility performance, low serum albumin concentration, and low serum total cholesterol concentration.

55. Similar findings were reported by Melamed *et al*., 2008, using NHANES III linked mortality data for 13,331 representative adults ≥ 20y. Serum 25(OH)D levels were collected between 1988-1994, and the participants were then passively followed for mortality via the NHANES III linked mortality files, until 2000 (median follow up 8.7 y). In multivariable analysis, the lowest 25(OH)D quartile compared to the highest quartile was associated with a 26% increased rate of all-cause mortality; see Table 4 below.

Table 4. Hazard Ratios of death referent to the highest quartile of serum 25(OH)D from Melamed *et al*., (2008)

	Unadjusted	Limited*	Fully‡	Fully (no diabetes or hypertension)
	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
25(OH)D nmol/L				
<44.5	1.78 (1.44-2.21)	1.52 (1.31, 1.77)	1.26 (1.08, 1.46)	1.28 (1.11, 1.48)
44.5-61	1.49 (1.24, 1.78)	1.11 (0.95, 1.31)	1.06 (0.89, 1.24)	1.06 (0.9,1.26)
61-80.1	1.14 (0.94-1.39)	0.92 (0.78, 1.08)	0.93 (0.79, 1.10)	0.94 (0.80, 1.12)
≥ 80.1	1.0	1.0	1.0	1.0

*Adjusted for age, sex, race, season,‡ Fully Adjusted Model includes age, sex, race, season, hypertension, history of prior CVD, diabetes, smoking, HDL cholesterol, total cholesterol, use of cholesterol medications, eGFR categories, serum albumin, log(albumin-creatinine ratio), log(CRP), BMI, physical activity level, vitamin D supplementation and low SES.

In a sub-group analysis, the association between serum 25(OH)D levels and all-cause mortality differed between genders being more pronounced in women; see Table 5 below:

Table 5. Hazard Ratios in men and women of death referent to the highest quartile of serum 25(OH)D from Melamed *et al*., (2008)

	Men	**Women**
	HR (95%CI)	HR (95%CI)
25 OHD nmol/L		
<44.5	1.04 (0.83-1.30)	1.51 (1.15, 1.98)
44.5-61	0.94 (0.75, 1.19)	1.27 (0.97, 1.66)
61-80.1	0.82 (0.64-1.05)	1.16 (0.87, 1.55)
≥ 80.1	1.0	1.0
P interaction 0.06		

56. However, in a study of 842 Australian nursing home residents (Sambrook *et al*., 2004), which investigated whether serum 25(OH)D was a significant predictor of time to death, it was found that higher serum 25(OH)D levels were protective (HR, 0.99; 95%CI 0.982-0.998). The data were analysed by univariate and multivariate models after correcting for age and gender. In analyses which corrected for health status, nutritional status and renal function, 25(OH)D was no longer associated with mortality. The 25(OH)D levels by survivor status were 27.9 nmol/L in the living subjects and 24.9 nmol/L in those that then died. (*p* =0.006).

57. Semba *et al*., 2009 reported that low serum 25(OH)D levels were associated with increased mortality in a study of 714 community dwelling women in the US aged 70-79; during the 7 year follow up period, 100 of the women died. Women in the lowest quartile of 25(OH)D concentrations (<38.25 nmol/L) were at higher risk compared to women in the highest quartile (>67.5 nmol/L) (HR 2.45, 95%CI 1.12-5.36, *P* = 0.02 in a multi-variate Cox proportional Hazards model adjusting for demographics, season, supplement use and conventional risk factors).

58. A number of additional studies of serum 25(OH)D levels and all-cause mortality are available in addition to those reviewed by IOM.

59. In a prospective study of NHANES III data (1988-1994), Ginde *et al*., (2009) investigated all-cause mortality in 3408 adults aged ≥ 65 years; the median follow up was 7.3 years. A total of 1493 deaths occurred during this period. Analysis of the fully adjusted data suggested there was an inverse relationship between all-cause mortality and serum 25(OH)D levels with no indication of an increase in mortality in the very highest levels. In the fully adjusted model (age, sex, race/ethnicity, poverty: income ratio, region, BMI, physical activity, smoking, asthma, COPD, renal function, hypertension, diabetes, hyperlipidaemia, MI, stroke and cancer) the HRs (95%CI were:

Table 6. Hazard Ratios of death referent to the highest quintile of serum 25(OH)D from Ginde *et al*., (2009)

Serum 25 OHD nmol/L	**HR (95% CI)**
< 25	1.83 (1.14-2.94)

25-49.9	1.47 (1.09-1.97)
50-74.9	1.21 (0.92-1.59)
75- 99.9	1.15 (0.86-1.53)
100	1.0 (ref)

60. A further study of data from NHANES was conducted by Ford *et al*., (2011) using data from the NHANES mortality study from 2002-2004, with mortality being compiled in 2006 (mean follow up 3.8 y). Of the 7531 participants aged ≥ 20 y there were 347 deaths. The mean unadjusted concentrations of vitamin D were 54.1 nmol/L among the participants that subsequently died, compared to 60.7 nmol/L in the survivors (p= 0.002). After adjustment for socio-demographic factors, the hazard ratios were 1.65 (95%CI 1.13-2.40) with a concentration of < 50 nmol/L 25(OH)D compared to those with a concentration ≥ 75 nmol/L. After more extensive adjustment, the HRs were 1.28 (95%CI 0.86-1.90) and 0.91 (0.63-1.33) respectively. The fully adjusted HR per 10 nmol/L 25(OH)D was 0.93 (0.86-1.01). The HRs did not vary by gender or among the three major racial or ethnic groups. As the regression models were adjusted for more variables, the association diminished.

Table 7. Hazard Ratios of death referent to the highest quartile of serum 25(OH)D from Ford *et al*., (2011)

		Model 1*	Model 2§	Model 3†	Model 4‡	Model 5¶
		HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)	HR (95%CI)
25(OH)D nmol/L	Deaths					
7-45	127/2362	1.74 (1.18-2.58)	1.24 (0.83-1.86)	1.57 (1.01-2.44)	1.39 (0.89, 2.17)	1.39 (0.90-2.14)
45-<60	93/1962	1.24 (0.83-1.86)	1.25 (0.84-1.85)	1.20 (0.80, 1.80)	1.13 (0.76-1.69)	1.12 (0.77-1.64)
60- <75	70/1708	0.93 (0.66-1.33)	0.92 (0.65-1.29)	0.90 (0.63-1.27)	0.85 (0.59-1.23)	0.83 (0.56-1.22)
≥ 75	57/1499	1.0	1.0	1.0	1.0	1.0

*Model 1, adjusted for age and 6 month examination period, § Model 2 as model 1 + gender, race/ethnicity, †Model 3 as model 2 + educational status, smoking status, alcohol intake, leisure time physical activity, vitamin and mineral supplement use,, ‡ Model 4 as model 3 + blood pressure, a range of biochemical parameters (including serum Ca) and waist circumference, ¶ Model 5 as model 4 +history of cardiovascular disease and/or diabetes

As with the earlier studies, this investigation was designed to establish whether low serum vitamin D was associated with an increased risk of all-cause mortality, but as with those considered by IOM (2011) there is a suggestion that the beneficial effect diminishes as serum 25(OH)D levels increase, with the HR being higher in the top quartile than the third quartile. The authors concluded that the findings gave only limited support to an inverse relationship between serum vitamin D levels and all-cause mortality; they did not consider the possibility of an increase in all-cause mortality at the highest levels.

61. Zittermann *et al*., (2012) conducted a meta-analysis to examine the relationship between vitamin D deficiency and mortality risk. The studies analysed included those by Visser *et al*. (2006); Jia *et al*. (2007), Melamed *et al*. (2008) and Semba *et al*. (2009) as above. For highest vs lowest categories of 25(OH)D, the summary relative risk (RR) for mortality was 0.71 (95%CI 0.50-0.91). In a parametric model, the estimated summary RRs (95% CI) of mortality were 0.86 (0.82-0.91), 0.77 (0.70-84) and 0.69 (0.60-0.78) for individuals with an increase of 12.5, 25 and 50 nmol/L serum 25 (OH)D respectively, from a median reference category of approximately 27 nmol/L. There was no significant decrease in mortality when an increase of 87.5 nmol/L above the reference category occurred. The model used indicates a U shaped response but with the RRs being below 1 but on an upward curve which, if it continued, would suggest an increased risk at levels above 87.5 nmol/L. The authors concluded that a non-linear decrease in mortality risk was apparent as circulating 25(OH)D increased, with an optimal serum concentration of 75-87.5 nmol/L.

Pancreatic cancer

62. A comparable U or reverse J shaped relationship was reported for some, but not all, observational studies investigating serum 25(OH)D intakes (and by inference 25(OH)D levels) and the risk of pancreatic cancer.

63. Skinner *et al*., (2006) reported data from 2 large prospective studies; 46,771 men aged 40-75 in the Health Professionals Follow-up Study (HPFS) and 75,427 women aged 38-65 in the Nurses' Health study. From these studies, 365 incident cases of pancreatic cancer were identified over 16 years of follow-up. The results suggested that higher vitamin D intakes were associated with a lower risk of pancreatic cancer. Compared with participants in the lowest category of vitamin D intake (<3.75 µg/day) pooled multivariate risks were:

Table 8. Relative Risks of pancreatic cancer referent to the highest quintile of serum 25(OH)D from Skinner *et al*., 2006

Vitamin D µg day	RR (95%CI**)**
<3.75	1.0
3.7.4-7.48	0.78 (0.59-1.01)
7.5- 11.23	0.57 (0.40-0.83)
12.25- 14.98	0.56 (0.36-0.87)
≥ 15	0.59 (0.40-0.88)
P trend = 0.01	

The model was adjusted for age, time period, total energy intake, smoking, diabetes, BMI, height, region of residence, parity and multi-vitamin supplement use

It was noted that 95% of men and 94% of women in the highest categories of vitamin D intake were supplement users; when these were excluded from the analysis, the inverse relationship between vitamin D intake and pancreatic cancer risk was still observed.

64. Similarly, in another study of 43,949 men from the HPFS cohort, Giovannucci *et al*, (2006) reported that a reduced risk of total cancer mortality and digestive cancer (which included pancreatic cancer) was associated with higher predicted serum levels of 25(OH)D[5].

65. In contrast, Stolzenberg-Solomon *et al*., (2006) found that in a nested case control in subjects from the Alpha-Tocopherol, Beta Carotene Cancer Prevention (ATBC) study cohort of Finnish male smokers aged 50-69 years at baseline, there was a positive association between higher serum 25(OH)D levels and the risk of pancreatic cancer. In this study, 200 incident cases were identified from the cohort and matched with 400 controls. Odds ratios were calculated using conditional logistic regression. Variables examined in analyses and as potential confounders were age, smoking history, education, residence in city, height, weight, BMI, blood pressure, a range of medical conditions including pancreatitis, dietary nutrient intakes from food, and supplements, alcohol intake, serum nutrients, occupational and leisure physical activity and season. Higher vitamin D concentrations were associated with a 3-fold increase in risk of pancreatic cancer (highest vs lowest quintile, >65.5 vs<32.0 nmol/L, OR 2.92; 95%CI 1.56-5.48, $P_{trend\ =}$ 0.001, after excluding cases diagnosed early during follow up).

