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A systematic review of the influence of skin pigmentation on changes in the concentrations of vitamin D and 25-hydroxyvitamin D in plasma/serum following experimental UV irradiation

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Abbreviations: 1,25$(OH)_2$D: 1,25-dihydroxyvitamin D, 25(OH)D: 25-hydroxyvitamin D, 7-DHC: 7-dehydrocholesterol; HPLC: high-performance liquid chromatography, MED: minimal erythema dose, SED: standard erythema dose, UV: ultraviolet; DBP: vitamin D binding protein

Tables: 2

Figure: 1

Abstract

Defining whether skin pigmentation influences vitamin D photosynthesis is important for delivering accurate public health messages. Current evidence is contradictory. We undertook a systematic review of the published literature to examine the association between skin pigmentation and change in blood concentrations of vitamin D and 25-hydroxyvitamin D following experimental UV irradiation. Twelve studies fulfilled the inclusion criteria: human study *in vivo* with non-diseased participants; controlled artificial UV radiation; vitamin D or 25-hydroxyvitamin D measured in serum or plasma; full text in English. In seven studies, vitamin D photosynthesis was reduced in dark-skinned compared with fairer-skinned individuals. In the remaining five studies, only one of which was published after 1990, there was no difference in vitamin D photosynthesis according to skin type. The disparities in these results may be due to small sample sizes and variations in study methodology, including the source, dose and frequency of UV irradiation, phototype classification, and analysis of vitamin D and 25-hydroxyvitamin D. Of these, the spectrum emitted by the UV lamps may be significant. No study considered potential modifying factors, such as relevant genetic polymorphisms. On balance, we conclude that pigmented skin has less effective photoproduction of vitamin D and 25-hydroxyvitamin D. The quantity of sun exposure needed for dark-skinned, compared with light-skinned, people to achieve vitamin D sufficiency remains uncertain.

Introduction

Exposure of the skin to solar ultraviolet B radiation (UVB, 280-315 nm) is a key source of vitamin D.[1] The irradiation converts 7-dehydrocholesterol (7-DHC) to previtamin D_3. 7-DHC is found mainly in the basal and spinous layers of the cutaneous epidermis although small amounts are also present in the outer layers of the epidermis (stratum corneum and granulosum) and in the dermis.[2] Previtamin D_3 undergoes a thermal isomerisation to form vitamin D_3, which is taken up into the blood. UV-induced vitamin D_3, as well as vitamin D obtained from the diet, is hydroxylated in the liver to form 25-hydroxyvitamin D [25(OH)D], the main circulating form of vitamin D. The serum concentration of 25(OH)D is currently considered to be the best measure of vitamin D status[3] and the consensus view is that the large majority of systemic vitamin D and its metabolites derives from the UV-induced synthesis in the skin[3]. 25(OH)D undergoes further hydroxylation in the kidney to form the active hormone, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. Vitamin D metabolites circulate in blood bound tightly to a vitamin D-binding protein (DBP)[4].

In 1922, Hess *et al.* showed that repeated daily UV irradiation for the treatment of rickets was more effective in white rats than in black rats, for a given dose of UV radiation.[5] This led to the notion that darker skin is less effective at vitamin D photosynthesis than lighter skin because melanin and 7-DHC compete for UV photons.[4] Melanin is produced by melanocytes in human skin located in the basal layer of the epidermis, and is stored in melanosomes. The melanosomes are exported to adjacent keratinocytes (primarily basal cells) where they can form a cap-like structure over the nuclei in response to UV radiation.[6, 7] Differences in skin pigmentation are due to differences in the number, size, composition and distribution of melanin pigments throughout the epidermis.

In many locations, serum 25(OH)D concentrations (as a measure of vitamin D status) are lower, on average, in dark-skinned compared with fair-skinned populations.[8-10] For example, in the United States, the mean serum 25(OH)D levels in all age groups of non-Hispanic whites was 1.2-1.7 times higher than in Mexican Americans and non-Hispanic blacks, and the mean in Mexican Americans was 1.3-1.4 times higher than in non-Hispanic blacks, after taking season and latitude of blood collection into account.[11] New Zealanders of European origins had the highest mean serum 25(OH)D concentration of 51 nmol/L, compared to 42 nmol/L in Maori and 37 nmol/L in Pacific Islanders.[12] Cultural/behavioural factors in addition to skin pigmentation are important in these differences.[13]

Several studies have evaluated the influence of skin pigmentation on vitamin D photosynthesis in experimental settings by measuring the relationship between exposure to a controlled dose or doses of UV radiation and levels of vitamin D or 25(OH)D in the serum of individuals with different skin colour. This experimental design has advantages over population-based studies in providing accurate data on the dose and spectrum of the UV radiation and area of the body surface irradiated. However the sample sizes are usually small and studies have not taken adequate account of factors causing individual variability, such as genetic polymorphisms and body mass index, resulting in lower discriminatory power.

