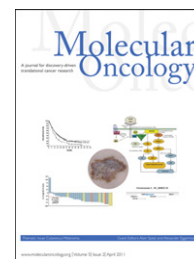


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Review

Melanoma and vitamin D

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ABSTRACT

Vitamin D is a fat-soluble steroid hormone, which is essential to health and for which epidemiological studies suggest a role in autoimmune disease, infections, cardiovascular disease and cancer. It is ingested in foods such as oily fish and supplements, so that average levels vary between countries, but most individuals worldwide make most of their vitamin D as a result of the effects of sun exposure on the skin. Many studies in different populations around the world have in recent years shown that sub-optimal levels of vitamin D (<70 nmol/L) are common. A series of epidemiological studies have suggested that low vitamin D levels increase the risk of cancers, particularly of the breast and gastrointestinal tracts, so that there has been much interest in understanding the effects of vitamin D on cancer cells. Vitamin D binds to the vitamin D receptor (VDR) resulting in transcription of a number of genes playing a role in inhibition of MAPK signalling, induction of apoptosis and cell-cycle inhibition, and therefore vitamin D has anti-proliferative and pro-apoptotic effects in cells of many lineages. It also has suppressive effects on adaptive immunity and is reported to promote innate immunity. Here we review data on vitamin D and melanoma. There are *in vitro* data, which suggest that vitamin D has the same anti-proliferative effects on melanoma cells as have been demonstrated in other cells. We have reported data to suggest that vitamin D levels at diagnosis have a role in determining outcome for melanoma patients. There is a curious relationship between melanoma risk and sun exposure where sunburn is causal but occupational sun exposure is not (at least in temperate climates). Seeking to understand this, we discuss data, which suggest (but by no means prove) that vitamin D might also have a role in susceptibility to melanoma. In conclusion, much remains unknown about vitamin D in general and certainly about vitamin D and melanoma. However, the effects of avoidance of suboptimal vitamin D levels on cancer cell proliferation are likely to be beneficial to the melanoma patient. The possible results of high vitamin D levels on the immune system remain unclear however and a source of some concern, but the data support the view that serum levels in the range 70–100 nmol/L might be a reasonable target for melanoma patients as much as for other members of the population.

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1. Vitamin D and health

Vitamin D is a fat-soluble steroid hormone, which binds to a receptor (the vitamin D receptor, VDR) via which it mediates its genetic effects (Reichrath et al., 2007a). Vitamin D has additional rapid action effects, which are mediated by effects on signalling pathways. Although much is now known about the role of vitamin D in determining health and the means by which it does so, much remains to be understood. This review takes an overview of what is known about vitamin D and health, as a background to considering the very much smaller amount of data on vitamin D and melanoma. Egan has discussed some of the limited data on vitamin D and melanoma (Egan, 2009).

It has long been known that vitamin D is crucial to bone health (the “classical” effects) but more recently it has been recognized to have pleiotropic effects, and is now held to play a role in autoimmune diseases, cardiovascular disease, susceptibility to infections and cancer and even in physiological ageing in animal studies (Keisala et al., 2009). Its pleiotropic effects are suggested perhaps by the work of Autier et al. who reported evidence of reduced overall mortality in individuals who took part in 18 randomized clinical trials of vitamin D supplementation (Autier and Gandini, 2007).

There are dietary sources of vitamin D (predominantly oily fish): it is postulated that the vitamin D originally derives primarily from synthesis by plankton, and is then concentrated by fish in the food chain (Holick, 2003). The content of vitamin D in fish therefore varies considerably so that there are, for example, high levels in oriental tuna and low levels in sole liver (Holick, 2003). Dietary sources also include supplements such as multivitamins or cod liver oil, and in some countries such as the USA (Holick, 2003), foods may be fortified.

Rickets, which resulted from vitamin D deficiency in industrial countries, has been recognized since the 17th century (Holick, 1981), and in the 19th century was known as the “English Disease”. It was recognized in the 1920s that rickets was caused by vitamin D deficiency and the condition was so common in the USA during the 1930s and 1940s, that many foods were fortified with synthetic vitamin D; even foods such as hot dogs, sodas, bread and beer (Holick, 2003). Synthetic vitamin D is produced by irradiation of yeasts (vitamin D₂) or lanolin (vitamin D₃). After World War II, vitamin D fortification was not sufficiently monitored and an outbreak of vitamin D intoxicification in children, leading to hypercalcaemia, led to the abandonment of fortification in many European countries (Holick, 2004). Rickets started to recur, in very recent years, especially in darker-skinned peoples living in less sunny countries, contributing to a resurgence of interest in the role of vitamin D in skeletal health and indeed in health generally.

As national diets vary considerably, the dietary contribution to serum levels is very variable. In countries where oily fish are a large part of the diet such as in Scandinavia and Japan, higher levels are expected, particularly compared with many other countries such as Scotland and Spain where intake is low. Even within countries however there is much variation in dietary patterns, understanding and assessing variation may be difficult: wild salmon, for example, is said to have higher levels of vitamin D than does farmed salmon.

The amount of vitamin D available from diet is also influenced by the preparation of the food, with smoked herring containing approximately 4 µg (160 IU) per 100 g and raw herring 40 µg (1600 IU) per 100 g. As mentioned above, food fortification policies also differ considerably between countries. Thus in many countries such as the UK, the diet is profoundly lacking in vitamin D.

Vitamin D supplementation has been shown to increase serum vitamin D levels as we reported in a study of melanoma cases (Newton-Bishop et al., 2009), but compliance with taking vitamin supplements is only moderate (48–95%) even in the controlled setting of randomized control trials (RCT) (Autier and Gandini, 2007). A 2009 study suggested that although supplements and fortified food explained some of the differences in intake between populations, they contributed relatively little to overall intake compared with the usual diet (Flynn et al., 2009). Flynn et al reported expected variation in levels between European countries with different diets. So, for high vitamin D consumers at the 95th percentile within each country, dietary intake ranged from 3.1 µg/d (<150 IU, Spain) to 17.3 µg/d (approx 750 IU, Finland) in men, and from 2.4 µg/d (100 IU, Spain) to 10.5 µg/d (400 IU, Finland) in women. There was no suggestion of excessively high levels in people with relatively high levels of intake in the diet who were also taking supplements. The higher levels observed in Finland, especially in men, were associated with a high-fish diet and vitamin D fortification of milk, which is semi-mandatory there (Flynn et al., 2009), but even in a Scandinavian country like Finland where the local diet is traditionally rich in oily fish the vitamin D daily intake (if taken as 400 IU) was still only sufficient in 5% of Finnish women (Flynn et al., 2009).

The principal source of vitamin D for most humans is therefore derived from a pro-vitamin in the epidermis, called 7-dehydrocholesterol, which requires photoactivation by ultraviolet B (UVB) in the range 290–315 nm (Holick, 2003). The skin is both the site of crucial steps in the synthesis of active metabolites of vitamin D, and a target organ, where it regulates growth and differentiation of cells (Reichrath, 2007b). In the winter however, less UVB penetrates the ozone layer so that beyond a latitude of 35°, little vitamin D synthesis occurs at that time of the year (Holick, 2003). In northern climes such as Canada, the period of time during which vitamin D synthesis does not occur is protracted: from October to March (Cantor and Shinohara, 2009). Following photoactivation, a series of further modifications occur in the skin, the liver and then the kidney to form hormonally active calcitriol (1 α ,25-dihydroxyvitamin D₃) (Figure 1). The precursors are transported between the skin and the liver, the kidney and other tissues by the carrier protein, vitamin D binding protein. Tissues other than the kidney are known also to be capable of the last hydroxylation step (Reichrath, 2007b) to produce calcitriol. Levels of calcitriol are closely regulated via this activation process but also by inactivation processes in which the cytochrome P450 isoform CYP24A1 plays a major part. Levels of CYP24A1 also vary considerably between cells of different lineage, so that in keratinocytes for example they are high and the enzyme results in limitation of vitamin D levels in the skin whereas the level of CYP24A1 is low in macrophages (Bikle, 2009). The CYP24A1 gene is very responsive to VDR