66. A nested case-control study was conducted in the US Prostate, Lung, Colorectal and Ovarian Screening Trial (PLCO) cohort of 152,810 men and women aged 55-74 years by the same group (Stolzenberg-Solomon, 2009). In the follow up period (11.7 years) 184 incident cases of pancreatic cancers were identified and matched with 368 controls. Blood samples and dietary information (including supplement use) were obtained at baseline. Odds ratios were calculated using conditional logistic regression, adjusting for smoking and BMI. Vitamin D concentrations were not associated with pancreatic cancer overall (highest vs lowest quintile, >82.3 vs <45.9 nmol/L, OR 1.45; 95%CI 0.66-3.15, $P_{trend\ =}$ 0.49).

67. To resolve these contrasting results, a pooled nested case-control study of several cohorts was conducted within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (1974-2006) (Stolzenberg-Solomon *et al*., 2010). In total, 952 incident cases of pancreatic cancer were identified during a median follow up period of 6.5 years and matched to 1,333 controls. Conditional logistic regression analysis was used to calculate adjusted odds ratios and 95% confidence intervals for pancreatic cancer. No significant associations were observed for participants with lower 25(OH)D status and pancreatic cancer. However, a high 25(OH)D concentration (≥ 100 nmol/L) was associated with a statistically significant 2-fold increase in pancreatic risk overall (OR 2.12, 95%CI 1.23-3.64).

Table 9. Relative Risks of pancreatic cancer referent to the highest sextile of serum 25(OH)D from Stolzenberg-Solomon *et al*., 2010

25(OH)D nmol/L	**RR (95%CI)**
<25	1.0

[5] The level of 25(OH)D was predicted from vitamin D intake assessed by food frequency questionnaire and a pilot study measuring 25(OH)D levels in 1095 men from the study.

25-37.5	1.04 (0.74-1.44)
37.5-50	1.10 (0.79-1.55)
50-75	1.06 (0.76-1.48)
75-100	1.08 (0.73-1.59)
≥ 100	2.24 (1.22-4.12)
***P* trend 0.14**	

The study cohorts were the ATBC study, Campaign against Cancer and Stroke (CLUE), the Cancer Prevention Study II Nutrition Cohort, the New York University Women's Health Study, the Multi-ethnic Cohort Study, the PLCO study, and the Shanghai Women's and Men's Health Studies. Median serum 25(OH)D levels were comparable between cases and controls, but ranged from 33.4 to 64.7 nmol/L in the different cohorts. The increased risk in the highest category of serum 25(OH)D persisted even after the exclusion of cases diagnosed within the first 2 years after blood draw, leaving 558 cases and 840 controls (OR 2.20, 95%CI 1.22-3.96). The odds ratios were similar when the analyses were restricted to US cohorts only or when each cohort was excluded in turn. There was no significant interaction by use of vitamin D supplements or multivitamins. The authors noted that this analysis differed from the previous 2 analyses by the use of clinically relevant cut-points.

68. The findings of Stolzenberg-Solomon were disputed by Baggerly and Garland (2012) who argued that the U shaped curve was a statistical artefact associated with the chosen cut-off point groupings and that there was no U shaped curve to be explained; they stated that merging the top 2 groups largely abolished the relationship.

Prostate cancer

69. Tuoihimaa *et al*., (2004) conducted a longitudinal nested case-control study on Nordic men using serum banks of 200,000 samples. Serum 25(OH)D levels from 622 prostate cancer case were compared with those from 1451 matched controls from the same cohort. The cohorts included in the study were the Helsinki Heart Project, the Janus project (Norway) and the Northern Sweden Health and Disease. It was reported that both low (≤ 19 nmol/L) and high (≥ 80 nmol/L) serum 25(OH)D were associated with an increased risk of prostate cancer as assessed by conditional logistic regression analysis. The mid-range serum concentrations of 40-60 nmol/L were associated with the lowest risk. The ORs for the countries combined are given below but when analysed for each different country, the pattern was similar, with a U shape being less apparent in the Norwegian cohort, but more marked in the Finnish one. *P* values are not given.

Table 10. Relative Risks of prostate cancer referent to the third quintile of serum 25(OH)D from Tuoihimaa *et al*., 2004

	All Countries	
25(OH)D level nmol/L	**Number of cases**	**OR (95%CI)**
≤ 19	19	1.5 (0.8-2.7)
20-39	69	1.3 (0.98 -1.7)
40-59	229	1 referent
60-79	138	1.2 (0.9-1.5)
≥ 80	67	1.7 (1.1-2.4)

The authors considered that the U shaped pattern would not have been apparent without using data from all 3 cohorts and that studies of homogenous populations might show different results depending on where the 25(OH)D values were centred.

70. In contrast, Faupel-Badger *et al*., (2007) reported that in a nested case-control study of men from the ATBC trial, no association between serum 25(OH)D and the risk of prostate cancer was found. In this study 296 cases were identified from the cohort of 29,133 men and matched with 297 controls. Conditional logistic regression analysis was used to estimate ORs and 95%CI for baseline serum 25(OH)D levels as below:

Table 11. Relative Risks of prostate cancer referent to the highest quartile of serum 25(OH)D from Faupel-Badger *et al*., 2007

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}
Cases/controls	83/75	69/73	57/74	87/75	
25(OH)D nmol/L	≤ 36.75	37- 47.05	18.83-23.98	>23.98	
Unadjusted OR (95%CI	1.00	0.81 (0.45-1.47)	0.57 (0.30-1.06)	0.87 (0.49-1.57)	0.99
Adjusted OR* (95%CI)	1.00	0.88 (0.48-1.61)	0.59 (0.31-1.11)	0.89 (0.49-1.62)	0.97

* Adjusted for age at randomisation, BMI and pack years of smoking

It was noted that the vitamin D status of the population was skewed towards the low end of the usual range with > 25% being deficient. The contrast with the results of Tuohimaa *et al*., (2004) and other authors was noted, but the study was considered consistent with other large studies suggesting that the relationship was inconsistent and cast doubt on the hypothesis that low vitamin D status was associated with prostate cancer.

Reverse J shaped curve- conclusions

71. For a number of endpoints, notably all-cause mortality, pancreatic cancer and prostate cancer some studies have shown a U or reverse J shaped dose response relationship between serum 25(OH)D and risk with higher risk in the lowest category of serum vitamin D levels than in the highest category.

72. The COT noted that the non-monotonic exposure-response relationships which had been observed for some end-points, such as all-cause mortality, were best described as reverse J- shape rather than U-shaped. The elevation of risk at higher serum levels might reflect confounding (some types of illness leading to increased serum levels and also predisposing to earlier death), and was not necessarily causal.

Setting a TUL

73. Numerous supplementation studies have been conducted on vitamin D using a range of dose regimes. The majority have used moderate doses (20- 100 µg/day) and adverse effects have not always been recorded in systematic way; information on dietary vitamin D intake is rarely collected. The systematic review conducted by IOM identified a range or placebo controlled double blinded studies where adverse effects could be assessed/ identified. These are summarised in Table 1 at Annex B. Additional studies considered in the main IOM report are summarised in Table 2 of Annex B.

74. Additional studies considered by EFSA not included in Tables 1 or 2 are included in Table 3 at Annex B.

The key studies used for TUL purposes

75. The key study used by IOM to establish an UL was Heaney *et al*., (2003) whilst EFSA used those by Heaney *et al*., (2003) and Barger-Lux *et al*., 1998 to establish a TUL.

76. Heaney *et al*., (2003) designed a study to investigate the relationship between steady state vitamin D_3 input and serum 25(OH)D concentration and to determine the proportion of the daily requirement met by tissue reserves. Doses of 0, 25, 125 or 250 µg/day were given to 67 healthy males for 20 weeks over the winter in Omaha, US. From a mean baseline value of 70 nmol/L, 25(OH)D concentrations increased in proportion to dose. The data provided on changes in serum calcium levels are limited, but the data provided in Figure 4 of the original paper indicate that in the 31 individuals in the top two dose groups, total serum calcium decreased, increased or stayed the same but no individuals had levels above the normal reference range after treatment. The IOM noted that vitamin D intakes of 125 µg/day achieved serum 25(OH)D levels of 100-150 nmol/L (but which did not exceed 150 nmol/L) after 160 days of administration.

77. In an open-label study by Barger-Lux *et al*., (1998) designed to investigate the relationship between oral dosing and changes in circulating levels of vitamin D_3, groups of 10-14 healthy men were given doses of 25, 250 or 1250 µg/day for 8 weeks. Limited data are provided, but from a baseline of 2.41 ± 0.07 mmol/L serum calcium (mean ± SD) no statistically significant changes in serum calcium were detected. The doses of 25, 250 and 1250 µg/day vitamin D resulted in mean ± SEM increases in serum 25(OH)D of 28.6 ± 5.3, 146.1 ± 12.0, and 643.0 ± 42.7 above the baseline level of 67 ± 25.

78. As noted previously, both IOM and EFSA established a TUL of 100 µg/day vitamin D. IOM used the Heaney *et al*., (2003) study taking the dose of 125 µg/day but setting the UL at 100 µg/day, a level 20% below the identified NOAEL which accounted for the uncertainties surrounding the data and for the use of a single report. EFSA also considered both the Heaney *et al*., (2008) and Barger-Lux *et al*., 1998 studies, identifying a NOAEL of 250 µg/day, applying an uncertainty factor of 2.5 to account for uncertainties surrounding variations in sensitivity to possible adverse effects of vitamin D in the long term and that the TUL of 100 µg/day was

based on only two studies in small samples of young men with minimal sun exposure.

Summary and conclusions on the TUL

79. As noted previously, the occurrence of hypercalcaemia is an appropriate endpoint to use to establish a TUL. The COT considers that the TUL of 100 µg vitamin D for adults set by EFSA and IOM is appropriate. The TUL does not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D are low and are not generally assessed in supplementation studies including those which informed the TUL.

Exceptions to the TUL

80. A number of population groups may be more sensitive to excess vitamin D either due to their life stage or as a result of medical conditions which pre-dispose to hypercalcaemia. These are considered below.

Children- Idiopathic Infantile Hypercalcaemia

81. Idiopathic infantile hypercalcaemia, was first observed in the 1950s when a small number of infants presented with failure to thrive, vomiting, dehydration, spikes of fever and nephrocalcinosis (reviewed Schlingmann *et al*., 2011). Laboratory investigations revealed severe hypercalcaemia and suppressed PTH levels. A number of infants died during the acute phase of hypercalcaemia. The relationship between the epidemic occurrence and Idiopathic infantile hypercalcaemia and increased doses of vitamin D (up to 100 µg/day) in infant formula and fortified milk in the UK was attributed to nutritional vitamin D intake. However, since most infants were unaffected this was not the only factor. It was suggested that an intrinsic hypersensitivity to vitamin D might be involved although it was unclear whether this was due to excessive activation of vitamin D or defective inactivation.

82. In 1957, the British Joint sub-committee on Welfare Foods recommended that the levels of vitamin D used to fortify National cod liver oil concentrate and National dried milk powder were reduced and that the manufacturers of infant cereals should also reduce the average vitamin D content. The vitamin D content of evaporated milk products was also reduced. A study by Bransby *et al*., 1964 reported that in 1960, vitamin D intakes in normal infants ranged from 6.25- 30 µg/day which were significantly lower than those in the 1950s, where intakes of 100 µg/day had been estimated, and it was further noted that the incidence of hypercalcaemia was almost halved, with little or no increase in the incidence of rickets. Occasional case reports of infantile hypercalcaemia have been published subsequently but these relate to particular genetic polymorphisms and are considered below in paragraph 94.