We conducted a systematic review of these experimental studies in order to clarify whether and to what extent differences in skin pigmentation alter the production of vitamin D and 25(OH)D in blood following exposure to UV radiation.

Methods

Literature search

PubMed was searched for papers published before 31 December 2014, using the terms: ("skin pigmentation" or "skin colour" OR "skin type") AND ("UVR" OR "UVB") AND ("vitamin D synthesis" OR "vitamin D production"). Criteria for inclusion were: 1) *in vivo* human studies of participants without any known underlying medical conditions; 2) controlled exposure to artificial UV radiation; 3) vitamin D or 25(OH)D measured in blood samples; 4) full text in English. Due to the small number of included studies, strict criteria were not applied to evaluate the quality of the evidence.[16] The assessment of risk of publication bias was also not included.

The electronic search resulted in 63 articles. Forty were excluded after screening the titles and abstracts as they were not relevant, such as studies that examined the photoprotective effect of 25(OH)D on UV-induced damage, or the efficacy of UVB phototherapy on skin disorders. The full texts for the 23 remaining articles were obtained and four additional articles were identified from the reference lists of these papers. After screening against the inclusion criteria, 12 reports were retained (Figure 1).

Data extraction

Data were extracted from the eligible publications, including study characteristics (location, month, numbers, age and skin type/ethnicity of participants), UV irradiation (source,

wavelength, dose, frequency and area of body surface area exposed), vitamin D and 25(OH)D measurement (timing of blood sample collection, metabolite measured and assay), and results. Studies reported the units of controlled UV irradiation dose as mJ/cm^2, SED or MED. One SED is equivalent to an erythemal effective radiant exposure of 10 mJ/cm^2 (100 J/m^2), and one MED is the dose of UV irradiation required to produce just perceptible redness. For example, a dose of 20 mJ/cm^2 (2 SED) would be expected to cause barely perceptible erythema - one MED - in people with pale white skin, Fitzpatrick skin type I.[17]

Due to the substantially different methodologies across the included studies, we were unable to conduct a formal meta-analysis. We thus provide a qualitative review of the findings including grouping studies with similar findings into two summary tables.

Results and Discussion

The 12 studies analysed were conducted in the Northern Hemisphere, between latitudes 40-55ºN and, where recorded, were undertaken during the winter months. Five concluded that skin pigmentation did not influence vitamin D production (Table 1, henceforth referred to as negative) and seven that it did (Table 2, henceforth referred to as positive). In the negative group, four reports were published prior to 1991 compared with one in the positive group. The negative group contained only one study with more than 20 participants[18] while the positive group contained only two with less than 30 participants. In addition to numbers of subjects, many other parameters varied considerably between studies. These are outlined and discussed below in an effort to understand why the published results have been so inconsistent.

Skin pigmentation. Two studies (both positive) used reflectance spectrometry to define constitutive skin pigmentation at an unexposed skin site, reporting the L* metric, and assessed change in vitamin D status according to this method of skin typing. In one case, all participants were South Asian,[19] in the other,[20] ethnicity was not reported but there was a wide range of skin reflectance from readings typical of a white person to those typical of a black African. Two additional studies measured skin type objectively, but did not use this to define comparator groups.[13, 18] Three positive[13, 21, 22] and three negative [23-25] studies defined the skin type according to ethnicity. The remaining studies (two positive, two negative) used the Fitzpatrick skin type classification[17] that assesses sun sensitivity rather than skin colour specifically. Note that Farrar *et al.*[13] compared south Asian, notionally skin type V, and White, notionally skin type I-IV, groups, and Bogh *et al.*[18] compared people of dark skin (V-VI) with light skin (I-IV), rather than according to skin type categories or ethnicity *per se*. It is possible that separation according to ethnicity provides additional consistency within the groups due to a closer similarity not just of skin type, but also of genetic type.