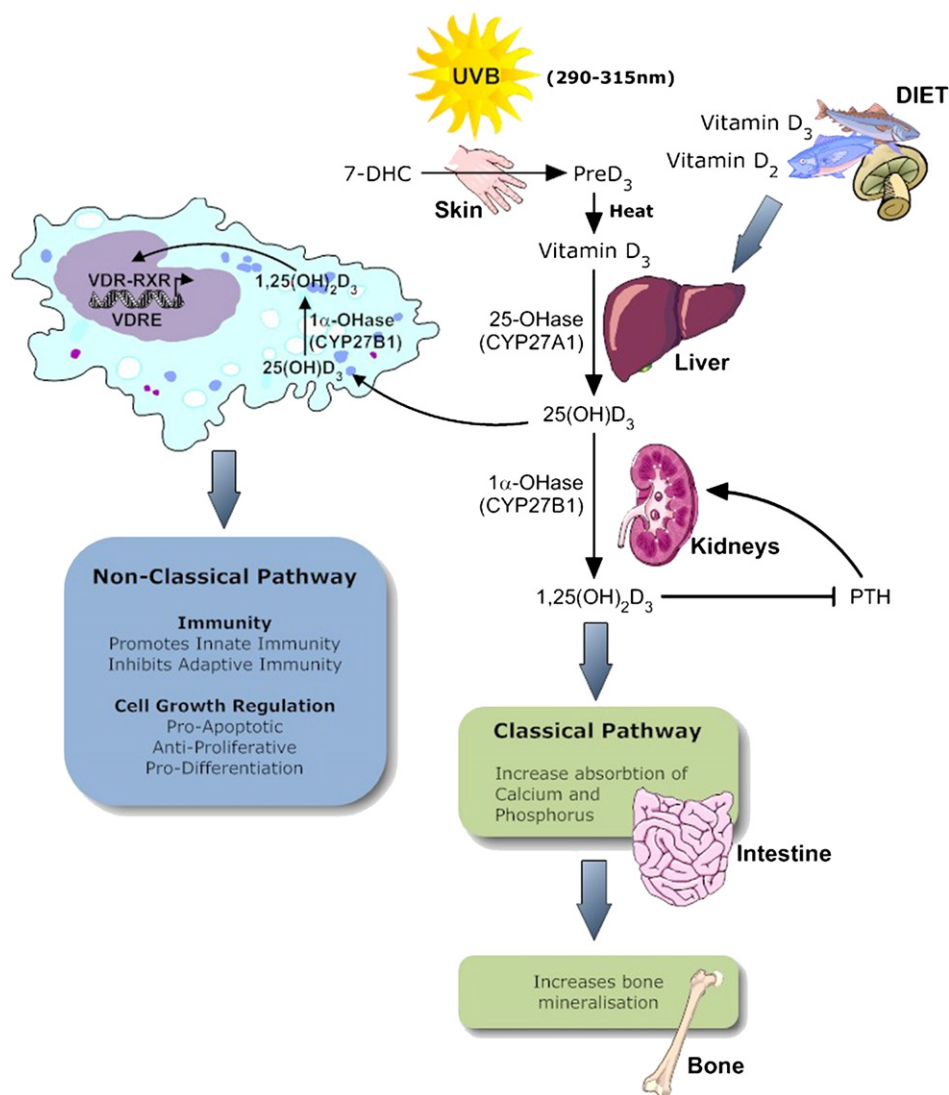


Figure 1 – Vitamin D Metabolism and Biological effects. Sun exposure (UVB) results in photoactivation of 7-dehydrocholesterol to pro- D_3 (cholecalciferol), then heat results in production of Vit D_3 . Endogenous and dietary Vit D_3 along with dietary D_2 (ergocalciferol) are metabolized by the liver and kidney to $1,25(OH)_2D_3$ (1,25 dihydrocholecalciferol) via the enzymes 25-OHase (25-hydroxylase) and 1α OHase (1α -hydroxylase) encoded by the genes *CYP27A1* and *CYP27B1* respectively. $1,25(OH)_2D_3$ has a negative feedback on PTH secretion and its classical effects are on intestinal absorption and bone mineralization. Many cells have the ability to synthesis 1,25 dihydrocholecalciferol locally, resulting in possible apocrine and paracrine non-classical effects on a variety of tissue and immune cells. Vitamin D forms a heterodimer with retinoid X receptor and binds to the nuclear vitamin D receptor (VDR) resulting in effects on gene expression.

regulation and it is suggested that variation in transcription of *CYP27A1* between cells will modulate variation in the effects of vitamin D between cells.

We will refer henceforth to $1\alpha,25$ -dihydroxyvitamin D_3 , as “vitamin D”.

2. Cellular effects of vitamin D

Vitamin D has non-genetic and genetic effects and, as above, the latter are mediated by binding to the vitamin D receptor (VDR), which is predominantly nuclear and is a member of the nuclear receptor superfamily. It is thought that interaction of the genetic and non-genetic pathways occurs, but the non-

genetic effects are less well understood, and interpretation of published data is made even more complex by the presence of tissue-specific variation in cellular responses to vitamin D (Campbell et al., 2010).

When vitamin D binds to the VDR, conformational changes occur in the ligand-binding domain which result in modification of co-regulatory molecules (such as DRIP, SRC, hairless and β -catenin in the skin (Bikle, 2010)), and ultimately in both modification of chromatin and transcription. Transcription generally requires that the activated VDR binds to so-called vitamin D response elements (VDREs) in target genes. The VDR forms a heterodimer, especially with the retinoid X receptor (RXR), which also binds to 9-cis-retinoic acid, a metabolite of vitamin A present in the serum.

The downstream target genes of VDR are many and therefore vitamin D has pleiotropic effects. Gene expression pathways have been generated in different cell types but the data are still relatively limited (Fleet, 2008). Vitamin D target genes include genes that may be upregulated, such as those coding for osteopontin, RANKL, Calbindin-9k, IGFBP and $\beta 3$ integrin, and others that are downregulated (including interleukin genes *IL1* and *IL12*, *TNF α* , *IFN- γ* and *EGF-R*) (Huotari and Herzig, 2008) and *TERT* (telomerase) (Hansen et al., 2001). Other authors have listed other signalling pathways downstream of the VDR (Hansen et al., 2001; Seifert et al., 2004), reviewed by Deeb et al. (2007). The literature is large and this review will not attempt to summarise the findings other than to comment that vitamin D appears to inhibit proliferation of cells, to increase apoptosis and to increase differentiation of cells in a number of ways. The review by Deeb et al. (2007) summarises the genetic and non-genetic effects as follows: “vitamin D appears to inhibit MAPK signalling through suppression of the epidermal growth factor receptor pathway (EGFR) (a pathway that is upregulated in melanoma), and insulin-like growth factor. It induces apoptosis via the IGF1R/PI3K-Akt signalling pathway and by inhibiting telomerase. It increases apoptosis by downregulating *BCL2*, inducing *BAX* and activating caspase cleavage and it results in perturbation of cell-cycle progression by targeting *p27* for degradation and via *MYC*. It increases the expression of E-cadherin, which ultimately leads to repression of the proto-oncogene of *MYC*”. The pathways targeted by vitamin D are therefore those known to be activated in melanoma (and in many cancers) so these data provide support for the view that vitamin D might have an anti-cancer effect on melanoma cells.

Further evidence that vitamin D might have an anti-cancer effect for melanoma would result from demonstration of changes in expression of genes downstream of vitamin D which are anti-proliferative or pro-apoptotic in an *in vitro* model. The genes which were demonstrated to show most upregulation in prostate cancer cells after incubation with vitamin D were *CYP24A1*, *IGFBP-3* (which encodes insulin growth factor binding protein 3) and *DUSP10* (dual specificity phosphatase 10), which inactivates MAPK signalling and thereby may mediate in part the anti-proliferative effects of vitamin D (Krishnan et al., 2003). Apart from Reichrath's work showing induction of *CYP24A1* mRNA in melanoma cell lines in response to vitamin D (discussed later), we are not aware of other similar work done specifically in melanoma cells as yet.