Growth and hypercalcaemia

83. Early studies (Jeans and Stearns, 1938) suggested that excess vitamin D could reduce linear growth in infants, but later work (Fomon *et al*., 1966) suggested

this was not observed at doses of up to 54 µg/day vitamin D/day, as did a large prospective study by Hyppönen *et al*., 2008 in which the growth of 10,060 Finnish children supplemented with 50 µg/day vitamin D was followed. Growth was not affected in breast fed children whose mothers were given 25 or 50 µg/day vitamin D from birth (Ala-Houhala *et al*., 1986). Where calcium levels were measured in these studies, they were unaffected by the supplementation. A number of investigations were also conducted in babies and infants to examine whether supplementation could improve vitamin D status; these include Ala-Houhala *et al*. (1986) but also Vervel *et al*. (1997); Zeghoud *et al*. (1997) and Gordon *et al*. (2008). Various regimens of vitamin D supplementation were used, the highest dose being 1250 µg twice a week but treatment related hypercalcaemia was not observed.

84. Fewer data are available for older children but treatment-related hypercalcaemia was not observed in groups of 8/9 children aged 10-17 years who received 350 µg/week vitamin D_3 in oil or ethanol for 8 weeks (Maalouf *et al*., 2008). Similar findings were reported in a second study by the same group (El Hajj Fuleihan *et al*., 2006) in which healthy girls and boys (n = 340) received a weekly dose of 35 or 350 µg vitamin D_3 or placebo for one year.

85. The IOM recommended TULs of 25 and 38 µg/day for infants aged 0-6 and 6-12 months respectively. Assessing the same database as IOM, the EFSA panel (2012) retained the previous TUL of 25 µg/day established by the SCF in 2003 for children aged 0-12 months.

86. IOM (2011) noted that there were no specific data available for age groups other than adults and infants. The IOM committee chose to scale down the adult UL of 100 µg for younger children to 62.5 µg/day for children aged 1-3 years and 75 µg/day for children aged 4-8 to be more consistent with the principle of graded tolerance with age. For children and adolescents aged 9-18, the ULs were the same as those for adults. Using the data from Maalouf *et al*., (2008) and El-Hajj Fuleihan *et al*., (2006) but considering it unlikely that adolescents in the phase of rapid bone formation would have a lower tolerance for vitamin D than adults, a tolerable upper level TUL of 100 µg/day was proposed for children aged 10-17 by EFSA. For children aged 1-10 years, it was noted that no new data had become available, but as it was considered unlikely that children in the phase of rapid bone formation would have a lower tolerance for vitamin D than adults but taking into account their smaller body size a TUL of 50 µg/day was agreed.

Older adults

87. Renal insufficiency occurs in a significant number of older adults (30% in North America cited in IOM, 2011) and may make them more sensitive to excess calcium or vitamin D. Decreased renal function simultaneously increases cardiovascular disease risk and impairs calcuric responses and calcium and phosphate homeostasis. Similarly, individuals using thiazide-based diuretics (a sizeable proportion of older adults) are more readily challenged by excess calcium and vitamin D due to a reduction in calcium excretion from the kidney. However, the vast majority of vitamin D supplementation trials have been conducted on older

adults including frail older adults and those living in residential care, suggesting that any sensitivity would have been identified.

88. Although IOM considered age groups 51-70 and >70 years, they did not adjust the UL. The reason for this is not discussed in the text. Similarly, the EFSA panel did not make any adjustments to their TUL for older adults.

Pregnancy and lactation

89. Data on the effects of excess vitamin D in pregnancy are limited. However, a few studies are available which investigate the effects of vitamin D supplementation in pregnancy, as a means of elevating vitamin D status in both mother and infants. Serum calcium is not always measured in such studies but where this has been done, hypercalcaemia has not been observed. However, the doses of vitamin D used have been generally modest with only Hollis and Wagner (2004), Wagner *et al*, (2006) and Hollis *et al*., (2011) and using doses of ≥100 µg; no adverse effects were observed in these studies. The data have been tabulated in Table 12 below.

90. Similarly, there are few data on the effects of supplementing breast-feeding mothers with vitamin D. The studies are generally designed to investigate ways of improving vitamin D status and serum calcium is not always measured. Where this has been done, hypercalcaemia has not been observed. See Table 13 below:

91. Neither IOM nor EFSA adjusted the TUL to take account of pregnancy or lactation.

Table 12 Summary of data from vitamin D supplementation trials in pregnant women.

Participants	**Dose (µg) and Duration of vitamin D**	**Baseline Ca mmol/L**	**Ca after treatment mmol/L**	**Serum calcium measurement method**	**25 (OH) D before treatment nmol/L**	**25 (OH)D after treatment nmol/L**	**Other adverse effects assessed or reported[6]**	**Reference**
350 women (black, white & Hispanic) 12-16 wks gestation Charleston, US	10 50 100 D_3/day Until delivery	No difference between groups[7]	No effect of treatment	Total	61.6 ± 27.1 58.3 ± 22.3 58.2 ± 21.8 mean ± SD	78.9 ± 36.5 98.3 ± 34.2 111.1 ± 40.4 Significant ($p< 0.0001$ by ANOVA). Neonatal levels correlated with maternal levels	No hyper-calcaemia or hypercalcuria, no adverse events reported. High drop out rate	Hollis *et al*., 2011
30 women/group (non-pregnant controls) Turkey	10 D_3/day + 1000 mg Ca, or No treatment (pregnancy and post-partum)		2.07 ± 0.3 (post-partum) 2.11±0.09	Total (?)[8]	Controls = 26.9 ± 17.7	47.8 ± 18.4 (1st trimester) 17.4 ±11.3 (post partum)		Haliloglu *et al*., 2011
Groups of 21, 27, 29 women. France	25/day last 3 mo 5000 x1 at 7 mo Controls		2.15 ± 0.09 (2.44 ± 0.14) 2.15 ± 0.11 (2.41 ± 0.20) 2.10 ± 0.11 (2.37 ± 0.11) mean± SD maternal (cord) . No			25.3 ± 7.7 (15.7 ± 5.1) 26.0 ± 6.4 (18.2 ± 5.2) 9.4 ± 4.9 (5.3 ± 2.5)	No differences in birth weights or maternal calcium excretion	Mallet *et al*., 1986

[6] Urinary calcium included in this column as it has generally been used to assess excess calcium.
[7] Limited data provided
[8] The table states Total (?), when there is no indication whether the calcium measure was corrected.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	Serum calcium measurement method	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[6]	Reference
			differences between groups by Mann Whitney					
40 women at end of 1st trimester France	25/day for last trimester or controls	2.25[9] (1.05) 2.3 (1.1) Total (ionised)	2.28 (1.03) 2.23 (1.03) No differences in cord blood <u>Infants day 4</u> Vit D 2.28±0.5 (1.25 ± 0.1) Control 2.1±0.0 (0.98 ± 0.03) Mean ± SEM	Total and ionised	27.5 25	62.4 27.5		Delvin *et al*., 1986
Indian Asian Middle Eastern, Black and Caucasian women, 60/group	1 x 5 mg dose 20 µg/day-wk 27 to delivery or, Placebo	within normal range	within normal range	Corrected	25 (21-38) 26 (20-37) 26 (21-41) Median (IQR)	34 (30-46) 42 (30-46) 27 (27-39) <u>Cord</u> 17 (14-22) 26(17-45)	No differences in birth outcomes	Yu *et al*., 2009

[9] Estimated from figure

Participants	**Dose (µg) and Duration of vitamin D**	**Baseline Ca mmol/L**	**Ca after treatment mmol/L**	**Serum calcium measurement method**	**25 (OH) D before treatment nmol/L**	**25 (OH)D after treatment nmol/L**	**Other adverse effects assessed or reported[6]**	**Reference**
UK						25 (18-34)		
British Asian women	25 µg/day-last trimester or, Placebo	2.42 ± 0.01 mean± sem	2.58 ± 0.02 2.51± 0.01 Cord blood 2.71± 0.02 2.65 ± 0.02	Corrected for albumin	20.1 ± 1.9	168.5 ± 12.5 16.2 ± 2.7 Cord blood 137.9 ± 10.8 10.2 ± 2.0	Hypocalcaemia in controls	Brooke *et al*., 1980
200 women India	30 µg/day + 375 mg Ca 20-24 wks-birth or, Placebo		Serum calcium significantly increased in treatment group.					Marya et al., 1987 (abstract only)

Table 13. Summary of data from vitamin D supplementation trials in lactating women.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	Serum calcium measurement method	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L		Other adverse effects assessed or reported[10]	Reference
18 women <1 mo post-	40		Within normal	Total	68.9 ± 8.2 Infants	90.1 ± 5.7 Infants		No vitamin D related	Hollis and Wagner

[10] Urinary calcium included in this column as it has generally been used to assess excess calcium.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	Serum calcium measurement method	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L		Other adverse effects assessed or reported[10]	Reference
partum and their breast-fed infants US.	90 D_2/day for 3 mo. Plus a multi-vitamin Supplement with 10 µg D_3		range.		19.7 ± 2.8 82.1 ± 6 Infants 33.4 ± 8.2 Mean ± SEM	87.4 ± 9.7 111.1 ± 9.7 Infants 76.9 ± 12.5 Mean ± SEM		adverse effects observed. No hypercalcuria measured.	2004b
19 women <1 mo post-partum and their breast-fed infants US.	150 D_3/day or placebo for 6 mo. Infants of placebo mothers also got 7.5/day supplement Plus a multi-vitamin Supplement with 10 µg D_3	2.4 ± 0.1 Infants 2.6 ± 0.1 2.3 ± 0.3 Infants 2.5 ± 0.1 Mean ± SEM	2.4 ± 0.1 Infants 2.6 ± 0.1 2.4 ± 0.1 Infants 2.5 ± 0.1	Total ?	84.9 Infants 34.9 80.4 Infants 32.5	95.9 Infants 107.3 146.7 Infants 114.8		No vitamin D related adverse effects observed. No hypercalcuria measured.	Wagner *et al*., 2006
Groups of 16-17 women post partum	50 25 or	2.3 Infants 2.7 2.4	2.4 Infants 2.5 2.4		30 Infants 22.5 25	118 Infants 44.9 74.9			Ala-Houhala[11] *et al*., 1986

[11] Calcium and 25(OH)D estimated from figure.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	Serum calcium measurement method	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L		Other adverse effects assessed or reported[10]	Reference
	0 D3/day for 15 weeks Infants of placebo mothers also got 10/day supplement	Infants 2.6 2.3 Infants 2.6	Infants 2.5 2.3 Infants 2.5		Infants 12.5 30 Infants 12.5	Infants 34.9 30 Infants 87.4			

Renal failure

92. Individuals with renal failure have been advised to use vitamin D cautiously (Medline plus, 2014) as it could increase serum calcium levels increasing the risk of arteriosclerosis. Although a Cochrane review meta-analysis has been conducted of vitamin D compound supplementation studies in patients with chronic kidney disease not requiring dialysis (Palmer, 2009a) which indicated an increased risk of hypercalcaemia, the compounds used in the analysis were the active forms of the vitamin such as $1,25(OH)_2D$ and not vitamin D_2 or D_3. Similarly, in a review of studies in patients with chronic kidney disease requiring dialysis (Palmer 2009b) the risk of hypercalcaemia was increased but again, the compounds used in the analysis were the active forms of the vitamin such as $1,25(OH)_2D$ and not vitamin D_2 or D_3.