UV sources and spectra. There was considerable variation across the studies in the UV source used and the resulting irradiation spectrum. We have been unable to obtain the exact spectral output of some of the older lamps. However, from the data available, it seems that, overall, there was a higher proportion of short-wave radiation in the lamps used in the negative group compared with the positive group. For example, the Theratkin,[24] FS-36T12,[25] FS72T12[26] and TL-12[18] sources all emit a significant amount of UVC (<290nm) radiation.[18, 27] Irradiation with short wavelength UVB and UVC results in higher maximum yield of pre-vitamin D_3 (up to 60% conversion) compared to sunlight-like exposure that lacks wavelengths below 290nm

and contains relatively more UVA (max. 10-15% conversion).[28] Moreover, Bjorn[29] has suggested that this type of radiation can induce the conversion of 7-DHC to previtamin D_3 in the superficial layers of the epidermis, above the melanocyte layer, and therefore is not affected by skin colour. In contrast, UV radiation of longer wavelength penetrates more deeply to reach the 7-DHC in the lower epidermal layers, where melanin is located in the winter months.[30] The result is a lower production of pre-vitamin D (and vitamin D metabolites) in more highly melanised skin. UVC is not present at the Earth's surface, suggesting that artificial UV sources should be filtered to omit such wavelengths when used in vitamin D studies.

The different spectra emitted by the lamps cannot fully account for all of the differences in the results. Thus both Clemens *et al.*[21] (positive) and Brazerol *et al.*[23] (negative) used the Westinghouse FS-40 lamp but with opposite findings. This may be explained by the different dosing regimes – a single dose of 54 mJ/cm^2 (positive) compared with an escalating dose biweekly for six weeks, starting at 30 mJ/cm^2 and rising to 48 mJ/cm^2 (negative). In addition the two studies by Matsuoka *et al.*[22, 26] used the FS72T12 lamp, with the same single, relatively low dose (27 mJ/cm^2) but reported opposite results, possibly related to the different way of classifying skin type (ethnicity in the positive study,[22] Fitzpatrick skin type in the negative study[26]). This may suggest that ethnicity is a better discriminator of differences in vitamin D photosynthesis than Fitzpatrick skin type, possibly because the former also takes account of modifying factors such as genotype. The combination of lamps used by Farrar *et al.*[13, 19] emit a spectrum similar to sunlight, starting from around 300 nm (with a small peak at 313 nm), as does the HOUV-A lamp used by Armas *et al.*.[20]

UV dose and frequency. Different dosing regimes were used in the 12 studies, ranging from a single dose to three-times weekly for 12 weeks. The dose was reported in standard erythema dose (SED), minimal erythema dose (MED) or as an unweighted physical dose, and was based on personal measured MED, self-reported skin type or ethnicity. Generally efforts were made to ensure that the exposure was sub-erythemal. Two methods were used to assess the effect of skin pigmentation on vitamin D production. In several studies a standard dose of UV radiation was given regardless of skin type (SED, physical dose), in which case the outcome is the difference in the concentration of a vitamin D metabolite assessed at a later time. In other studies, a dose of UV radiation according to skin type (MED) was given. Although the action spectra for erythema and vitamin D differ, the MED provides the best available estimate of skin type relevant to UV-induced production of vitamin D. In this second method, the outcome focuses more on the difference in the absolute dose of UV radiation that is required to achieve a similar change in concentration of the vitamin D metabolite. Both methods provide valid results but cannot be compared. In general, there was no consistent pattern in terms of dose across the negative vs. the positive studies that could account for the difference in the results. The use of repeated doses of irradiation will lead to progressively greater tanning, with altered distribution and increased synthesis of melanin in the epidermis, as well as thickening of the stratum corneum. Less adaptation will occur in highly pigmented skin than in lightly pigmented skin. Thus it would not be possible under these circumstances to determine the relationship between constitutive skin colour and 25(OH)D production. On the other hand, exposure to a single dose gives no information about the influence of tanning on vitamin D production.

Body area irradiated. While Barth *et al.*[31] reported that a higher level of 25(OH)D was produced if more of the body area was irradiated, Bogh *et al.*[32] found that this was only the

case for small doses of UV radiation (less than 1.5 SED). In the 12 studies reviewed here, the body area exposed to irradiation varied from 24% to full body. No consistency relating to surface area irradiated was observed which distinguished the positive and negative reports. Full body exposure, used in 8 of the 12 studies, is not usually practical in daily life.

Assessment of vitamin D and 25(OH)D. Serum or plasma levels of vitamin D and 25(OH)D were measured by HPLC, competitive protein binding, radioimmunoassay, immunoassay (Liaison) and liquid chromatography-tandem mass spectroscopy across the 12 reports, but with no systematic difference between the positive and negative studies. Two of the older negative[22, 25] and one older positive[26] studies measured only serum vitamin D, while the remaining studies measured concentrations of 25(OH)D either on serum or plasma. Vitamin D levels are low in serum, so that the inconsistent results could be due to measurement error, particularly in combination with the small sample sizes. The concentration of 25(OH)D in serum or plasma is considered the best measure of vitamin D status: it is present at levels that can be reliably assessed (nmol/L), has a half-life of about two weeks in the circulation, and takes account of both UV-induced synthesis in the skin as well as dietary intake.[33] In most locations, over 90% of systemic 25(OH)D derives from UV-induced synthesis of vitamin D in the skin.[1] The assessment of 25(OH)D is not straightforward, with care required to ensure consistent results, even when using the same assay method.[34, 35] The displacement of 25(OH)D from the DBP varies between different assays.[36] This factor could be significant as the binding affinities of 25(OH)D to the DBP may differ between phototypes. Another critical variable could be the pre-irradiation concentration of 25(OH)D. In the majority of the 12 reports, the starting 25(OH)D values, when assessed, were lower in the deeply pigmented people than in the people with fair skin. Serum 25(OH)D levels rise more quickly following UV irradiation in individuals with a low starting level compared to those with higher starting