Animal studies of VDR knockout mice showed that the mice were smaller than the controls. The older VDR knockout mice also had a reduced lifespan, wrinkled skin (probably due to reduced fat deposits), vestibular problems (Keisala et al., 2009), and reduced expression of *NF- κ B*, *FGF-23*, *p53*, *klotho* and *IGF1R*.

The “classical” targets of vitamin D are osteoblasts, cells of the small intestine and kidney cells, so that for many years the only significant effects of vitamin D deficiency were thought to be on the bones (Figure 1). In more recent times however, additional major effects have been established, including anti-proliferative effects on cells and effects on the immune system. The possible relationship between vitamin D levels and melanoma may relate to these effects.

The anti-proliferative effects of vitamin D have been demonstrated *in vitro* in cells of many types such that vitamin D analogues have been used to treat a number of cancers, albeit with as yet limited success. In skin diseases characterized by immunologically driven hyperproliferation such as psoriasis however vitamin D analogues such as calcipotriene have proved effective and are widely used. It has proved more difficult to use vitamin D analogues effectively to treat cancer partly because of hypercalcaemic toxicity of high doses of vitamin D analogues, but also at least in part postulated to be because of genetic changes in the cancer cells which reverse its anti-proliferative effects (Holick, 2008).

3. Vitamin D and the immune system

A number of different immuno-competent immune cells express the VDR such as activated T- and B-lymphocytes and dendritic cells, and it is known that vitamin D plays a role in macrophage function (Holick, 2008). Overall, vitamin D is thought to inhibit adaptive immunity and to promote innate immunity (Bikle, 2009). The incidence of a number of diseases postulated to be immunologically mediated appears to be negatively correlated with sunlight exposure, such as multiple sclerosis (Smolders et al., 2008). These epidemiological observations lead to the suggestion that vitamin D may play a role in autoimmune diseases. The immune effects of vitamin D appear to be complex but many appear to result in suppression of adaptive immune responses, particularly of the T helper (Th1)-driven immune response (Froicu et al., 2006), probably mediated in part by reduced antigen presentation by dendritic cells (Enioutina et al., 2009). Moreover, there are data to suggest that vitamin D, at least in VDR knockout mice, reduces the Th1 activation in proportion to Th2 responses (Cantorna et al., 2004). Vitamin D also appears to increase regulatory T cells (Tregs) (Urry et al., 2009) with increased *Foxp3* expression, resulting in blockage of Th1 responses (Bikle, 2009). Th1 activation is crucial for cell-mediated immune responses, which are held to be important for anti-cancer responses in man, and indeed many authors propose that a Th2 dominant response to cancer has an adverse effect. There is therefore a view that high levels of vitamin D might be harmful for cancer patients as a result of suppression of host mediated adaptive immune responses to cancer cells.

The effects of vitamin D on immunity however are complex. In colonic epithelium, vitamin D is reported to play a role in the control of the innate immune system (Froicu and Cantorna, 2007), and there is a reasonable amount of evidence to suggest that innate immunological responses to tuberculosis are dependent on vitamin D. Thus there is also a possibility that higher vitamin D levels could promote beneficial innate immunological responses to cancer cells. The innate immune responses involve sensing of foreign antigens (such as bacterial antigens) via toll-like receptors (TLR), and the induction of cell killing, in part via the release of reactive oxygen species (Bikle, 2009). Vitamin D appears to be involved in these responses and it is of note that TLR agonists such as imiquimod have activity against melanoma in which the drug appears capable of inducing apoptosis (Schon et al.,

2004). There are some data additionally that the drug may modify the Th1/Th2 balance (Green et al., 2008).

That vitamin D clearly has a number of effects on the immune system is therefore both a concern for cancer patients (if the data are relevant which suggest that higher doses of vitamin D suppress Th1 responses), and a hope, if promotion of innate immunity results from increased vitamin D levels.

4. Serum levels of vitamin D and the determinants of those levels

If vitamin D is important to health, then what are the optimal levels in the serum and how can they be achieved?

Vitamin D levels are measured in nmol/L or ng/ml by different laboratories but we will use nmol/L in this article. Although $1\alpha,25$ -dihydroxyvitamin D_3 is the active form of vitamin D, it has a short half life (<4 h) and therefore laboratories measure 25-hydroxyvitamin D. Levels of 25-hydroxyvitamin D are linearly inversely correlated with parathyroid hormone (PTH) levels. Although most commercial assays have an upper level of 125 nmol/L, people out in the sun all the time such as lifeguards may have levels higher than 250 nmol/L (Holick, 2004). Vitamin D intoxication occurs at more than 325 nmol/L. It is unclear what the optimal level of vitamin D might be, but PTH levels have been reported to plateau at around 78 nmol/L (although this figure is contested (Bjorkman et al., 2009)) so that the lower limit of optimal is usually quoted non-the-less as around 78 nmol/L. In our own hospital laboratory in Leeds a level of 60 nmol/L is judged to be the lower end of the optimal range and others have suggested a level of around 75 nmol/L (Holick, 2004; Ingraham et al., 2008). There are some data to suggest that, in study populations at least, vitamin D levels tend to remain fairly steady over time (Hofmann et al., 2010) although with some seasonal variation as we have observed in melanoma patients (Figure 2). Despite the reports of blood levels in lifeguards described above, serum levels of vitamin D are said to be tightly regulated (Huotari and Herzig, 2008) which suggests that maintaining an “optimal” level is important to human health.

As most humans depend on UV-induced synthesis of vitamin D, those with black or Asian skin living in less sunny areas of the world tend to have lower levels. For white skinned people, there has been the view expressed that comparatively little sun exposure such as “5–15 min of exposure between 1000 and 1500 h during the spring, summer and autumn” is usually enough sun exposure for individuals with type II or III skin (Holick, 2004). In recent times however, many authors from around the world have reported sub-optimal levels in many different populations as summarized in Table 1. The high prevalence of sub-optimal levels might suggest that in real life rather than under laboratory conditions, rather more sun exposure is required. Furthermore, within white skinned populations, somewhat surprisingly, we and others have shown that in the very fair, vitamin D levels are actually lower than in brunettes for example (Glass et al., 2009; Randerson-Moor et al., 2009), which is probably behavioural, related to the sun avoidance measures required to avoid sunburn. There may therefore be particular difficulties for fair skinned peoples in achieving optimal vitamin D levels.

Vitamin D is stored in fat: indeed it is stored there in the summer and released during the winter months. Individuals with a high body mass index (BMI) tend to have lower serum levels of vitamin D which is postulated to be explained by excess fat acting as an irreversible sink for vitamin D (Holick, 2004) although there is also some support for the view that overweight individuals sunbathe less (Kull et al., 2009). Inactivity (Brock et al., 2010) and low intake as well as obesity, were recently reported as determinants of low levels in an Australian sample.

Levels are also genetically determined. Genetic associations with African ancestry were shown to correlate with lower vitamin D levels (Signorello et al., 2010), and recent genome wide association studies have identified single nucleotide polymorphisms (SNPs) near genes coding for pathways involved in cholesterol synthesis, hydroxylation, and vitamin D transport as determinants of vitamin D levels (Ahn et al., 2010; Wang et al., 2010).

Vitamin D levels are therefore related to skin type (reported tendency to burn), BMI, diet, supplementation, patterns of sun exposure (and therefore likely latitude) and genetic variation. It is not therefore easy as yet to give practical advice to different populations, which will enable individuals to achieve “optimal” levels whilst avoiding skin cancer.

5. The epidemiology of vitamin D and cancer

Epidemiological studies of the incidence of cancers in relation to latitude and altitude by Apperly (1941) and then by Garland and Garland (1980) first suggested a relationship between vitamin D and death rates from cancers, particularly breast and colon cancer.