Genetic polymorphisms

93. A number of genetic polymorphisms exist at various points in the metabolic pathway of vitamin D metabolism and in calcium regulation overall. These include polymorphisms in the calcium sensing receptor, vitamin D binding protein and the vitamin D receptor. These affect factors such as calcium absorption and the "set point" of the calcium sensing receptor, but it is unclear whether these polymorphisms affect either vitamin D metabolism or the response to vitamin supplementation (polymorphisms related to serum calcium were reviewed by Kapur *et al*., 2010).

94. The best documented genetic polymorphism is a de-activating polymorphism in CYP24A1 which prevents the breakdown of $1,25(OH)_2D$, the active form of vitamin D. This polymorphism is associated with cases of infantile hypercalcaemia (IIH) with a variety of mutations in the gene having been reported. A number of cases have been reported, generally occurring in response to vitamin D supplementation (reviewed Schlingmann *et al*., 2011 also Fencl *et al*., 2013). Although the majority of IIH cases have been reported in infants, 3 cases in adults have also been reported (Tebben *et al*., 2012; Streeten *et al*., 2012). An additional case was diagnosed in infancy but the patient presented again as an adult (Meusberger *et al*., 2013). Although the numbers involved are small, the condition appears to be milder in adults. The available case reports are tabulated below:

Table 14 Case reports of IIH

Patient	Supplemental dose µg	Reference
6 patients aged 6-8 months 4 patients aged 5 wk-7 months	125/day from birth 15,000 1-3 doses	Schlingmann *et al*., 2011
Infant 4 months	16.5/day	Fencl *et al*., 2013
Infant 10 months	Unknown	Dauber *et al*., 2012
Male 44 years	Unknown	Streeten *et al*., 2012
Male 39 years	Unknown	Tebben *et al*., 2012
Infant 3 months (presented again as an adult aged 29 years)	20/day	Meusberger *et al*., 2013

Conditions which pre-dispose to hypercalcaemia.

Asymptomatic normocalcaemic primary hyperparathyroidism.

95. Primary hyperparathyroidism is a disorder of mineral metabolism characterised by incompletely regulated, excessive secretion of parathyroid hormone (PTH) from one or more of the parathyroid glands. It is the third most common endocrine disorder and is most common in post-menopausal women (Fraser *et al*, 2009). The prevalence of primary hyperparathyroidism is estimated to be 1/1000, 4.3/1000 and 3/1000 in the US, Sweden and Norway respectively but may be higher in certain other populations (eg 22/1000 in a Finnish population aged 55-75y).

96. Primary hyperparathyroidism is largely asymptomatic, often being diagnosed following an incidental diagnosis of hypercalcaemia. However, more recently described are patients with normal total and ionised calcium levels but consistently elevated PTH levels, a "forme fruste" (mild, attenuated form) of the condition. These normocalcaemic patients differ from those with mild hypercalcaemia who are occasionally normocalcaemic, as they are always normocalcaemic (Bilezikian and Silverberg, 2010).

97. In normocalcaemic hyperparathyroidism (Bilezikian and Silverberg, 2010) serum ionised calcium levels are normal. Since vitamin D deficiency may be associated with elevated PTH and normal serum calcium; correction of the vitamin D deficiency should normalise PTH levels in these patients. However, occasionally, when vitamin D deficiency is corrected, a normocalcaemic patient becomes hypercalcaemic and the presentation becomes that of traditional hypercalcaemic hyperparathyroidism. The asymptomatic condition is part of the spectrum of primary hyperparathyroidism (Eastell *et al*., 2009) but in order to make the diagnosis, other causes of secondary hyperparathyroidism should be excluded.

98. In a study by Maruani *et al* (2003) of 178 people with primary hyperparathyroidism, 34 individuals had normal calcium levels and 144 had hypercalcaemia. Patients with normocalcaemia had, on average, a lower excess of parathyroid hormone than those with hypercalcaemia. They also had lower fasting urine calcium excretion and renal tubular calcium reabsorption. In addition, normocalcaemic patients differed by having lower values of markers of bone turnover and plasma 1,25 $(OH)_2D$ and higher values of renal phosphate threshold[12].

99. It is unclear how many individuals in the general population could have asymptomatic hyperparathyroidism. However, it is possible that this could explain the occasional sporadic occurrence of hypercalcaemia which is reported in vitamin D intervention trials. Hypercalcaemia is considered in detail in paragraphs 31-32.

Granulomatous disease

100. Granulomas are small patches of red and swollen tissue. They are compact centrally organized collections of macrophages and epithelioid cells encircled by

[12] Renal phosphate threshold is the assessment of renal phosphate reabsorption under differing circumstances.

lymphocytes and generally form to confine pathogens, restrict inflammation and protect surrounding tissue. Macrophages subject to chronic cytokine stimulation, differentiate into epithelioid cells, gain secretory and bactericidal capability, lose some phagocytic capacity and fuse to form giant cells. In more mature granulomas, fibroblasts and collagen encase the ball-like clusters and in some cases sclerosis ensues, altering organ architecture and function (Iannuzzi *et al*., 2007).

101. Hypercalcaemia has been described in almost all granulomatous diseases such as sarcoidosis, berrylliosis (chronic beryllium disease- a granulomatous disease occurring as a result of occupational exposure to beryllium), tuberculosis (TB) and leprosy (Nayak-Rao, 2013).

Sarcoidosis

102. Sarcoidosis is a rare condition which causes granulomas to develop in the organs of the body, usually the skin or the lungs (NHS choices, 2013). It usually develops below the age of 40, and most commonly occurs in people aged 20-40. However, in Scandinavia the incidence in women appears to be bimodal with a peak at 25-29 years of age and a second peak at 65-69 years of age. Symptoms can include tender red bumps on the skin, persistent cough and shortness of breath. Systemic symptoms may include fatigue, weight loss and night sweats.

103. The incidence of sarcoidosis varies widely, the highest annual incidence occurs in Northern Europe (5-40 cases per 100,000 people), with a lower incidence in Japan (1-2 cases per 100,000 people). The condition is more common in black Americans compared to white Americans and it is more likely to be chronic and fatal in this group. Females are more likely to have sarcoidosis, regardless of ethnic group. The cause of sarcoidosis is uncertain (Iannuzzi *et al*., 2007) and may result from immune responses to environmental triggers (including mycobacterial antigens) as well as being influenced by genetic factors.

104. The granulomas may resolve with little consequence, but pulmonary fibrosis occurs in 20-25% of patients. The majority of patients will have a remission within a decade with few or no consequences, however, up to one third of patients, have unrelenting disease leading to clinically significant organ impairment. Sarcoidosis is fatal in fewer than 5% of patients, usually resulting from pulmonary fibrosis with respiratory failure or cardiac involvement (Iannuzzi *et al*., 2007).

105. Calcium metabolism is disturbed in sarcoidosis because sarcoidal macrophages have 25-hydroxyvitamin D-1-α hydroxylase which converts 25 hydroxyvitamin D (25(OH)D) to the more active vitamin D metabolite $1,25(OH)_2D$ (Iannuzzi *et al*., 2007) but this is not regulated by PTH or phosphorus as would be the case in the kidney. The enzyme is also less sensitive to product inhibition by $1,25(OH)_2D$ than renal 1-hydroxylase. Inflammatory mediators such as interleukin 2 may stimulate the further production of $1,25(OH)_2D$. Hypercalcuria occurs in 40% of patients, hypercalcaemia in 11% and renal calculi in 10%. Intra-renal calcium deposits may be so severe that renal failure ensues. The incidence of hypercalcaemia in sarcoidosis patients has also been stated to range from 2-63% (quoted Sharma, 1996).

106. Several case reports have been published which indicate that exposure to vitamin D from diet and/or exposure to sunlight has precipitated hypercalcaemia in sarcoidosis patients (Harrell and Fisher, 1939; Demetriou *et al*, 2010; Hassler *et al*., 2012; Amrein *et al*., 2011; Nayak-Rao, 2013).

Other granulomatous conditions

107. Other granulomatous conditions may be associated with hypercalcaemia, including granulomatous myositis (Zhang *et al*., 2012) and disseminated giant cell polymyositis (Kallas *et al*., 2010). As with sarcoidosis this is thought to be related to excessive 1 α-hydroxylase activity and may be unmasked by low dose vitamin D supplementation.

Tuberculosis

108. TB is considered to be a granulomatous disease as granulomas may form in response to the mycobacterial infection. Hypercalcaemia is thought to occur in some cases of TB although the precise incidence is unclear (Isaacs *et al.,* 1987). For example, in a study by Roussos *et al* (2001) mean (± SD) albumin adjusted serum calcium concentration and ionized serum calcium concentration were significantly higher in patients with TB (2.49 ± 0.21 mmol/L and 1.27 ± 0.02 mmol/L) compared to the controls (2.36 ± 0.11 mmol/L and 1.19 ± 0.02 mmol/L). Hypercalcaemia was detected in 22 patients with TB (25%) but only 3 showed symptoms. Similarly, corrected serum calcium was significantly higher in Chinese patients with untreated pulmonary TB compared to matched controls (2.33 ± 0.07 vs 2.20 ±0.09 mmol/L) despite lower calcium intakes (Chan *et al*., 1996). There were no significant differences in serum 25(OH)D or $1,25(OH)_2D$ levels between the groups.

109. The best known work on TB and hypercalcaemia was the study by Narang *et al*., (1984) which explored whether vitamin D could have a role in the treatment of tuberculosis. 150 subjects were divided into 5 groups of 30 individuals depending on the severity of their condition as well as groups of healthy controls and controls with chronic obstructive pulmonary disease. The groups were further divided to receive doses of 10, 20, 30, 60 and 95 µg of vitamin D/day for 3-6 months. Serum calcium was normal in all groups at baseline (but was non-significantly lower in the active tuberculosis group) and increased in all groups following treatment with all doses of vitamin D. The rise was significant in patients with active tuberculosis at all vitamin D doses, but was significant in the other groups only at doses of 60 µg/day or greater (and did not indicate hypercalcaemia ≥2.97 mmol/L). However, 19 of the 30 patients with active tuberculosis developed hypercalcaemia, though of these only 2 developed symptoms (nausea, vomiting and abdominal pain). The sputum conversion from initial positivity to negativity in the cases that developed hypercalcaemia paralleled a return to normocalcaemia. The mechanism for the production of hypercalcaemia was unclear but the authors concluded that anti-tubercular therapy should not be supported with vitamin D as the patients appeared to be particularly sensitive to it. This effect is considered consistent with the known effect of granulomatous disease on 1 α-hydroxylase activity (IOM, 2011).

110. Sharma *et al*. (1981) reported that 10/94 Indian TB patients had hypercalcaemia and that these 10 patients were receiving a higher level of vitamin D supplementation that the normocalcaemic patients. It was noted that there was a

correlation between daily vitamin D intake and the degree and duration of the hypercalcaemia. There was no indication of hypercalcaemia in matched controls with chronic obstructive pulmonary disease.