levels.[19] In addition, in each individual there is a tendency for a plateau to be reached.[37] Whether the level of this plateau varies between people of different ethnicities or with differences in skin pigmentation is largely unknown. Therefore the UV dose used needs to be chosen carefully to ensure this plateau is not reached in the participants of different skin colour. In some cases, particularly where the UV dose was repeated several times, this could have happened. The studies by Bogh *et al.*[18] and Brazerol *et al.*[23] are possible examples.

Following an oral bolus of vitamin D, there was considerable variability between individuals in the change in 25(OH)D levels in the blood,[38] suggestive of fast and slow responders. This may also apply to UV-induced vitamin D production and changes in 25(OH)D level, lowering the discriminatory power and adding to the variability in the results, especially for small studies.

Timing of vitamin D measurement. Concentration of vitamin D or 25(OH)D was measured at baseline, and then with a wide range of intervals post-irradiation – from 1 hour to 1 week after the irradiation, on varying schedules – daily, weekly, and at various times after the completion of the irradiation schedule. Certainly more information can be gathered if the assessment takes place at various time points throughout a repeated-exposures schedule or at several times following a single exposure. It is notable that in the study reported by Bogh *et al*,[18] the measurement of serum 25(OH)D was made only at baseline and at two days after the last of four UV exposures, and not throughout. Hence, there are no data on possible differences in the rates at which final levels were reached, and whether these differed according to skin type.

Other contributing factors. The criteria used to recruit the subjects into the different groups were not provided in any of the 12 reports. The majority of the studies gave no information regarding diet, body mass index or the general health of the participants, particularly any underlying liver or renal disease. Genetic differences in the epidermal content and distribution of 7-DHC, the vitamin D receptor, the DBP, enzymes that lead to the production and degradation of the vitamin D metabolites, and the different pigment genes that control the ratio of eumelanin to pheomelanin and melanin content, were not considered, but are likely to be important.[39, 40]

Conclusions

Careful analysis of the 12 reports that were eligible for inclusion in this systematic review failed to reveal any consistent factor which could distinguish the positive and negative studies. However, on balance, we found the results of the positive studies more convincing than those of the negative studies. We thus conclude that having pigmented skin reduces the effectiveness of UV-induced production of vitamin D and 25(OH)D in blood compared with having fair skin. One possibility to partially explain the discrepant results was the type of UV source used. Those lamps that emitted a higher proportion of UV-C wavelengths tended to result in negative findings (no difference according to skin type). As such wavelengths are not found naturally at the Earth's surface, future studies should ensure that the lamps selected mimic as closely as possible the solar UV spectrum with the UV-C wavelengths filtered out.

Based on our analysis of the studies included here, the "perfect" study to generate evidence for public health messages would measure skin pigmentation objectively using a measure of skin reflectance, rather than relying on a proxy such as ethnicity, self-reported skin type, or Fitzpatrick skin type. The source of UV radiation should have a spectral output that simulates normal sunlight and the irradiation schedule should deliver suberythemal doses at a frequency likely to be relevant to normal daily living. The body area irradiated should be similarly realistic; serum 25(OH)D levels should be measured at baseline and at regular intervals during and after the UV irradiation regime, using a standardised assay with excellent performance on accuracy and precision metrics. Most current sun exposure messages for optimal vitamin D status are given for skin type II (Fitzpatrick) with multipliers for other skin types, based largely on differences in average MED across the different skin types.[41] Currently there is insufficient evidence on the effectiveness of vitamin D production for different skin types, with different doses (and spectral variation by time of day, time of year and location) of UV radiation to be able to frame any evidence-based messages on optimal sun exposure for vitamin D sufficiency.

Conflict of Interest

The authors declare no conflicts of interest

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References

1. M. F. Holick, Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease, *Am J Clin Nutr*, 2004, **80**, 1678S-1688S.

2. M. F. Holick, J. A. MacLaughlin, M. B. Clark, S. A. Holick, J. T. Potts, Jr., R. R. Anderson, I. H. Blank, J. A. Parrish and P. Elias, Photosynthesis of previtamin D_3 in human skin and the physiologic consequences, *Science*, 1980, **210**, 203-205.