There are few data on the role of vitamin D in survival from melanoma other than those published by our group, which are discussed below. There are a series of studies however in other cancers which are broadly summarized in Table 2: indeed the literature is extensive and cannot reasonably be exhaustively covered here. The studies have been “association”

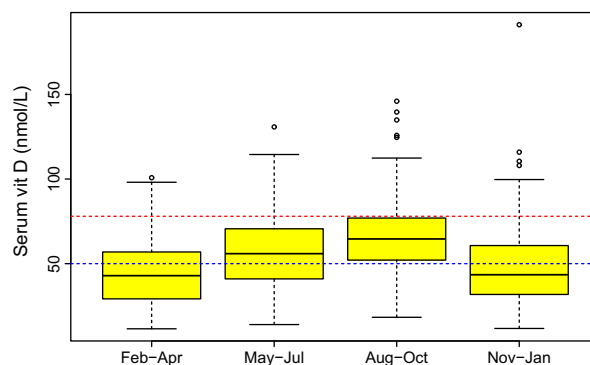


Figure 2 – Variation in serum vitamin D levels by season of venepuncture in the Leeds Melanoma Cohort. A level of 78 nmol/L indicated by red line is the level that PTH stabilisation is expected and < 50 nmol/L below the blue line is used by some studies to define vitamin D insufficient which illustrates how many melanoma patients have sub-optimal levels at diagnosis.

Table 1 – Vitamin D serum levels in various populations at various latitudes.

Country of study	Latitude	Population	Number studied	Serum level in nmol/L	Factors influencing serum levels	Reference
Norway	65–71°N	Older females 44–59 years	300	Overall <37.5 13.7% <50.0 38% Jan/Feb <37.5 23.3%	Low UV exposure Vit D intake significant predictor of serum Vit D level ($p = .0003$)	(Brustad et al., 2003)
Denmark	55°N	Perimenopausal females 45–58 years	2016	<25 7% <50 39.7%	Avoidance of direct sun, no vitamin supplements, low dietary Vit d	(Brot et al., 2001)
Netherlands	52°N	Older male and female longitudinal aging study	1235	<25 10.6% <50 47%	Mean age 75.4 ± 6.5 years	(Snijder et al., 2006)
United Kingdom	51°N	Caucasian females	1414	<25 3.78% <50 25.73% <75 57.29%	Fair skinned	(Glass et al., 2009)
United Kingdom	50–57°N	45 year old white male and female	7437	Winter/Spring <25 15.5% <40 46.6% <75 87.1% Summer/Autumn <25 3.2% <40 15.4% <75 60.9%	Seasonal variation, regional variation from North to south UK. Higher levels with vitamin supplements or oily fish intake	(Hypponen and Power, 2007)
Italy	45°N	Postmenopausal females screening osteoporosis clinic	570	<30 28% Winter (Nov–May) <30 38.5% Summer (Jun–Nov) <30 12.5%	Most frequent in women >70 years <30 51% May–Dec	(Bettica et al., 1999)
France	43–51°N	General urban	1569	North <30 29% Mediterranean Coast <30 7% North 43 ± 21 South 81 ± 27	Low Vit D intake, less daytime sunshine in north. PTH rises with Vit D < 78 nmol/L	(Chapuy et al., 1997)
Canada	43°N	Healthy 18–30 years, mixed ethnicity	107	Wintertime <25 25.5% <50 73.6% <75 93.4%	Skin pigment inverse relationship with Vit D levels	(Gozdzik et al., 2008)
USA	32–45°N	Males, healthy, six U.S. centres	1606	<50 26% <75 72%	Increased rate of deficiency if >80 years, BMI >25, low Vit D intake, N latitude,	(Orwoll et al., 2009)
USA	25–47°N	NHANES III Non-institutionalised, adolescents and adults population	18,875	Winter/median 32°N <25 1–5% <50 13–40% <62.5 25–57% Summer/median 39°N <25 <1–3% <50 8–34% <62.5 21–58%	Insufficiency more prevalent in Mexican Americans and non-Hispanic blacks compared with non-Hispanic whites	(Looker et al., 2002)

USA	25–47°N	NHANES III Non-institutionalised, African American women (1546) and white women (1426) 15–49 years	2972	African American <25 12.2 ± 1.7% ≤37.5 42.4 ± 3.1% White American <25 4.2 ± 0.7% ≤37.5 4.2 ± 0.7% Winter/spring <50 40.5% <50 37.4% <50 67.3%	Determinants of serum levels in African American: no supplements, diet, season, BMI, urban living, oral contraceptive	(Nesby-O'Dell et al., 2002)
Australia Queensland	27°S	General female population <60 years of age	167		Latitude and seasonal variation explained 1/5 of variation	(van der Mei et al., 2007)
Geelong	38°S		561			
Tasmania	41–43°S		432			
1 ng/mL = 2.5 nmol/L.						
NHANES = National Health and Nutrition Examination Surveys; BMI = Body mass index.						

studies, in which the relationship between lower vitamin D levels and cancer risk is reported (and these are essentially retrospective studies therefore subject to bias) and cohort studies. In cohort studies healthy participants either have their vitamin D levels measured at entry to the study or have their intake estimated and the long-term risk of cancer determined. Vitamin D levels tend to be higher in thinner healthier individuals and it must be said that observational or association studies of either type are always subject to the worry that the association seen with vitamin D might be merely a marker for other causal health related factors which are not identified.

The epidemiological data listed in Table 2 are certainly nonetheless persuasive for some cancers. Other authors have reported studies designed to bring together the evidence for particular cancers. Chen et al. (2010), recently reported a meta-analysis of 11 studies on vitamin D intake, 7 studies on circulating 25(OH)D levels, 3 studies of circulating 1,25(OH)₂D levels, and 15 studies on calcium intake and breast cancer risk. From the meta-analysis, there was a significant inverse relationship between vitamin D intake and breast cancer risk, with an overall relative risk (RR) of high versus low vitamin D intake for breast cancer of 0.91 (95% CI = 0.85–0.97). The highest quantile of circulating 25(OH)D was found to be associated with a 45% (OR = 0.55, 95% CI = 0.38–0.80) decrease in breast cancer when compared with the lowest quantile. No significant association for the circulating 1,25(OH)₂D level and breast cancer was found (OR = 0.99, 95% CI = 0.68–1.44). For calcium, a 19% (RR = 0.81, 95% CI = 0.72–0.90) decrease in breast cancer risk was found for those with the highest quantile of calcium intake compared to the lowest quantile. These results provide good evidence that vitamin D and calcium have a chemopreventive effect against breast cancer. Rhee et al. (2009) also recently reviewed a number of studies of colorectal cancer and concluded that prospective studies showed fairly uniform reduction in risk in relation to higher vitamin D levels. In an important, broader study reported by Giovannucci et al. (2006) a model which predicted vitamin D levels was used to compute a predicted 25(OH)D level for each of 47,800 men in the Health Professionals Cohort. 4286 incident cancers (excluding organ-confined prostate cancer and non-melanoma skin cancer) and 2025 deaths from cancer were then observed prospectively and correlated with predicted vitamin D levels. In multivariable models, an increment of 25 nmol/L in predicted 25(OH)D level was associated with a 17% reduction in total cancer incidence (multivariable relative risk [RR] = 0.83, 95% confidence interval [CI] = 0.74–0.92), a 29% reduction in total cancer mortality (RR = 0.71, 95% CI = 0.60–0.83), and a 45% reduction in digestive-system cancer mortality (RR = 0.55, 95% CI = 0.41–0.74). The absolute annual rate of total cancer was 758 per 100,000 men in the bottom decile of predicted 25(OH)D, and 674 per 100,000 men for the top decile. The respective rates were 326 per 100,000 and 277 per 100,000 for total cancer mortality, and 128 per 100,000 and 78 per 100,000 for digestive-system cancer mortality. The authors concluded that low levels of vitamin D may be associated with increased cancer incidence and mortality in men, particularly for digestive-system cancers. There was no protective effect observed for melanoma, indeed there was a non-significant positive

Table 2 – Examples of epidemiological data looking at associations between vitamin D levels and cancer.