111. In contrast, in a small study by Fuss *et al*. (1988)[13] 11 patients with TB were given 50 µg 25(OH)D/day. Following treatment, corrected serum calcium and $1,25(OH)_2D$ levels were lower without any change in vitamin D binding protein levels. The authors concluded that hypercalcaemia was unusual in TB.

112. In a randomised double blind placebo controlled trial study by Wejse *et al*. (2009) patients in Guinea-Bissau with TB who were starting treatment were given 2.5 mg vitamin D or placebo at the start of the trial and at 5 and 8 months. Clinical severity score and 12 month mortality were unaffected by vitamin D treatment. Mild hypercalcaemia was reported in both treatment and placebo patients. At 8 months, the mean serum calcium levels were slightly higher in the treated group (2.17 and 2.19 mmol/L respectively) but no cases of hypercalcaemia occurred.

113. Martineau *et al.* (2011) conducted a randomised double blind placebo controlled trial to investigate the effect of high dose vitamin D during the intensive phase of anti-microbial treatment for pulmonary TB in the UK. The patients received 2.5 mg vitamin D at baseline, 14, 28 and 42 days after starting standard treatment. The treatment increased 25(OH)D levels but did not affect sputum conversion time. Mean serum calcium decreased during the study, but the rate of decline was not affected by allocation. Mild hypercalcaemia was reported in two individuals in the treatment group (2.68 and 2.72mmol/L corrected for protein) after 2 doses of 2.5 mg vitamin D and none in the controls. It was suggested that the decline could be due to a reduction in granulomatous burden in patients responding to treatment, leading to a decline in extra-renal 1-alpha hydroxylation of 25(OH)D to $1,25(OH)_2D$ and thus a fall in serum $1,25(OH)_2D$ concentrations.

Summary and conclusions- vulnerable groups

114. Idiopathic infantile hypercalcaemia was described in infants given excess vitamin D in infant milk and other milk products. The incidence of this condition has declined, although sporadic cases have been reported occurring as a result of a genetic polymorphism in an inactivating enzyme increasing sensitivity to vitamin D supplementation. Excess vitamin D has also been associated with growth retardation in some older studies. The TULs set to protect children in general may be adequate to protect against the IIH phenomenon which occurred through infant feeding in the 1950s but which might not be protective of the few individuals with a genetic predisposition.

115. There are limited other data on older children and adolescents but the results of RCTs and other available data as described above do not indicate that children are particularly sensitive to vitamin D.

116. The COT agreed that although the data were limited, there was no indication that pregnant or lactating women would be sensitive to vitamin D and that the TUL would need adjusting.

[13] Abstract only available.

117. Asymptomatic normocalcaemic primary hyperparathyroidism occurs when normocalcaemic patients have elevated PTH, prompting concerns that these individuals could be precipitated into hypercalcaemia by vitamin D supplementation. The prevalence of primary hyperparathyroidism ranges from 1/1000 to 22/1000 depending on the population but it is unknown whether there is also a significant undiagnosed normocalcaemic population who could be at risk. It is possible that such an occurrence could explain the sporadic findings of hypercalcaemia in individuals or the occasional elevation of serum calcium measured in some vitamin D intervention studies, though it should be noted that the majority of studies do not measure serum calcium.

118. Similarly, a number of granulomatous conditions may be associated with hypercalcaemia and/or hypercalcuria often due to extra-renal 1 hydroxylase activity which increases the production of $1,25(OH)_2D$. This has been reported to be exacerbated by exogenous vitamin D exposure from either UV exposure or supplementation and has been described in several case reports. The hypercalcaemia in the case reports often appears to have been precipitated by a large loading dose of vitamin D used to treat deficiency. There are few studies which allow a threshold dose for the promotion of hypercalcaemia to be identified, however, in the study by Narang *et al*. (1984) where graded doses were used, 95 µg/day vitamin D was considered to be the LOAEL for the occurrence of hypercalcaemia in patients with TB. The results of further work in this area have been inconsistent.

119. It is unclear how many individuals this may affect. The incidence of sarcoidosis has been estimated to be 5-40 cases per 100,000 people, but how many of these individuals could be at risk of hypercalcaemia is unknown. Using data from 2011, the incidence of TB in the UK has been estimated to be 13.9 cases per 100,000 people (Public Health England, 2013).

120. COT concluded that the TUL might not be protective of individuals with conditions that predispose to hypercalcaemia.

Single doses of vitamin D

121. SACN asked for the COT to consider the possible effects of high single doses of vitamin D which are sometimes used to improve vitamin D status in populations where compliance with a more regular schedule may be poor. The concern partly related to the findings of Sanders *et al*. (2010).

Sanders et al. (2010).

122. In this study, 2,256 community dwelling Australian women aged ≥ 70 years, considered to be at a high risk of fracture, were randomly assigned to receive a single dose of 12,500 µg vitamin D_3 or placebo for 3-5y. Women in the treatment group had 171 fractures compared to 135 in the controls; 837 women in the treatment group fell 2,892 times (rate 83.4 per 100 person years) compared to 769 women in the placebo group who fell 2,512 times (rate 72.7 per 100 person years). The incidence rate ratio (IRR) was 1.26; 95%CI 1.00-1.59 p= 0.047. The IRR;95%CI

for fracture was 1.15;1.02-1.30 *p*= 0.03. A temporal pattern was observed by a *post hoc* analysis. The IRR;95%CI of falling in the vitamin D group was 1.31;1.12-1.54 in the first 3 months and 1.13;0.99-1.29 in the following 9 months. A comparable pattern was observed for fractures but this was not statistically significant. There were no differences in the incidence of adverse events between the two groups.

123. In a sub-study, 137 participants (75 from the vitamin D group and 58 from the placebo group) underwent serial blood sampling for 25(OH)D and PTH levels. At baseline, the median 25(OH)D concentration was 49 nmol/L with fewer than 3% of participants having levels lower than 25 nmol/L. In the vitamin D group, 25(OH)D levels increased to 120 nmol/L 1 month after dosing, were approximately 90 nmol/L at 3 months and remained higher than the placebo group 12 months after dosing. Data on calcium levels were not provided.

124. The authors noted the limitations of the study; particularly that it had not been possible to assess biochemical parameters in all of the participants. However they considered that the participants would not have reached the “toxic” 25(OH)D level of 375 to 400 nmol/L because the highest level measured 1 month after dosing in the sub-study was 208 nmol/L. Since 25(OH)D levels were thought to peak 7-21 days post-treatment and decrease slowly thereafter, it was considered likely that the peak levels were only marginally higher than the 1 month values. It was also considered that the incremental increase was likely to be lower in individuals that were vitamin D replete before supplementation. The authors discussed the contrasting results from other studies where comparable annual doses of vitamin D were given, either in 4 monthly doses or single doses. It was suggested that the dose regime rather than the total dose was the factor determining the outcome, since the 3 month period following administration of the dose, was the time where the risk of fracture was most increased.

125. As noted previously a number of case reports of vitamin D toxicity have been published (see Table 1) these suggest that such cases are generally associated with serum 25(OH)D levels > than 400 nmol/L and more usually > 700 nmol/L. From this it appears that serum levels of ≤ 300 nmol/L25(OH)D would be unlikely to result in adverse effects

126. The studies in which large occasional doses of vitamin D have been used are tabulated below (Table 15). These show that where such doses are used, mean serum 25(OH)D levels increase substantially (up to about 100 nmol/L above baseline depending on dose, but usually less) but do not approach the levels measured in case reports. However, data on the distribution of the changes in levels are not always provided so it is not possible to clearly establish the highest individual levels.

Table 15. Changes in serum 25(OH)D levels following single doses of vitamin D

Dose (µg)	Population	25(OH)D Change from baseline (nmol/L)	Time of measurement (day)	Reference
15,000 D_3	48 young people with vitamin D deficiency (25-56y) Italy	+ 153 + 151 + 116	3 15 30	Cipriani *et al*., 2010
15,000 vitamin D_2 **or D_3**	24 patients with vitamin D deficiency Italy	+ 15.22 + 30.95	30 30	Cipriani *et al*., 2013
15,000 vitamin D_2	43 infants East Germany	+ 400 (median)	15	Markestad *et al*., 1987
12,500	2256 community dwelling women, ≥ 70y Australia	+ 70 + 40	1 mo 3 mo	Sanders *et al*., 2010
12,500 **12,500 + 1250/month**	19, 22 elderly people (≥ 65 y) hospitalised at time of recruitment, largely independent thereafter. New Zealand	+ 58 + 58 + 20 (estimated from Fig 2)	1 mo 1 mo 3 mo	Bacon *et al.,* 2009
7500	14/group elderly subjects with secondary hyperparathyroidism. Brazil	+ 50 + 37 +30	1 mo 2 mo 3 +mo	Premaor *et al*., 2008
7500 D_2	8/group Elderly women	79.87	3	Romagnoli *et al*., 2008

D_3	Italy	62.4 43.28 ± 11.93 25.26 ± 16.85 94.85 104.83 119.38 ± 18.22 70.03 ± 20.8 (mean ± SD) or estimated from figures	7 30 60 3 7 30 60	
7500	32 outpatients 49 Elderly in-patients (69-94y) New Zealand	+ 35 + 44 inpatients	Various but average of 17 weeks.	Wu *et al*., 2003
3750 given every 3 months or placebo	686 community dwelling women aged >70 y Subset of 40 analysed Australia	+ 37.44 higher than placebo overall	3, 6 and 9 months	Glendenning *et al*., 2010
2500	Elderly people in residential home	+ 62	2 weeks	Weisman *et al*., 1986. Abstract and proceedings[14].
2500	30 subjects (20 aged 61-84y and 10 aged 27-47y) US	+ 37 33 30 27 25 15	Measured at intervals, peak occurred at day 7, rest of values estimated from figure Day 14 21 30 35 60	Ilahi *et al*., 2008
1750	34 pregnant 27 non-pregnant women	32 28	Day 0, 2, 4, 7, and then weekly for up to 10 weeks. Different sampling schedules	Roth *et al*., 2012

[14] There appear to be 2 references for the same study, one being a conference proceedings.

	Bangladesh		to minimise burden on volunteers.	
1250 of D_2, D_3 or placebo **US**	10 Healthy male volunteers/group (20-61y)	+ 12 + 17 -	Day 0, 1, 3, 5-7, 14 and 28.	Armas *et al*., 2004

D_2- Ergocalciferol

D_3 Cholecalciferol

Summary and discussion- single doses

127. The increased risk of fracture and falls reported by Sanders *et al*., 2010 are of interest and cannot be discounted. However these need to be replicated before further conclusions can be drawn.

128. Although data from controlled studies where high, occasional doses have been used do not suggest that serum 25(OH)D would reach the level associated with vitamin D toxicity. The exception to this is the study by Markestad *et al*., (1986) where infants aged 1-20 months were given doses of 15,000 µg vitamin D_2 every 3 months. In this study, median 25(OH)D levels increased by up to 400 nmol/L 2 weeks after dosing with individual increases being as high as 1000 nmol/L above the level prior to each dose; however 25(OH)D concentrations did not accumulate. Hypercalcaemia was observed in 34% of the infants. The study was conducted to investigate the possible adverse effects of an established prophylaxis programme. Data on the distribution of individual changes in serum 25(OH)D are generally lacking in the other available studies.

129. A single dose of 7500 µg every 3 months or less would not be expected to result in serum 25(OH)D levels > 300 nmol/L but the risk of this occurring in some individuals would become higher as the dose level increased.