3. M. F. Holick, Vitamin D deficiency, *The New England journal of medicine*, 2007, **357**, 266-281.

4. M. F. Holick, J. A. MacLaughlin and S. H. Doppelt, Regulation of cutaneous previtamin D_3 photosynthesis in man: skin pigment is not an essential regulator, *Science*, 1981, **211**, 590-593.

5. A. F. Hess, L. J. Unger and A. M. Pappenheimer, Experimental rickets in rats : Vii. the prevention of rickets by sunlight, by the rays of the mercury vapor lamp, and by the carbon arc lamp, *J Exp Med*, 1922, **36**, 427-446.

6. J. Y. Lin and D. E. Fisher, Melanocyte biology and skin pigmentation, *Nature*, 2007, **445**, 843-850.

7. M. Brenner and V. J. Hearing, The protective role of melanin against UV damage in human skin, *Photochem Photobiol*, 2008, **84**, 539-549.

8. S. S. Harris and B. Dawson-Hughes, Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women, *Am J Clin Nutr*, 1998, **67**, 1232-1236.

9. S. Nesby-O'Dell, K. S. Scanlon, M. E. Cogswell, C. Gillespie, B. W. Hollis, A. C. Looker, C. Allen, C. Doughertly, E. W. Gunter and B. A. Bowman, Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994, *Am J Clin Nutr*, 2002, **76**, 187-192.

10. A. Mithal, D. A. Wahl, J. P. Bonjour, P. Burckhardt, B. Dawson-Hughes, J. A. Eisman, G. E. Fuleihan, R. G. Josse, P. Lips, J. Morales-Torres and I. C. S. Advisors, Global vitamin D status and determinants of hypovitaminosis D, *Osteoporos Int*, 2009, **20**, 1807-1820.

11. A. C. Looker, B. Dawson-Hughes, M. S. Calvo, E. W. Gunter and N. R. Sahyoun, Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III, *Bone*, 2002, **30**, 771-777.

12. J. E. P. Rockell, C. M. Skeaff, S. M. Williams and T. J. Green, Serum 25-hydroxyvitamin D concentrations of New Zealanders aged 15 years and older, *Osteoporos Int*, 2006, **17**, 1382-1389.

13. M. D. Farrar, R. Kift, S. J. Felton, J. L. Berry, M. T. Durkin, D. Allan, A. Vail, A. R. Webb and L. E. Rhodes, Recommended summer sunlight exposure amounts fail to produce sufficient vitamin D status in UK adults of South Asian origin, *Am J Clin Nutr*, 2011, **94**, 1219-1224.

14. P. Autier, M. Boniol, C. Pizot and P. Mullie, Vitamin D status and ill health: a systematic review, *Lancet Diabetes Endocrinol*, 2014, **2**, 76-89.

15. E. Theodoratou, I. Tzoulaki, L. Zgaga and J. P. A. Ioannidis, Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials, *Brit Med J*, 2014, **348**.

16. D. L. Weed, Methodologic guidelines for review papers, *J Natl Cancer Inst*, 1997, **89**, 6-7.

17. T. B. Fitzpatrick, The validity and practicality of sun-reactive skin types I through VI, *Arch Dermatol*, 1988, **124**, 869-871.

18. M. K. B. Bogh, A. V. Schmedes, P. A. Philipsen, E. Thieden and H. C. Wulf, Vitamin D production after UVB exposure depends on baseline vitamin D and total cholesterol but not on skin pigmentation, *J Invest Dermatol*, 2010, **130**, 546-553.

19. M. D. Farrar, A. R. Webb, R. Kift, M. T. Durkin, D. Allan, A. Herbert, J. L. Berry and L. E. Rhodes, Efficacy of a dose range of simulated sunlight exposures in raising vitamin D status in South Asian adults: implications for targeted guidance on sun exposure, *Am J Clin Nutr*, 2013, **97**, 1210-1216.

20. L. A. G. Armas, S. Dowell, M. Akhter, S. Duthuluru, C. Huerter, B. W. Hollis, R. Lund and R. P. Heaney, Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: The effect of UVB dose and skin color, *J Am Acad Dermatol*, 2007, **57**, 588-593.

21. T. L. Clemens, J. S. Adams, S. L. Henderson and M. F. Holick, Increased skin pigment reduces the capacity of skin to synthesise vitamin D_3, *Lancet*, 1982, **1**, 74-76.