	Population	Cancer type	Number of cases/controls	Vitamin D benefit	Comment	Reference
<i>(a) Cancer risk</i>						
Prospective case-cohort study (nested in osteoporotic fractures in men study)	U.S.A. All ethnicity, 1217 non-Hispanic whites	Prostate	297/1351 ≥65 years	No	No association found between Vit D serum levels and subsequent risk of prostate cancer	(Barnett et al., 2010)
Prospective case-control study (nested within prostate, lung, colorectal and ovarian cancer screening trial)	U.S.A. non-hispanic white	Prostate	749/781	No	No association with Vit D and decreased risk of prostate cancer, higher circulating levels may be associated with increased risk of aggressive disease	(Ahn et al., 2008)
Prospective case-control study (nested European prospective investigation into cancer and nutrition)	Europeans	Prostate	652/752	No	The risk of prostate cancer did not vary significantly with Vit D levels	(Travis et al., 2009)
Prospective case-control study (nested α -tocopherol, β -carotene prevention study)	Finnish	Prostate	296/296	No	No difference in Vit D levels between cases and controls	(Faupel-Badger et al., 2007)
Prospective case-control study (nested nutritional prevention cancer trial)	U.S.A. Caucasians	Prostate	83/166	No	No difference in prostate cancer risk	(Jacobs et al., 2004)
Prospective case-control study (nested European prospective investigation into cancer and nutrition)	Europeans	Colorectal	1248/1248	Yes	Strong inverse association between levels of pre-diagnostic Vit D levels and risk of colorectal cancer	(Jenab et al., 2010)
Prospective case-control study (nested nurses health study)	U.S.A.	Colorectal	193/386	Yes	25(OH)D levels but not 1,25-dihydroxyvit D levels were associated in an inverse linear manner with risk of colorectal cancer (distal colon and rectal)	(Feskanich et al., 2004)
Prospective case-control study (nested α -tocopherol, β -carotene prevention study)	Finnish men	Colorectal	146/292	Yes	25(OH)D levels but not 1,25-dihydroxyvit D levels were associated in an inverse linear manner with risk of colorectal cancer (most marked for rectal cancer 55 cases; RR by quartile = 1.00, .93, .77, .37; trend P = 0.06)	(Tangrea et al., 1997)
Prospective case-control study (nested cancer prevention study-II nutrition cohort)	U.S.A.	Breast	516/516	No	No association between 25(OH)D levels and breast cancer risk	(McCullough et al., 2009)
Case series	U.K. Caucasians	Breast	279	Yes	Serum levels of 25(OH)D are significantly higher in patients with early stage breast cancer than those with locally advanced or metastatic	(Palmieri et al., 2006)
Prospective study (third national health and nutrition examination survey)	U.S.A.	Breast	28 breast cancer deaths	No	No association between Vit D levels and risk of death from breast cancer	(Freedman et al., 2007)
Prospective case-control study (nested nurses health study)	U.S.A.	Breast	701/724	Trend to benefit	Higher levels of both 25(OH)D (RR 0.73) and 1,25(OH)2D (RR 0.76) were associated with non significant lower risk of breast cancer. For both metabolites, the association was stronger ≥60 years	(Bertone-Johnson et al., 2005)

Pool nested case-control study (Cohort consortium vitamin D pooling project of rarer cancers)	Multiple geographical location	Pancreatic	952/1333	Negative benefit	High 25(OH)D level (≥ 100 nmol/L) was associated with a significant 2-fold increase in pancreatic cancer risk overall (OR = 2.12)	(Stolzenberg-Solomon et al., 2010)
Prospective case-control study (nested α -tocopherol, β -carotene prevention study)	Finnish Male smokers	Pancreatic	200/400	Negative benefit	3-fold increase risk of pancreatic cancer with high Vit D levels (highest versus lowest quintile, >65.5 versus <32.0 nmol/L; OR, 2.92; 95% CI, 1.56–5.48, $P_{\text{trend}} = 0.001$)	(Stolzenberg-Solomon et al., 2006)
(b) Outcome Prospective study (nested physicians health study)	U.S.A.	Prostate	492/664	Yes	Pre-diagnostic Vit D levels tended to be inversely associated with risk of aggressive prostate cancer at diagnosis, especially men >65 years	(Li et al., 2007)
Association study	Norway	Prostate	160	Yes	Serum Vit D levels 50–80 nmol/L and >80 compared to <50 had better prognosis (cause specific mortality) (RR0.33; RR0.16)	(Tretli et al., 2009)
Prospective cohort study	Japan	Colorectal	257	Yes	Higher Vit D levels were associated with better overall survival (HR, 0.91, $P = .027$)	(Mezawa et al., 2010)

relationship, but the measure used to predict vitamin D level in the cohort was derived from such variables as skin colour and latitude of residency which would likely confound such a test as skin color and latitude of residence are risk factors for melanoma.

The only conclusive evidence that supplemental vitamin D should be adopted as a means of prevention, would be long-term data showing benefit in the context of randomized clinical trials. The difficulties are numerous however in reaching this goal and the data so far are of limited value. A study from the Women's Health Initiative, for example, failed to report evidence of reduced risk of colon cancer in 36,000 women randomised to receiving 400 IU of vitamin D and calcium per day for an average of 7 years (Wactawski-Wende et al., 2006): however the study was criticized for too small doses, poor compliance and inadequate control for vitamin D in the placebo group (Bikle, 2009). There was some evidence for a preventive effect for all cancers in a short study of 1179 women in residential homes given 1100 IU vitamin D and calcium daily over four years (Lappe et al., 2007).

6. Vitamin D and melanocytes: *in vitro* data

There are data on *in vitro* models of melanoma in relation to added vitamin D but they are few in comparison with those produced in cells of other cell lineages. We have therefore started by summarizing the type of evidence of anti-proliferative effects reported, accepting that this cannot be an exhaustive list (Table 3).

Vitamin D was shown to inhibit proliferation and induce differentiation in melanoma cells in the late 1990s (Danielsson et al., 1998, 1999), and variable degrees of inhibition of proliferation were reported more recently (Reichrath et al., 2007b). Subsequently however certain melanoma cell lines have been demonstrated to be vitamin D resistant. The Reichrath group compared a vitamin D resistant melanoma cell line (SKMel 5) and a vitamin D responsive melanoma cell line (MeWo cells) to explore determinants of vitamin D resistance. They showed that both cell lines had mRNA expression for the VDR gene and metabolizing enzymes such as CYP24A1 (24-OHase). However, in response to vitamin D only the MeWo cells responded with a dose dependent increase in mRNA for the VDR and CYP24A1 genes as well as suppressing cell line proliferation (up to approximately 50%). (Seifert et al., 2004) The conclusion of the Reichrath group was that unresponsiveness to vitamin D was mediated by failure of VDR-mediated transcription. After a breast cancer research group reported that amplification at chromosome 20q13.2 (the location of the 24-OHase gene, CYP24A1) might explain vitamin D resistance in breast cancer cells (Albertson et al., 2000), the Reichrath group sought amplification of the CYP24A1 *in vivo* using Southern blotting in a small number of fresh tissue cutaneous malignant melanoma samples but did not demonstrate upregulation of 24-OHase (Reichrath et al., 2004). Further work in other melanoma resistant and responsive cell lines to study vitamin D anti-proliferative effects, has also demonstrated support for a defect in VDR-mediated transcription (Reichrath et al., 2007b).

Table 3 – *In vitro* studies looking at the effect of vitamin D on cancer cells.