Summary

130. The Scientific Advisory Committee on Nutrition are revising the Dietary Reference Values for vitamin D and the COT have been asked to consider the potential adverse effects of high intakes both as regular intake and occasional doses.

Previous assessments

131. An extensive review of vitamin D was undertaken in 2011 by the US Institute of Medicine (IOM, 2011) who established an Upper Level (UL) of 100 µg/day vitamin D for adults. The IOM noted the paucity of long term studies investigating the effects of vitamin D intakes above 250 µg per day or maintenance of serum 25-hydroxyvitamin D (25(OH)D) above 250 nmol/L. The IOM also stated that the data suggested that it was unlikely that symptoms of toxicity would be observed at intakes below 250 µg/day, but that adverse effects would be observed at or above 1250 µg/day consumed for weeks or months.

132. In 2012, EFSA (2012) established a tolerable upper level (TUL, equivalent to the term UL used by the IOM) for adults of 100 µg vitamin D per day. A TUL applies to all groups of the general population (excluding those receiving the nutrient under medical supervision), including sensitive individuals, throughout life stages such as pregnancy - except in some cases discrete, identifiable sub-populations (e.g. those

with genetic predisposition or certain disease states) that may be especially vulnerable to one or more adverse effects).

133. In principle, separate ULs or TULs can be set for discrete sub-groups such as children. The IOM established ULs of 25, 38, 63, 75 and 100 µg/day vitamin D for infants aged 0-6 months and 6-12 months and children aged 1-3y, 4-9 y and 9-18y respectively. EFSA established TULs of 25, 50 and 100 µg/day vitamin D for infants aged up to 1 year, children aged 1-10 and 11-17 respectively.

Vitamin D and calcium metabolism

134. Vitamin D is a pro-hormone, occurring as ergocalciferol (vitamin D_2), which is formed in fungi in response to UV irradiation, and cholecalciferol (vitamin D_3), which is formed in the skin in response to UV.

135. Vitamin D undergoes hydroxylation to 25(OH)D which circulates in the plasma bound to albumin or DBP. Further hydroxylation in the kidney results in the formation of the active form of the vitamin, $1,25(OH)_2D$.

136. Vitamin D elevates the levels of plasma calcium and phosphorus in a complex feedback system. Vitamin D increases calcium levels by stimulating intestinal calcium absorption, mobilising calcium from bone via parathyroid hormone (PTH) and stimulating renal reabsorption. However, it is possible that it may have roles in addition to this classic action.

137. The best indicator of vitamin D status is serum 25-hydroxyvitamin D (25(OH)D). In interpreting the results of supplementation studies using vitamin D it should be noted that normal circulating levels of 25(OH)D are in the range 25-200 nmol/L (Jones., 2008).

D2 and D3

138. The difference in potency between vitamin D_2 and D_3 is uncertain. There is some suggestion that vitamin D_2 is less toxic than vitamin D_3 and also less effective at increasing serum levels, though this may only be apparent at high doses. The differences, if any, may be due to minor differences in metabolism.

Hypercalcaemia and vitamin D toxicity

139. Excess levels of vitamin D are associated with the occurrence of hypercalcaemia and hypercalciuria. Vitamin D promotes the absorption of calcium from the gut as well as calcium resorption from bone, therefore sustained high levels of vitamin D exposure and thus hypercalcaemia can result in calcium deposition in soft tissues, diffuse demineralisation of bones and irreversible renal and cardiovascular toxicity. These effects have been observed in a number of case reports of vitamin D intoxication; hypercalcaemia has also been reported in a few individuals taking part in vitamin D supplementation studies. Vitamin D intoxication has resulted from supplementation, fortification or medical treatment and has often arisen through errors in dosing. Cases of vitamin D intoxication arising from conventional dietary intake or UV exposure have not been documented.

140. Free (ionised) calcium is the biologically active form of calcium, but calcium is also present in serum bound to protein or complexed with anions. Since total calcium might not accurately reflect calcium status, it is sometimes adjusted for protein or albumin concentration to improve its reliability. The majority of studies where serum calcium is measured report total or total corrected calcium.

141. Total calcium in the blood and extracellular fluid is maintained at a concentration of 2.5 mmol/L (range 2.25-2.6 mmol/L) and ionised calcium at (1.1-1.4 mmol/L). Hypercalcaemia is generally considered to be when total calcium concentration is greater than 2.75 mmol/L. However, hypercalcaemia also occurs when ionised calcium alone becomes elevated.

142. The correlation between total serum calcium and ionised serum calcium/ calcium status is not perfect and whilst this might have clinical implications for the individual, it would still be sufficient to detect treatment related changes in a population or group of individuals.

143. The animal data generally support the findings from case reports and studies in humans. In the majority of studies, hypercalcaemia and calcification of soft tissues was observed. Clinical signs such as anorexia, weight loss, weakness, lethargy, polyuria and polydipsia are also reported in animals treated with excess vitamin D. Rodents seem to be able tolerate higher levels of vitamin D than other species, including humans. In reproductive studies, adverse effects such as foetal loss, reduced foetal growth and bone lesions occurred at doses where maternal toxicity was apparent or where significant disruption of calcium and phosphate homeostasis was observed in the mothers.

Other adverse effects

144. Adverse effects from vitamin D unrelated to hypercalcaemia have not been reliably documented

Dose response relationships

145. In the 2011 IOM review, it was noted that serum vitamin D concentrations had a reverse J or U shaped dose-response relationship with some endpoints, such as all-cause mortality and certain cancers notably pancreatic and prostate cancer, with a flattening of the dose-response curve and a slight increase in the end point being observed at the highest serum 25(OH)D levels. Given this and taking into account data on African-Americans[15], IOM considered that serum 25(OH)D levels should not exceed 125 nmol/L. However, the COT considered that the slight elevation in risk at higher 25(OH)D levels might reflect confounding, with some types of illness leading to increased serum levels and also pre-disposing to earlier death.

[15] The IOM noted that emerging data suggested that there was a positive association between serum 25(OH)D levels and calcified atherosclerotic plaque in the aorta and carotid arteries of African Americans and that the risk for all-cause mortality among non-Hispanic blacks compared to whites, occurred at lower levels of 25(OH)D. The IOM stated the data were limited and might eventually be explained by factors other than serum 25(OH)D but they were concerning and increased uncertainty.

Single doses of vitamin D

146. A number of case reports of vitamin D intoxication have been published, these suggest that such cases are generally associated with serum 25(OH)D levels > than 400 nmol/L and more usually > 700 nmol/L. Although intoxication cases may have resulted from multiple doses, the resulting serum 25(OH)D levels may be helpful in assessing single dose studies. From these data it appears that levels of 300 nmol/L would be unlikely to result in adverse effects.

147. A study by Sanders *et al*., (2010) in which a large annual dose of vitamin D was associated with an increased risk of falls and fracture cannot be discounted but the results need to be replicated before conclusions can be drawn.

148. The studies in which large occasional doses of vitamin D have been used show substantial increases in mean serum 25(OH)D levels (up to about 100 nmol/L above baseline depending on dose, but usually less) but these do not approach the levels generally measured in case reports of vitamin D toxicity. However, data on the distribution of the changes in levels are not always provided so it is not possible to clearly establish the highest individual changes.

149. Data from controlled studies where high, occasional doses of vitamin D have been used do not suggest that serum 25(OH)D would reach the levels associated with vitamin D toxicity. However, the data are generally reported as group means with little information on the distribution of individual serum 25(OH)D levels. In adults, a single dose of 7500 µg vitamin D every 3 months or less would not be expected to result in serum 25(OH)D levels > 300 nmol/L but the risk of this occurring in some individuals would be higher as the doses used increased.

Conclusions

150. The COT have drawn the following conclusions:

a) Excess vitamin D intake can result in hypercalcaemia, leading to bone demineralisation, soft tissue calcification and renal damage. This may occur as a result of acute and chronic exposure. This is an appropriate endpoint to assess the effects of high exposure to vitamin D, since adverse effects unrelated to elevated calcium have not been reliably documented.

b) The TULs of 100 µg/day vitamin D for adults and children aged 11-17, 50 µg/day for children aged 1-10 and 25 µg/day for infants, as recommended by EFSA, are appropriate. The TUL does not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D are low and are not generally assessed in supplementation studies including those which informed the TUL.

c) The TUL may not apply to individuals with some health conditions such as normocalcaemic hyperparathyroidism and granulomatous conditions including sarcoidosis and TB which predispose to hypercalcaemia.

d) The US IOM identified a U or reverse J shaped dose-response relationship between serum 25(OH)D and conditions such as all-cause mortality, pancreatic and prostate cancers suggesting that the risk was elevated at high as well as low serum levels of vitamin D. However this relationship may be due to confounding.

e) Case reports of vitamin D toxicity are associated with serum 25(OH)D levels > 300nmol/L and more usually 600-1000 nmol/L. In adults, a single dose of 7500 µg vitamin D every 3 months or more would not be expected to result in serum 25(OH)D levels > 300 nmol/L but the risk of this occurring in some individuals would be higher as the doses used increased.

Secretariat
August 2014

GLOSSARY

ATBC- Alpha Tocepherol Beta Carotene
BMI – Body Mass Index
Ca- Calcium
CKD – Chronic kidney disease
CI- Confidence Intervals
CLUE – Campaign against Cancer and Stroke
COMA – Committee on the Medical Aspects of food policy
COPD- Chronic Obstructive Pulmonary Disease
CRP-C Reactive Protein
CYP- Cytochrome P
DBP- Vitamin D Binding Protein
DRV- Dietary Reference Value
$1,25(OH)_2D$ -1,25-dihydroxyvitamin D_2
EC- European Commission
EFSA- European Food Safety Authority
EU-European Union
EVM- Expert Group on Vitamins and Minerals
GFR- Glomerular Filtration Rate
GI – Gastrointestinal
HR-Hazard Ratio
HPFS-Health Professionals Follow up Study
25(OH)D - 25-hydroxyvitamin D
IIH-Idiopathic Infantile Hypercalcaemia
IOM – Institute of Medicine
IRR-Incidence Rate Ratio
IU-International Units
Kcal-kilocalories
μg/day-micrograms/day
mg/day- milligrams/day
MI- Myocardial Infarction
mmol/L-millimoles/Litre

NDA- EFSA Panel on Dietetic Products, Nutrition and Allergies
NDNS- national Diet and Nutrition Survey
NHANES-National Health and Nutrition Examination Survey
NHS- National Health Service
ng/ml- Nanograms/millilitre
nmol/L- nanomoles/Litre
OR – Odds Ratio
PLCO- Prostate, Lung, Colorectal and Ovarian Screening Trial
PTH- Parathyroid Hormone
RR-Relative Risk
RCTs – Randomised Controlled Trials
SACN- Scientific Advisory Committee on Nutrition
SCF- Scientific Committee on Food
SD- standard Deviation
SEM- Standard error of the mean
TB-Tuberculosis
TUL- Tolerable Upper Level
UK-United Kingdom
UL - Upper Level
US-United States
UV-Ultraviolet
VDR-Vitamin D Receptor
VDRE- Vitamin D Responsive Elements

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

SACN Review of vitamin D. Adverse effects of high levels.

1st draft statement.