22. L. Y. Matsuoka, J. Wortsman, J. G. Haddad, P. Kolm and B. W. Hollis, Racial pigmentation and the cutaneous synthesis of vitamin-D, *Arch Dermatol*, 1991, **127**, 536-538.

23. W. F. Brazerol, A. J. McPhee, F. Mimouni, B. L. Specker and R. C. Tsang, Serial ultraviolet B exposure and serum 25 hydroxyvitamin D response in young adult American blacks and whites: no racial differences, *J Am Coll Nutr*, 1988, **7**, 111-118.

24. T. C. Stamp, Factors in human vitamin D nutrition and in the production and cure of classical rickets, *Proc Nutr Soc*, 1975, **34**, 119-130.

25. C. W. Lo, P. W. Paris and M. F. Holick, Indian and Pakistani immigrants have the same capacity as Caucasians to produce vitamin D in response to ultraviolet irradiation, *Am J Clin Nutr*, 1986, **44**, 683-685.

26. L. Y. Matsuoka, J. Wortsman, J. G. Haddad and B. W. Hollis, Skin types and epidermal photosynthesis of vitamin-D_3, *J Am Acad Dermatol*, 1990, **23**, 525-526.

27. A. C. Green, Premature ageing of the skin in a Queensland population, *Med J Aust*, 1991, **155**, 473-474, 477-478.

28. M. F. Holick, The photobiology of vitamin-D and its consequences for humans, *Ann Ny Acad Sci*, 1985, **453**, 1-13.

29. L. O. Bjorn, Vitamin D synthesis may be independent of skin pigmentation only with UV of short wavelength, *J Invest Dermatol*, 2010, **130**, 2848-2850.

30. T. Tadokoro, Y. Yamaguchi, J. Batzer, S. G. Coelho, B. Z. Zmudzka, S. A. Miller, R. Wolber, J. Z. Beer and V. J. Hearing, Mechanisms of skin tanning in different racial/ethnic groups in response to ultraviolet radiation, *J Invest Dermatol*, 2005, **124**, 1326-1332.

31. J. Barth, B. Gerlach, P. Knuschke and B. Lehmann, Serum 25(OH)D_3 and ultraviolet exposure of residents in an old people's home in Germany, *Photodermatol Photoimmunol Photomed*, 1992, **9**, 229-231.

32. M. K. Bogh, A. V. Schmedes, P. A. Philipsen, E. Thieden and H. C. Wulf, Interdependence between body surface area and ultraviolet B dose in vitamin D production: a randomized controlled trial, *Br J Dermatol*, 2011, **164**, 163-169.

33. M. F. Holick, Resurrection of vitamin D deficiency and rickets, *Journal of Clinical Investigation*, 2006, **116**, 2062-2072.

34. C. T. Sempos, H. W. Vesper, K. W. Phinney, L. M. Thienpont, P. M. Coates and Vdsp, Vitamin D status as an international issue: National surveys and the problem of standardization, *Scand J Clin Lab Inv*, 2012, **72**, 32-40.

35. F. Granado-Lorencio, I. Blanco-Navarro and B. Perez-Sacrsitan, Critical evaluation of assays for vitamin D status, *Curr Opin Clin Nutr*, 2013, **16**, 734-740.

36. W. D. Fraser and A. M. Milan, Vitamin D assays: past and present debates, difficulties, and developments, *Calcified Tissue Int*, 2013, **92**, 118-127.

37. A. R. Webb, B. R. Decosta and M. F. Holick, Sunlight regulates the cutaneous production of vitamin-D_3 by causing its photodegradation, *J Clin Endocrinol Metab*, 1989, **68**, 882-887.

38. R. P. Heaney, K. M. Davies, T. C. Chen, M. F. Holick and M. J. Barger-Lux, Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol, *Am J Clin Nutr*, 2003, **77**, 204-210.

39. R. Saternus, S. Pilz, S. Graber, M. Kleber, W. Marz, T. Vogt and J. Reichrath, A closer look at evolution: Variants (SNPs) of genes involved in skin pigmentation, including EXOC2, TYR, TYRP1, and DCT, are associated with 25(OH)D serum concentration, *Endocrinology*, 2015, **156**, 39-47.

40. A. Slominski and A. E. Postlethwaite, Skin under the sun: when melanin pigment meets vitamin D, *Endocrinology*, 2015, **156**, 1-4.

375 41. A. J. Samanek, E. J. Croager, P. Gies, E. Milne, R. Prince, A. J. McMichael, R. M.
376 Lucas and T. Slevin, Estimates of beneficial and harmful sun exposure times during
377 the year for major Australian population centres, *Med J Australia*, 2006, **184**, 338-
378 341.