Type of cancer	Model studied	Cell line used	Vitamin D or analogue	Findings	Conclusion	Reference
Prostate	Mouse model	PC-3	16-23-D ₃	Mean tumour volume 15% smaller than in controls – modest anti-proliferative effect	Antiproliferative	(Schwartz et al., 1995)
Prostate	Cell culture	Human Tissue samples	1,25(OH) ₂ D ₃	Benign and malignant epithelial cells growth was inhibited with half maximal inhibition at 1 nmol and complete inhibition at 25 nmol	Antiproliferative	(Peehl et al., 1994)
Prostate	Cell culture	DU 145, PC-3, LNCaP	1,25(OH) ₂ D 16-23-D ₃	PC-3, LNCaP were poorly invasive ± Vit d, PC-3 cells – highly invasive and markedly inhibited by 1,25(OH) ₂ D and 16-23-D ₃ , dose and time dependent	Decreased Invasiveness	(Schwartz et al., 1997)
Breast	Cell culture	MCF-7, BT-20	1,25(OH) ₂ D ₃	Reduced proliferation irrespective of oestrogen status of cell line	Probably Antiproliferative	(Chouvet et al., 1986)
Breast	Rats	MCF-7	Calcipotriol, (MC903), 1,25(OH) ₂ D ₃ , 1α(OH)D ₃	1α(OH)D ₃ (0.25, 1.25 μg/kg) response rate (rr) 25% (rr = 50% regression of tumour) but ↑Ca ⁺² . Calcipotriol 17% rr without severe ↑Ca ⁺²	Antiproliferative	(Colston et al., 1992)
Breast	Rats Cell culture	Nitrosomethyl urea induced rat mammary tumours MCF-7 cell line	1,25(OH) ₂ D ₃ EB1089	↑Ca ⁺² No significant inhibition of tumour progression with 1,25(OH) ₂ D ₃ (0.5 μg/kg). EB1089 tumour suppression no ↑Ca ⁺² . EB1089 increased expression of TRPM-2 apoptosis gene, ↑p21, ↑p53, ↓bcl-2 in MCF-7 cell cultures	Antiproliferative analogue in vivo Possible role in apoptosis in vitro	(James et al., 1996)
Colon	Cell culture	HT-29 (human colon carcinoma cell line HT-29 differentiates into functional enterocytes)	1,25(OH) ₂ D ₃	Promotes differentiation of HT-29 cells as measured by maltase activity	Prodifferentiation	(Brehier and Thomasset, 1988)
Colon	Cell culture	HT-29 Biopsy normal mucosa and ademoas	1,25(OH) ₂ D ₃ Synthetic Analogues	Inhibit proliferation (CCPR, Ki-67) in normal and premalignant rectal epithelium and suppress growth HT-29 colon cell line	Antiproliferative	(Thomas et al., 1992)
Colon	Cell culture	SW480	1,25(OH) ₂ D ₃ and Multiple nonhypercalcemic synthetic analogues	1α,25(OH) ₂ D ₃ induces E-cadherin and modulates beta-catenin-TCF-4 target genes promoting the differentiation of colon carcinoma cells	Pro differentiation	(Palmer et al., 2001)
Pancreas	Cell culture and mice	GER xenografts immunodeficient mice	EB1089	GER cells dose dependent growth inhibition. No ↑p53. In vivo 5 μg/kg 3/week significant growth inhibition	Antiproliferative	(Colston et al., 1997)
Pancreas	Cell culture	AsPc-1, BxPc-3, T3M-4	EB1089, CB1093	Growth arrest correlated with increased proportion of cells in G0/G1 phase	Antiproliferative antiapoptotic effect	(Pettersson et al., 2000)
Pancreas	Cell culture	BxPC-3 Hs700T Hs766T AsPC-1	1,25(OH) ₂ D ₃ , 25(OH) ₂ D ₃	25(OH) ₂ D ₃ inhibited the growth of 3/4 pancreatic cell lines that correlated with induction of p21, p27 and cell-cycle arrest at the G1/S checkpoint	Antiproliferative	(Schwartz et al., 2004)

16-23-D₃ = Dihydroxy-16-ene-23-yne-cholecalciferol; 1,25(OH)₂D = 1,25(OH)₂D₃ = 1,25-dihydroxyvitamin D₃ = calcitriol; EB1089 = 1(S),3(R)-dihydroxy-20(R)-(5'-ethyl-5'-hydroxy-hepta-1'(E),3'(E)-dien-1'-yl)-9,10-secopregna-5(Z),7(E),10(19)-triene; TRMP-2 = Testosterone-repressed prostatic message-2 also known as clusterin; CCPR = Crypt cell production rate.

There are, in summary, some *in vitro* data, to suggest that vitamin D has anti-proliferative effects on cultured melanoma cells, as it is reported to have in other cancers, but the data are limited and further research is needed.

7. Survival from melanoma

A relatively simple way to look at a potential relationship between vitamin D and cancer mortality is to correlate death rates from that cancer by ultraviolet flux, or at least latitude as a surrogate. This would, for melanoma, be extremely difficult to assess, given that both vitamin D levels and melanoma risk are related to factors such as skin colour and sun exposure. In 2005 however, Berwick *et al.* (2005) reported a study in which the presence of solar elastosis (dermal sun damage) in melanoma excision specimens appeared to be associated with a better prognosis for melanoma patients. One interpretation of this finding was that chronic sun damage might induce a less aggressive form of melanoma, but another possible explanation is that vitamin D levels related to chronic sun exposure might be protective for relapse even if sun exposure is related to its aetiology.

If the anti-proliferative effect of vitamin D was important in modifying outcome for melanoma patients, we might first postulate that prognosis would be better in countries with higher sun exposure than in countries with less. In fact, outcome for patients diagnosed with melanoma in Australia is better than for those diagnosed in the UK. Both groups of melanoma patients are genetically similar as the melanoma population in Australia is largely of UK origin ethnically as seen in analyses reported in the GenoMEL genome wide association study (Bishop *et al.*, 2009). We therefore thought it possible to compare determinants of outcome in the two populations with similar genetic background but potentially different environmental influences. We therefore carried out a study collaboratively between cancer registries in the UK and Australia (Downing *et al.*, 2008) to better understand the determinants of outcome in both countries. Patients diagnosed with invasive melanoma between 1993 and 2003 in Yorkshire ($n = 4170$) and New South Wales (NSW, $n = 30,520$) were identified from cancer registry databases and prognostic information (age, sex, socioeconomic background, tumour site and Breslow thickness) was extracted. Age-standardised incidence rates, 5-year relative survival and relative excess risk of death were calculated. Between 1993–1995 and 2001–2003, the incidence of melanoma increased in both areas. These increases were mainly seen in patients with tumours with a Breslow thickness ≤ 1 mm. Five-year relative survival was 86.9% (95% CI 85.2–88.5) in Yorkshire and 88.6% (95% CI 88.1–89.1) in NSW. Compared with that observed in NSW, survival in Yorkshire was lower for males and for those living in the most deprived areas. After adjustment for differences in prognostic factors, the relative excess risk of death in Yorkshire compared to that in NSW reduced from 1.36 (95% CI 1.20–1.53) to 1.11 (95% CI 0.99–1.23). There was a small residual suggestion of reduced risk for death in Australia, but differences in tumour thickness appeared to be the most important factor. We therefore concluded that the small difference in outcome for melanoma patients in the UK and

Australia likely results from earlier presentation with thinner tumours in Australia where the surveillance for early detection of skin cancers is heavily promoted. Thus this study provided no real support for a protective effect of vitamin D, if one assumes indeed that vitamin D levels are on average higher in Australian melanoma patients than in UK patients.

Nonetheless we explored the possible effects of vitamin D level in UK melanoma patients. A pilot retrospective study of 271 patients with melanoma suggested that vitamin D may protect against recurrence of melanoma (Newton-Bishop *et al.*, 2009). We tested these findings in a survival analysis in a cohort of 872 patients recruited to the Leeds Melanoma Cohort (median follow-up, 4.7 years). In the retrospective study, self-reports of taking vitamin D supplements were non-significantly correlated with a reduced risk of melanoma relapse (odds ratio = 0.6; 95% CI, 0.4–1.1; $P = .09$). Non-relapsers had higher mean 25-hydroxyvitamin D₃ levels than relapsers (49 versus 46 nmol/L; $P = .3$; not statistically significant). In the cohort (prospective) study, higher 25-hydroxyvitamin D₃ levels were associated with lower Breslow thickness at diagnosis ($P = .002$), and were independently protective of relapse and death: the hazard ratio for relapse-free survival (RFS) was 0.79 (95% CI, 0.64–0.96; $P = .01$) for a 20 nmol/L increase in serum level. A survival curve for the cohort with longer follow up than previously reported is shown in Figure 3. There was evidence of interaction between the vitamin D receptor gene (VDR) BsmI genotype and serum 25-hydroxyvitamin D₃ levels on RFS in the data reported in 2009. We concluded that the results from the retrospective study were consistent with a role for vitamin D in melanoma outcome. The cohort study tested this hypothesis, providing evidence that higher 25-hydroxyvitamin D₃ levels, at diagnosis, were associated with both thinner tumours and better survival from melanoma, independent of Breslow thickness. The data urgently need to be validated in additional mature sample sets and importantly the effects of vitamin D level in the follow up period must be investigated.