Annex A

Search strategy

Members agreed to use the 2011, IOM report as a bibliographic source. Relevant references were taken from this document and from the EFSA panel report published in 2012.

An update search was carried out by the secretariat as below:

Scientific/medical name(s): 1,25 dihydroxycholecalciferol, 25 hydroxycholecalciferol, 1,25 dihydroxyvitamin D

Search 1:

"vitamin D" OR "vitamin D2" OR "vitamin D3" OR ergocalciferol* OR cholecalciferol*

AND

intake*

AND

"adverse health outcome*" OR "adverse health effect*" OR toxic*

Search 2:

serum OR "blood level*"

AND

"vitamin D" OR "25 hydroxyvitamin D" OR "25-hydroxy vit D" OR "1,25 dihydroxyvitamin D" OR "25-hydroxy vit D" OR "plasma vit D" OR 25OHD OR 25-OHD OR 25OHD3 OR "25(OH)D3" OR 25-OHD3 or "25-(OH)D3" OR "25(OH)D" OR "25-(OH)D" OR "25-OH-D" OR 25-hydroxycholecalciferol OR 25-hydroxyergocalciferol OR calcidiol OR calcifediol

AND

“adverse health effect*” OR “adverse health outcome*” OR toxic*

from Google Scholar; FoodlineWeb; PubMed; IngentaConnect; Thomson Reuters Web of Knowledge: ISI Web of Science

Secretariat

August 2014

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

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Annex B

Tables summarising the supplementation studies considered by IOM and EFSA are summarised in the attached Tables.

Table 1 contains the RCTs identified in the systematic review conducted for IOM.

Table 2 contains additional studies considered by IOM

Table 3 contains studies considered by the EFSA panel.

Secretariat

July 2014

Table 1. RCTs able to assess adverse effects as identified in the Ottawa review for IOM

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
56 Infants (20,20 & 16/group) with vit D deficient rickets + 20 controls, mean age =10 mo Turkey	3750 75000 15000 Single dose	1.96 ± 0.34 (patients with rickets) 2.22 ± 0.12 (controls) Mean ± SD	↑ 0.41 ± 0.29 ↑ 0.55 ± 0.44 ↑ 0.61 ± 0.49	0.016 ± 0.0063 0.015 ± 0.0047 0.015 ± 0.004		Hypercalcaemia in 2/20 at 75,000 and 6/20 at 15,000 µg/day 30d after treatment (significant p<0.05). [17]Ca/Cr ↑after treatment, persisting at top dose.	Cesur *et al*., (2003)
Healthy neonates 15, 15 and 30/group Algeria	2500 at 0, 3 and 6 mo, 5000 x1 at birth, 15,000[18] x1 at 15 d	2.35 ± 0.25 2.33 ± 0.28 2.2 ± 0.23 Mean ± SD	2.38 ± 0.08 2.38 ± 0.1 2.52 ± 0.13- at 0.5 mo, significantly increased (p <0.005) from baseline and lower doses By Mann Whitney	All < 25 nmol/L	92 ± 42 150 ± 55 307 ± 160	No hyper-calcaemia but transient elevation in Ca in top dose.	Zeghoud (1994)

[16] Urinary calcium included in this column as it has generally been used to assess excess calcium.
[17] Student's t test, Wilcoxon signed ranks test and Mann-Whitney U tests are the specified methods, but unclear what was used when.
[18] 15 mg data from an earlier study but included in paper

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
			U test				
Female children and adolescents (55-58/group) Lebanon	Placebo 35 Vitamin D_3 350 Vitamin D_3/week for 1 year	2.5 ± 1 2.48 ± 0.8 2.48 ± 1 Mean ± SD		34.9 ± 17.5 34.9 ± 22.5 34.9 ± 19.9 Mean ± SD	40 ± 19.9 43 ± 14.9 95 ± 77 3/55 in top dose had >250	No hyper-calcaemia in treatment group.	Fuleihan *et al*., 2006
Healthy adults (28-33/group; no placebo) Canada	25 100/day vitamin D_3 0 (no supp) for 2-5 months		All within normal range (2.2-2.6) and no significant change from baseline by repeated measures ANOVA.	40.7 ± 15.4 Mean (SD)	68.7 ± 16.9 96.4 ± 14.6 Plateau after 3 mo 46.7 ± 17.8 Controls (summer)	More incidences of elevated urinary calcium: creatinine in top dose but not significant by repeated measures ANOVA.	Vieth *et al*., 2001
Adult endocrine outpatients, 53-55y (64-66/group to start) Some patients continued on to a 2nd study plus additional new patients Canada	Study 1 15 100 Study 2 15 100 for 2 mo Overlapping design meant that the duration of	1.23[19]	1.23 1.235 122, 1.235 After 2 and 6 mo treatment. No significant differences	48 ± 9 39 ± 9	79 ± 30 112 ± 41	No hyper-calcaemia	Vieth *et al*, 2004

[19] Data estimated from figure.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
	dose was 2-15 mo		between groups or over time. (? t test)				
208 post-menopausal African American women (104/group) Long Island, US	20/day vitamin D_3 for 2y then 50 µg/day for 3rd year or, placebo + Ca supplements to ensure Ca intake of 1200-1500 mg/day	2.2	2.31 (both groups) at 2 years. 2.34 (controls) and 2.38 (treated) at 3 years. No difference by mixed model ANOVA. Mean serum Ca 0.062 mmol/L higher in top serum vitamin D quartile	46.9 (95%CI 43.9-50.9)	70.8 (66.4-76.1) within 3 mo of treatment. Further increased to 86. (80.1-94.1) within 3 mo of dose increase. No sig change in controls.	6 mild hyper-calcaemia in treatment group, 2 in controls. 3 elevated urinary Ca (but no difference in 24h urine Ca per kg bw) (placebo-92.0, 107.4, 100.6 and treatment 86.3, 118.8 and 113 mg/day) at 0,2 and 3y	Talwar *et al*., 2007 Aloia *et al*., 2005
Older women in residential care, mean age 85 (60-62/group) Switzerland	20 D_3/day + 1200 mg Ca placebo + 1200 mg Ca for 12 weeks	2.34 (2.28-2.39) 2.32 (2.23-2.39 Median (IQR))	2.34 (2.25-2.42) 2.28 (2.22-2.34) No difference by Wilcoxon rank test	30.8 29	65.5 28.5	No hypercalcaemia	Bischoff (2003)
48 women with osteoporosis or osteopenia (mean age 70 y)	20 D_3/day + 1000 mg Ca + 10 mg alendronate or				65.0 35.0	No hypercalcaemia No hypercalcuria but urinary Ca/cr from baseline in treatment	*Brazier *et al*, 2002

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
	placebo + 1000 mg Ca + 10 mg for 3 mo					group by day 30	
192 women with vitamin D insufficiency (mean age 65 y) France	20 D_3/day + 1000 mg Ca or placebo For 1 year	2.19 2.17 Median	2.29 (2.19-2.39) 2.27 (2.19-2.42) Median (Q1-3). Not significant by *t* test	18.25 17.5	71.8 26.8	Hypercalcaemia 7/95 11/96 (controls) 24h urinary Ca/cr sig increased in treatment group (<0.001)	Brazier *et al*., 2005
3270 Healthy women in senior facilities (1,634-1,636 /group) Age 84 ± 6y France	20 µg D_3/day + 1200 mg Ca or, placebo for 1.5 y	2.29 ± 0.09 2.29 ± 0.1 Mean ± SD	2.30 ± 0.08 2.25 ± 0.09 (sig lower than baseline by ANOVA)	40 ± 27.5 3 2.0 ± 22.47	105 ± 22.5 27. 5 ± 17.5	No hypercalcaemia or renal calculi. More GI effects in treatment group	Chapuy *et al*., 1992
Healthy women in senior facilities with low Ca and Vit D intakes (190-194/group) France	20 D_3 + 1200 mg Ca (fixed combination) or 20 µg D_3 + 1200 mg Ca (separate) or, placebo/day for 2 y	2.31 ± 0.11 2.29 ± 0.12 2.3 ± 0.11 Mean ± SD	2.31 ± 0.12 2.32 ± 0.13 2.27 ± 0.13 Significantly higher in treated	21.22 ± 13.23 22.46± 16.47 22.71 ± 17.22	75[20] 80 15 Significantly higher in	No hyper-calcaemia (3 but unrelated to treatment) Non sig increase in hyper-calcuria More GI effects in treatment group but also not sig.	Chapuy *et al*., 2002

[20]Data taken from figure.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
			groups, p< 0.05 by ANCOVA		treatment groups than placebo.		
Elderly patients in hospital (mean age 82y) (41/group) UK	22.5/day or placebo up to 40 wks	2.32 ± 0.02 2.34 ± 0.02 Mean ± SEM		17.63 ±2.05 16.60 ±2.10	110[21] 17	1/41 hyper-calcaemia (attributed to hyper-parathyroidism)	Corless *et al.,* 1985
Healthy older men and women (mean age 70-72y) (187-202/group completed) US	36 µg D_3 + 500 mg calcium/day or Placebo For 3 years	1.25 ± 0.05, 1.25 ± 0.05 (men, women) 1.25 ± 0.05, 1.28 ± 0.03 (men, women) Mean ± SD	↑0.03±0.05, ↑0.05±0.05, (men, women) **sig** (p< 0.005 for men only) by t test ↑0 ± 0.03, ↑0 ±0.05, (men, women)	82.37 ± 40.7 71.64 ± 33.2 (men, women) 83.87 ±33.6 61.15 ± 25.7 (men, women	↑29.45 ± 29.0 ↑40.2 ± 35.7 (men, women) ↓6.7 ± 25.5 ↑1.7 ±20.2 (men, women)	No hyper-calcaemia (small ↑ in serum Ca). 1hyper-calcuria, 24 h urinary Ca/cr increased	Dawson-Hughes *et al*., 1997
Adults 1306-1343/group Scotland	20 D_3/day or 20 D_3/day + 1000 mg Ca or, 1000 mg Ca or, Placebo for 5 years			37.9 ± 16.2 (sample of 60)	↑24.2 ± 21.7 ↑24.5 ±17.2 ↑3.5 ± 14.2 ↑7.8 ± 18	21 cases hypercalcaemia but no differences between groups. No differences in renal stones, GI effects, adverse events, renal insufficiency, mortality	Grant *et al.,* (2005) RECORD trial

[21] Data taken from figure

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
Adults 36-39/group in hospital. Mean age 81.2y UK	7500 D_2 /month (i.m.) or 7500 µg D_2/month (i.m.) + 1000 mg Ca/d, or 20 D_2 + 1000 mg/d Ca (tablet) or placebo for 1 year	2.38 (2.0-2.6) 2.37 (2.0-2.6) 2.35 (2.0-2.6) 2.39 (2.0-2.6)	2.46 2.45 2.42 2.40	28 (10-67) 30 (12-85) 29 (6-75) 30 (12-128) Mean (range)	40 44 50 27	No hyper-calcaemia, no change in serum Ca by χ^2	Harwood *et al*., 2004.
Adults (18,106 or 18,176 /group)	10 + 1000 mg Ca or placebo for 7 years				46 48.4	Increase in renal stones. Slight increase in GI effects.	Jackson *et al*., 2006
Elderly women. Free living (25 intervention, 27 controls) Institutionalised (30 intervention, 33 controls) Finland	45/day vitamin D + 1558 mg Ca for 11 weeks (winter) or, no treatment 45/day vitamin D + 1558 mg Ca for 11 weeks (winter) or, no treatment	2.44 (2.3-2.6) and 2.49 (2.4-2.6) 2.59 (2.4-2.8) and 2.56 (2.4-2.8) (intervention and controls respectively) No significant	2.40 (2.3-2.5) and 2.41 (2.3-2.6) 2.58 (2.4-2.8) and 2.73 (2.5-2.9) (intervention and controls respectively)	Mean 38.5 Mean 24.1	23.3 controls 80.7 (treatment) 10.4 controls 64.4 (treatment) Both changes significant (P<0.001)by 2	Mild GI effects in 9/25 free living. No change in creatinine levels	Honkanen *et al*., 1990