379 42. T. C. Chen, F. Chimeh, Z. R. Lu, J. Mathieu, K. S. Person, A. Q. Zhang, N. Kohn, S.
380 Martinello, R. Berkowitz and M. F. Holick, Factors that influence the cutaneous
381 synthesis and dietary sources of vitamin D, *Arch Biochem Biophys*, 2007, **460**, 213-
382 217.

383 43. F. Libon, E. Cavalier and A. F. Nikkels, Skin color is relevant to vitamin D synthesis,
384 *Dermatology*, 2013, **227**, 250-254.

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Table 1 Summary of studies reporting that skin pigmentation does not influence the synthesis of vitamin D and 25(OH)D

Reference	Study characteristics				UV radiation				Metabolite measurement		Results
	Location	Study month	No of participants Ethnicity/skin type (Age)	Skin pigmentation measurement	UV source	UV wavelength (peak) [λ<290 nm]	UV dose/frequency	Body area	Time of blood collection	Metabolite measured and assay	
Stamp, 1975[24]	London, UK	NR	2 Asian (14 years), 3 White, 1 Black (age unknown)	No	Theratkin	290-350 nm (290 nm), [Yes]	Once per day for 24 days; mainly suberythemal	Full body	Every 2-3 days	Plasma 25(OH)D, Competitive protein-binding radioassay	Rate of increase same in all subjects
Lo et al., 1986[25]	Boston, USA	NR	6 Asian, 4 Caucasian (18-30 years)	No	FS-36T12 UVB:HO	280-450 nm (313 nm) [Yes]	1.5 personal MEDs, single dose	Full body	0,1,2,3,6,9 days after exposure	Serum vitamin D, HPLC	No difference in baseline and post-exposure serum vitamin D between Asians and Caucasians
Brazerol et al., 1988[23]	Cincinnati, USA	Jan-Feb	13 White, 7 Black (22-35 years)	No	FS-40	280-450 nm (313 nm) [Yes]	30 mJ/cm^2, 10% increase for each of the next 5 exposures, then 48 mJ/cm^2 for remaining 6 exposures, given biweekly	Full body	1 week before, weekly for 6 weeks, and 4 days after the last exposure	Serum 25(OH)D, Competitive protein-binding radioassay	25(OH)D response similar in black and white participants
Matsuoka et al., 1990[26]	Philadelphia, USA	Dec-Mar	2 I, 4 II, 6 III, 4 IV (about 20 years)	No	FS-72T12	260-330 nm (313 nm) [Yes]	27 mJ/cm^2, single dose	Full body	1 hour before, 24 hours after exposure	Serum vitamin D_3, HPLC	No difference in rate of increase in vitamin D between skin types
Bogh et al., 2010[18]	Copenhagen, Denmark	Jan - Mar	4 I, 5 II, 4 III, 5 IV, 7 V, 3 VI (19-48 years)	Reflectance meter on exposed and unexposed body sites (PPF)	TL-12	280-350 nm (300-325 nm) [Yes]	3 SEDs, 4 times in one week	24%	Baseline, 2 days after last exposure	Serum 25(OH)D, Liquid chromatography-tandem mass spectrometry	No correlations between increase in 25(OH)D and constitutive or facultative skin PPF; no difference in increase in 25(OH)D between V-VI and I-IV groups (9 matched pairs)

Abbreviations: HPLC: high-performance liquid chromatography; MED: minimal erythema dose; No: Number; NR: not reported; PPF: pigmentation protection factor; SED: standard erythema dose; UK: United Kingdom; USA: United States of America; UV: ultraviolet; λ: wavelength.
Roman numerals denote Fitzpatrick skin type.

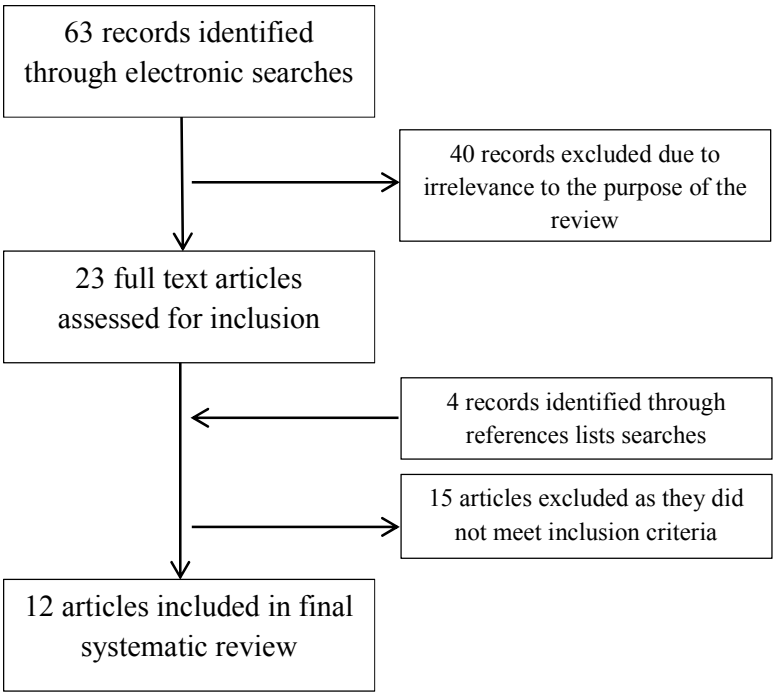
Table 2 Summary of studies reporting that skin pigmentation does influence the synthesis of vitamin D and 25(OH)D