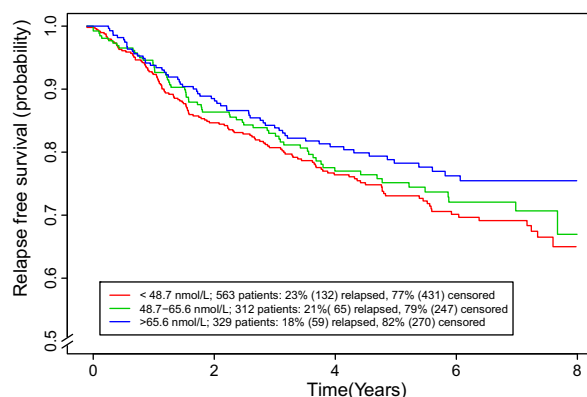


Figure 3 – Up to date (Sept 2010) Kaplan-Meier curves for relapse free survival probabilities for serum vitamin D levels (nmol/L) at interview (categorized based on tertile cutoff) in the Leeds Melanoma Cohort. The text box shows the overall proportion of relapsers by vitamin D category.

Nonetheless in the interim the data do suggest a relationship between higher vitamin D levels and thinner tumours and better outcome for melanoma patients in the UK. At the beginning of this section we described a comparative study between patients diagnosed with melanoma in Australia and the UK, and when we published these data, we concluded that the difference between the two countries with regards to outcome was related to Breslow thickness. In view of our more recent data in which tumour thickness was related to vitamin D level (Newton-Bishop et al., 2009), we cannot exclude the possibility that outcome is better for melanoma patients in Australia presenting with thinner tumours at least in part, as a result of postulated higher vitamin D levels in that sunnier country.

We will next discuss the very limited data on the relationship between vitamin D and melanoma susceptibility.

8. Vitamin D and susceptibility to melanoma

Although epidemiological studies have established a clear relationship between sun exposure and melanoma risk (Gandini et al., 2005), the nature of this relationship has proved complex. Indeed the complexities (largely related to the lack of a simple dose/risk relationship between sun exposure and melanoma risk) have complicated public education programmes internationally. We will explore possible explanations for these complexities and speculate as to whether vitamin D might play a role.

For most carcinogens there is a dose/risk relationship, so that higher levels of exposure are associated with higher levels of risk. The analogy for melanoma would be that the more sun exposure a fair skinned person has through life, the higher would be the risk of melanoma. One would then predict that outdoor workers would be at greatest risk of melanoma. In many studies however there was no evidence of such relationship, and indeed there is a suggestion from individual studies and from a meta-analysis that occupational sun exposure might actually be associated with a lower risk (Gandini et al., 2005). We reported a pooled data analysis, which did not show a protective effect of measures of total lifetime sun exposure, but confirmed that it was sun bathing which was associated with the highest risk at all latitudes. For studies in temperate climates there was no measurable relationship between total lifetime sun exposure and risk (Chang et al., 2009). At all latitudes, a history of sunburn was consistently associated with increased risk (sunburn being a marker both of genetically determined susceptibility and exposure). Again therefore intermittent sun exposure associated with sun bathing and sunburn was confirmed as the dominant risk factor for melanoma rather than cumulative sun exposures at most latitudes.

This complex relationship has not yet been explained but seminal animal work by Kripke and colleagues has led to the view that sunburn related immune suppression is important for carcinogenesis for melanoma (Jeevan et al., 1992; Noonan et al., 1981; Wang et al., 1990). The strong relationship between sunburn history and melanoma risk for humans, coupled with the lack of a simple dose response relationship between sun exposure and risk, suggested also that there is something particular about inflammation related to sunburn,

and melanoma tumorigenesis. For melanoma, chronic sun exposure (in this hypothesis) would be less important, at least in temperate climates, than it is for non-melanoma skin cancer.

There is some evidence that chronic sun exposure protects from sunburn by inducing photoadaptation with sun exposure related tanning and epidermal thickening affording some protection. The most prevalent current hypothesis is that the indoor worker (not adapted to sun exposure) suffers sunburn during holidays, which induces DNA damage and immunosuppression, leading over time to an increased risk of melanoma on intermittently covered body sites. The outdoor worker, or people habitually exposed to moderate sun exposure, may become photoadapted so that burning (and melanoma) are less likely consequences.

We have recently reported a large case-control study of melanoma in the north of England (Newton-Bishop et al., 2010). In this study we showed that the phenotypes associated with a tendency to sunburn and reported sunburn at ≥ 20 years of age were associated with increased melanoma risk. Overall higher average weekend sun exposure in warmer months was however actually protective for melanoma (OR 0.67, 95% CI 0.50–0.89 for highest versus lowest tertile of exposure) (Newton-Bishop et al., 2010), lending some support for the hypothesis that moderate sun exposure can actually be protective for melanoma.

What is the explanation for a possible protective effect of habitual sun exposure? Would it result from photoadaptation, or because sun exposure increases vitamin D levels? There are limited data to inform this but there are some which suggest that vitamin D itself has photoprotective effects (Damian et al., 2010; Gorman et al., 2007; Gupta et al., 2007). Conflicting data exist however that vitamin D may actually be a mediator via which sunburn results in systemic immunosuppression (Gorman et al., 2007). Mason et al. (2010) have reported evidence that vitamin D reduces UV-induced DNA damage *in vitro* and in mouse and human skin, and reduces UV-induced immunosuppression as a result, although other data suggest that vitamin D has an immunosuppressive effect when analogues were applied topically to irradiated skin (Damian et al., 2010). A recent review published by Mason's group describes the complexity of what is currently known about the putative role of vitamin D as a cutaneous defence molecule (Dixon et al., 2010). We would suggest that the complexity of those responses is such that currently the data are difficult to interpret.

If vitamin D was playing a role in melanoma prevention, then although sunburn is causal for melanoma, vitamin D intake might be protective. The possible role of vitamin D as reported dietary intake in melanoma risk was reported by Gandini et al. (2009) in a meta-analysis. They excluded one study on the basis of heterogeneity and in the remaining studies showed some evidence of a protective effect for highest versus lowest intake, SRR 0.63 (95% CI 0.42–0.94). A small previous study had failed to show lower levels of vitamin D in melanoma patients than in healthy individuals (Reichrath and Querings, 2004), and a tiny study from 1992 showed a 5% lower level of vitamin D in 23 healthy donors who later went on to develop a melanoma (Cornwell et al., 1992). This was, not surprisingly, a non-significant effect but shows the

long-standing interest in the field at least. [Asgari et al. \(2009\)](#) however explored this in a large prospective cohort. They examined whether dietary and supplemental vitamin D intake was associated with melanoma risk among 68,611 men and women who were participants in the Vitamins and Lifestyle cohort study. Participants reported dietary vitamin D intake over the preceding year and 10-year use of multivitamin and individual vitamin D supplements on a baseline questionnaire. After follow-up through 2006, 455 incident melanomas were identified through linkage to the Surveillance, Epidemiology, and End Results cancer registry. There was a suggestion in these data of a decreased risk for high supplemental use $RR = 0.77$ (95% CI 0.34–1.72) compared with the lowest quartile, but they did not see a protective effect of combined dietary and supplemental intake (1.05, 95% CI = 0.79–1.40). There was therefore no support from this large study for the suggestion that vitamin D levels might be protective for melanoma, but in this study the authors were not able to correct for known risk factors of melanoma risk such as reported sunburn or sun exposure. Overall therefore the data available to assess the role of vitamin D and melanoma risk are few.