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
		changes by 2 way ANOVA Mean (95%CI)	Mean(95%CI)		way ANOVA		
Adults, mean age ≥ 65y (32-33/group) Connecticut, US	50 vitamin D + 500 mg Ca/d or placebo (500 mg Ca) for 11 weeks			65 ± 16.7 58.9 ± 18.7	87.1 ± 13.7 56.4 ± 17	No hypercalcaemia or hyper-alcuria	Kenny *et al*., 2003
Elderly institutionalised women 124/group Switzerland	11 D_3 + 1000 mg calcium or placebo for 2 years	Mean ± SD	2.27 ± 0.09 2.27 ± 0.09,	29.7 ± 3 29.2 ± 3	↑123% ↓ 51 % 74.5 and 20.8 at 1 y and 66.3 and 14.3 at 2 y	1 hypercalcaemia 6 withdrawals in treatment group due to GI effects	*Krieg *et al*., 1999.
Institutionalised elderly men and women (81y senior home) 84 y nursing home) (70-72/group) Netherlands	10 D_3/day or 20 D_3/day for 1 year				Increased to >40 (16 ng/ml)	1 hypercalcaemia unrelated to treatment non sig increase in Ca/cr and sig increase in serum creatinine.	Lips *et al*., (1988)
Post-	125 /day + 500	2.33 (2.33-	2.4 (2.3-2.48).	Median	Median	No hypercalcaemia	Mastaglia *et al.,*

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[16]	Reference
menopausal women aged 50-70y (12-13/group) Argentina	mg Ca, or 250 µg D_2/day + 500 mg Ca or 500 mg Ca for 3 months	2.4) Both treatment groups	Both treatment groups. No individual value outside normal. Sig increase in top group alone ($p<0.05$ by Mann-Whitney test)	42.0 (23.7-45) 32.5 (27.5-45.0)	77.5 (66.2-56.2) 97.7 (79.3-123.1)	No difference in hyper-calcuria between groups	2006

Table 2. Studies considered in main IOM (2011) report where no hypercalcaemia was documented.

Some studies reported instances of but these were considered to be unrelated to treatment

NB. Studies marked with * are where only the abstract is available

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25(OH)D before treatment nmol/L	25(OH)D after treatment nmol/L	Other adverse effects assessed or reported[22]	Reference
60 subjects. UK and St Louis, US	45 250, 500 1000 for 4wks. 35 40 150 220 500 1000 2000 3000 for ≥ 4 months	Unclear whether Ca was measured at any point.		7.5-60[23]	50 92.5 162.5 300 - 70 120 200 350 500 700 900		Stamp *et al*., 1977
449 Elderly subjects (in a review of 11 smaller studies)	10-20/day High dose 2500 /year					Hypercalcaemia in 3 (2/3 with pre-disposing cause)	Byrne *et al*., 1995

[22] Urinary calcium included in this column as it has generally been used to assess excess calcium.
[23] 25(OH)D values estimated from figures.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25(OH)D before treatment nmol/L	25(OH)D after treatment nmol/L	Other adverse effects assessed or reported[22]	Reference
Elderly women. Free living 25 intervention, 27 controls) Institutionalised 30 intervention, 33 controls Finland	45 vitamin D + 1558 mg Ca or no treatment for 11 weeks (winter)	Mean(95%CI) 2.44 (2.3-2.6) 2.49 (2.4-2.6) 2.59 (2.4-2.8) 2.56 (2.4-2.8)	Mean(95%CI) 2.40 (2.3-2.5) 2.41 (2.3-2.6) 2.58 (2.4-2.8) 2.73 (2.5-2.9) No significant changes by 2 way ANOVA	Mean 38.5 Mean 24.1	23.3 controls 80.7 (treatment) 10.4 controls 64.4 (treatment) Both changes significant (P<0.001) way ANOVA	Mild GI effects in 9/25 free living. No change in creatinine levels	Honkanen *et al*., 1990
109 subjects (> 60 or 65y) UK	40 µg /ml oil or oil placebo. Doses varied, up to 3000 µg vitamin D_2 or D_3 or up to 250 µg 25(OH)D Varying durations, some over 4 months	2.430 (2.110-2.650) 2.411 (2.241-2.481) Mean ± 2 SD	2.431 (2.111-2.651) 2.416 (2.246-2.486) No change in mean serum calcium, but increased (Mann-Whitney U test, p= 0.01) in treatment group when corrected			Hyper-calcaemia in 2/63	Johnson *et al*., 1980
Patients on anti-	250 µg/day				110		*Davie et al.,

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25(OH)D before treatment nmol/L	25(OH)D after treatment nmol/L	Other adverse effects assessed or reported[22]	Reference
convulsants England	for 10 weeks						1982

Table 3. Additional studies considered by EFSA

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[24]	Reference
200 Healthy overweight adults	83 /day vitamin D_3 or placebo for 12 mo during weight loss programme.	2.36 2.38	2.38 2.40	30.0 ± 17.5 30.3 ± 20.1	85.5 ± 57.5 42.0 ± 35.0		Zitterman *et al*., 2009
150 subjects with TB, 30 groups (I-V) at different stages of infection. Further divided for dose (A-E) 6/group) + controls India	10 20 30 60 95 For 15d,1, 2 and 3 mo	A 2.4, B 2.38 C 2.38, D 2.43 E 2.46 (Controls)[25]	2.5, 2.43, 2.66, 2.62, 2.83 P<0.02 in top dose groups			Hypercalcaemia where infection active	Narang *et al*., 1984
208 post-menopausal African American women Long Island, US	20/day vitamin D_3 for 2y, then 50 /day for 3rd year, or placebo + Ca supplements to ensure Ca intake of 1200-1500 mg/day	2.2	2.31 (both groups) at 2 years. 2.34 (controls) and 2.38 (treated) at 3 years. No difference by mixed model ANOVA. Mean serum Ca 0.062 mmol/L	46.9±20.6 43.2 ± 16.8	71.4 ± 21.5, 65.9 ± 22.4, 87.2 ± 27.0 (3, 24 & 27 mo) 39.1 ± 18.2,41.6 ±18.1, 45.2 ± 21.4 (3, 24 & 27 mo)	276 mild hypercalcaemia, 3 ↑ urinary Ca (but no difference in 24h urine Ca/kg bw) (placebo- 92.0, 107.4, 100.6 and treatment 86.3, 118.8 and 113 mg/day) at 0,2 and 3y	Aloia *et al*., 2008

[24] Urinary calcium included in this column as it has generally been used to assess excess calcium.
[25] Results in TB groups discussed in TOX/2012/23

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[24]	Reference
			higher in top serum vitamin D quartile				
Healthy adults (28-33/group; no placebo) 25 comparable subjects Canada	25 100 /day vitamin D_3 0 (no supp) for 2-5 months		All within normal range (2.2-2.6) and no significant change from baseline by repeated measures ANOVA.	40.7 ± 15.4 (mean ± SD)	68.7 ± 16.9 (40-100) 96.4 ± 14.6 (69-125) Plateau after 3 mo 46.7 ± 17.8 Controls (summer)	More incidences of elevated urinary calcium:creatinine in top dose but not significant by repeated measures ANOVA.	Vieth *et al*., 2001
19 Healthy pre-menopausal (22-49y) women Denmark	100/day vitamin D_2 or 100/day vitamin D_3 For 8 weeks	2.46 ± 0.03 2.46 ± 0.02 Mean ± SEM	2.46 ± 0.01 2.51 ± 0.02 P<0.02 by Mann Whitney test	75.1 (55.1 -95.6). 77.4 (46.2 -100.3).	88.6 (19.7-120.6) 113.3 (88.6-148.3) P<0.001 by Wilcoxon's test	Significant increase in urinary calcium excretion with D_3	Tjellesen *et al*., 1986
163 Healthy post-menopausal white women (20-21/group) Nebraska, US	10 20 40 60 80 100 120/day vitamin D_3 or Placebo for 1 year	2.37 (0.075) Mean ± SD		38.2 (9.4) Mean ± SD	Modelled as curve, plateau at 112 nmol/L at 80-120 µg/day	1 0 1 1 1 1 5 0 4 2 1 0 1 0 2 1 1 0 Individuals with serum Ca ≥ 2.5 or ≥2.7, normalised after re-testing	Gallagher *et al*., 2012

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[24]	Reference
45 nursing home residents Romania	Bread fortified with 125 µg vitamin D_3 + 800 mg calcium for 1y	2.29 ± 0.15 Mean ± SD	2.28 ± 0.15	28.8 ± 9.9	126.4 ± 37.3	No apparent adverse effects	Mocanu *et al*., 2009
438 overweight or obese subjects 21-27Y Norway	1000 500/week Placebo for 1 year			58	140 101	Slight increases in systolic blood pressure	*Jorde *et al*., 2010
297 post-menopausal women Norway	162.5 20 µg/day For 1 y			71 ± 23 71 ± 22 Mean ± SD	185 ± 34 89 ± 17 Mean ± SD	No difference between groups	*Grimnes *et al*., 2012
12 healthy men Sweden	450 vitamin D_3 or controls 3 x week for 7 weeks.	2.47 2.50	2.50 2.44 No difference by unpaired t test	38 ± 4 37 ± 2	123 ± 5 (significant increase) 48 ± 3	No side effects recorded. No sig change in urinary Ca excretion (but became sig when paired t test used. 4.8 and 6.2 mmol/24h in test and 4.2 and 4.5 mmol/24h in controls.	Berlin *et al*., 1986
67 healthy men Omaha, US	0 25, 125 250 vitamin D_3 /day for 20 weeks		Limited data presented but stated that no value rose above the normal range.	70.3	↓11.4 ±4.4 ↑12.0 ± 4.0 ↑91.3 ±9.4 ↑158.4 ± 16.7		Heaney *et al*., 2003
31 patients with corticosteroid induced osteopenia	1125 vitamin D_2 2x week for 24 wks + sodium fluoride and calcium phosphate or				Increased in treatment group		*Rickers *et al*., 1982

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported[24]	Reference
	control for 24 weeks						
163 patients with spinal crush fracture osteoporosis Denmark	450 D_2 + sodium fluoride and calcium phosphate /day for 5 y					1125 µg vitamin D_2 2xweek for 24 weeks + sodium fluoride and calcium phosphate or control for 24 weeks	Hasling *et al*., 1987
116 healthy men Boston, US	25, 250 or 1250 vitamin D_3 /day for 8 weeks,	2.41		67 ± 25	↑28.6 ↑146.1 ↑643.0	2 hypercalcaemia cases in 2 µg/day 1,25 (OH_2)D group. Resolved in restricting intake of Ca rich food	Barger-Lux *et al*., 1998

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