Reference	Study characteristics				UV radiation				Metabolite measurement		Results
	Location	Study month	No of participants Ethnicity/skin type (Age)	Skin pigmentation measurement	UV source	UV wavelength (peak) [λ<290 nm]	UV dose/frequency	Body area	Time of blood collection	Metabolite measured and assay	
Clemens et al., 1982[21]	Boston, USA	NR	2 Caucasians (mean 29 years), 3 Black (mean 33 years)	No	FS-40	280-450 nm (313 nm) [Yes]	54 mJ/cm^2, single dose	Full body	Daily for 21 days after treatment	Serum vitamin D, 25(OH)D, HPLC	Vitamin D and 25(OH)D increased in Whites, no increase in Blacks
Matsuoka et al., 1991[22]	Philadelphia, USA	Nov - Feb	8 white, 8 oriental, 7 indian, 8 black (in 30s)	No	FS-72T12	260-330 nm (303 nm) [Yes]	27 mJ/cm^2, single dose	Full body	1 hour before, 24 hours after exposure	Serum vitamin D_3, HPLC	Basal serum vitamin D same in all groups, post-exposure level higher in Whites than Indians and Blacks, higher in Orientals than Indians and Blacks
Chen et al., 2007[42]	Boston, USA	Winter	II, III, IV, V, numbers and age unknown	No	Tanning bed, similar output to sunlight	NR	0.75 personal MED, 3 times per week, 12 weeks	Full body	Baseline, during and at completion of the study	Serum 25(OH)D, Competitive protein binding	Percentage increase in 25(OH)D:320 for II; 287 for III; 225 for IV, 140 for V
Armas et al., 2007[20]	Omaha, USA	Sep - Jun, 72% Nov - Mar	72 (19-49 years)	Reflective meter (L*); Upper inner arm	HOUVA II	[No]	20-80 mJ/cm^2, depending on the self-reported skin type, 3 times per week, 4 weeks	90%	Baseline, weekly for 4 weeks and then at eighth week	Serum 25(OH)D, Liquid phase radioimunoassay	The lighter the skin tone, the greater the rates of increase in serum 25(OH)D with UVB dose
Farrar et al., 2011[13]	Manchester, UK	Jan - Feb	15 V (South Asians), 109 I-IV (white) (20-60 years)	Spectrophotometer (L*) (used only to measure change in skin colour over course of irradiation)	Arimed B and Cleo Natural lamps, similar to sunlight, 95% UVA and 5% UVB	290-400 nm (313 nm) [No]	1.3 SEDs, 3 times per week, 6 weeks	35%	Weekly	Serum 25(OH)D, HPLC	Increase greater in I-IV than in V
Farrar et al., 2013[19]	Manchester, UK	Jan - Feb	10 South Asian, V, (mean 26 years)	Spectrophotometer (L*) (unexposed buttock)	Arimed B and Cleo Natural lamps, similar to sunlight, 95% UVA and 5% UVB	290-400 nm (313 nm) [No]	3.25 SEDs, 3 times per week, 6 weeks	35%	Weekly	Serum 25(OH)D, HPLC	Increase more rapidly in those with lighter skin over the first 3 weeks
Libon et al., 2013[43]	Liege, Belgium	Feb	19 II, 1 III, 11 VI, (19-41 years)	No	Waldmann 8001 k	290-320 nm [No]	22 mJ/cm^2, single dose	Full body	Before, days 2 and 6	Serum 25(OH)D, DiaSorin Liaison Total Vitamin D method	High 25(OH)D levels in fair subjects compared with Blacks on day 6

Abbreviations: HPLC: high-performance liquid chromatography; MED: minimal erythema dose; No: numbers of participants; NR: not reported; SED: standard erythema dose; UK: United Kingdom; USA: United Sates of America; UV: ultraviolet.; λ: wavelength

Roman numerals denote Fitzpatrick skin type

Figure 1 Flow chart of literature search



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