The global burden of disease attributed to sunlight exposure is estimated to be only 0.1% of the total and the likely negative health effects resulting from very low levels of vitamin D as a result of very low levels of sun exposure, are estimated to be much greater ([Lucas et al., 2008](#)) therefore the advice to avoid excessive sun exposure must be carefully delivered. The epidemiology relevant to primary prevention of melanoma currently remains incompletely understood. The above summarized data suggest that sunburn is aetiological for melanoma, and that sunny holidays and sun bathing are causal. It also suggests that chronic sun exposure is much less clearly related to risk in many temperate areas of the world, such as Europe, compared with hot countries such as Australia. Given that occupational sun exposure has been reported as protective for melanoma, that vitamin D is reported to have anti-proliferative effects in melanoma cells, and the reported photoprotective and anti-proliferative effects of vitamin D, interest has arisen in a possible role for vitamin D in melanoma prevention. If vitamin D was preventative for melanoma then one might hypothesise that melanoma incidence would be higher in areas of the world where sun exposure is less but this relationship would likely be confounded by the effect of sun exposure itself and therefore there is no easy means of using incidence data to address this possibility.

In order to further investigate this hypothesis, a number of groups looked at inheritance of polymorphisms in the gene coding for VDR as determinants of melanoma risk ([Halsall et al., 2004](#); [Han et al., 2006](#); [Li et al., 2008](#); [Moon et al., 2006](#); [Santonocito et al., 2007](#)). We in Leeds also looked at these and reported the data in a meta-analysis in conjunction with published data from other smaller data sets (2159 cases and 2429 controls) ([Randerson-Moor et al., 2009](#)). In the meta-analysis, the VDR FokI T allele was associated with increased melanoma risk OR 1.19 (95% CI 1.05, 1.35), and the BsmI A allele was associated with a reduced risk OR 0.82 (95% CI 0.71, 0.94), in each instance assuming a dominant model. The association between the FokI T allele and

melanoma was confirmed in a second Leeds case (299) control (560) series, OR 1.42 (1.06–1.91), $p = .02$. In our reported study however there was no significant case-control difference in vitamin D levels. These association case study data therefore provide some genetic evidence to support for the view that vitamin D might have an effect on susceptibility to melanoma.

In summary then, there are as yet no clear answers as to whether vitamin D might have a role in protection of melanoma but there are sufficient data to suggest that we should continue to explore the possibility. There is no doubt however that sunburn causes melanoma and the incidence of melanoma continues to increase worldwide. Public health advice must therefore remain that sunburn MUST be avoided.

Box 1

What we do know

- That vitamin D is crucial to health
- That many individuals world wide have sub-optimal levels
- That melanoma patients in the UK often have low levels soon after diagnosis
- If they keep out of the sun after diagnosis then many melanoma patients are likely to become severely vitamin D depleted
- That it would therefore be sensible to avoid having low levels (less than 60 nmol/L) of vitamin D in the serum although we would like to know what the optimal dose would be
- Melanoma patients should avoid excessive amounts of vitamin D because of anxieties around immune suppression and levels higher than 90 to 100 nmol/L might best be avoided

Box 2 Summary of what currently remains unknown about vitamin D and cancer but needs to be defined to ensure advice to melanoma patients and the general public is safe and accurate.

What we don't yet know

- The optimal serum level of vitamin D for the prevention of cancer: we think it's around 60 to 100 nmol/L
- How to achieve a level of this around the year in individuals of different diet, sun exposure patterns, body mass index and age
- We don't know whether high levels of vitamin D are harmful by suppressing immunity or possibly by other mechanisms
- So we don't yet know what supplemental dose of vitamin D to suggest for various populations
- Which inherited genes modify the effects of the vitamin D on risk

9. Future research

In Boxes 1 and 2 we have summarized what we do know and what we don't know about vitamin D but the reality is that there remains much to learn.

The optimal levels of vitamin D required to prevent cancer or to improve outcomes are unclear. Indeed, although it is known with some certainty that low levels of vitamin D are associated with myocardial infarction, congestive heart disease and calcific aortic stenosis, the exact doses recommended are unclear (Zittermann and Gummert, 2010). The shape of the dose response curves for risk of cardiovascular disease (Wang et al., 2008) and for better survival for breast cancer patients (Goodwin et al., 2009), are both suggestive of a U-shaped effect. That is that there are concerns that high doses of vitamin D may also be harmful, and there is an urgent need to understand the effects of higher blood levels, particularly on host immune responses to melanoma and cancer in general.

The evidence however is persuasive that levels lower than around 75 nmol/L are sub-optimal for many aspects of health and should be avoided. Research directed towards better establishing optimal levels is urgently needed and understanding how those levels should be attained in phenotypically variable individuals. It is established that there is genetic variation in genes coding for key proteins such as the vitamin D binding protein (Wang et al., 2010) which affect vitamin D levels, and that there is variation due to BMI, age, skin colour, diet and sun exposure. We therefore do not know as yet how best to advise different populations as to how to balance the need to avoid melanoma with the need to avoid vitamin D deficiency. It is clear however that rapid progress is now taking place in understanding these factors which will likely lead to improved health internationally in the relatively near future.

10. Recommendations currently for melanoma patients

In the UK at least, it has been shown that melanoma patients around the time of diagnosis, commonly have sub-optimal levels of vitamin D, and moreover that lower levels of vitamin D were both associated with thicker tumours and poorer survival even when adjusted for thickness and other factors having an effect on outcome such as age and sex (Newton-Bishop et al., 2009). Whilst it will require a randomized clinical trial to establish whether increasing blood levels of vitamin D after diagnosis might improve survival, the established harmful effects of sub-optimal levels of vitamin D on health in general (Autier and Gandini, 2007) lead us to the view that melanoma patients should avoid vitamin D deficiency. A common practice is for melanoma patients to reduce their sun exposure after a diagnosis and this is sensible, as sunburn-induced immunosuppression is potentially harmful, and the patient wishes to reduce their probability of another primary. However, if they do so, it is likely that their vitamin D levels will fall. In a small study reported by McCombie et al. (2009) the majority of patients who had had non-melanoma skin cancer had levels lower than 50 nmol/L after winter even in Sydney. It

would seem sensible therefore to supplement the diet with vitamin D.

Many experts suggest that a minimum of 1000 IU of vitamin D daily is necessary to achieve serum levels of around 70 nmol/L (Holick et al., 2007; Reichrath, 2007a). The National Academy of Sciences suggests somewhat lower levels ranging from 200 IU up till 50 years of age and 400 IU for older individuals, (these levels are usually found in multivitamin tablets). It seems to us however that there is still much to learn about what are optimal levels and how they might be achieved at different latitudes, in people of different ages, skin types, diets and BMI. In the healthy population many authors have suggested that sun exposure is the best means of maintaining healthy levels of vitamin D. We have demonstrated however, as have others, that people with fair skin (those at risk of melanoma) tend to have lower levels of vitamin D than other "white skinned" people (Glass et al., 2009; Malvy et al., 2000) which has been attributed to sun avoidance (Malvy et al., 2000), probably in order to avoid a burn. We would therefore recommend that fair skinned melanoma patients, and probably all fair skinned peoples at risk of melanoma (those with red hair, increased numbers of melanocytic naevi, previous melanoma or a family history of melanoma), should avoid sun exposure sufficient to avoid burning and to supplement their diet with vitamin D. As high levels of vitamin D may have currently unforeseen effects on melanoma patients, we would recommend aiming for serum levels around 70 nmol/L rather than aiming for very high levels. Clearly however urgent research is needed to establish the optimal level and how it might be achieved. Good dietary sources of vitamin D include oily fish, fortified foods, cod liver oil and dietary supplements in multivitamins. Vitamin D₃ is thought to be more effective than vitamin D₂. Mushrooms are the only vegan source of vitamin D other than the sun. On-line information for melanoma patients can be found at www.genomel.org.

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