Vitamin D3 Synthesis: A self-limiting process in natural sunlight.

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Introduction

Our understanding of the process by which UVB exposure enables almost all vertebrate animals to synthesise vitamin D3 has developed steadily since the pioneering work of Dr. Michael Holick and his colleagues at the beginning of the 1980s. We now have a fairly clear picture of the process and of the role vitamin D3 plays in calcium metabolism; but we are only just beginning to understand the importance of vitamin D3 intracellularly: its involvement in gene transcription and cell signalling. Vitamin D is not really a vitamin; it is a seco-steroid hormone, with multiple functions throughout the body.

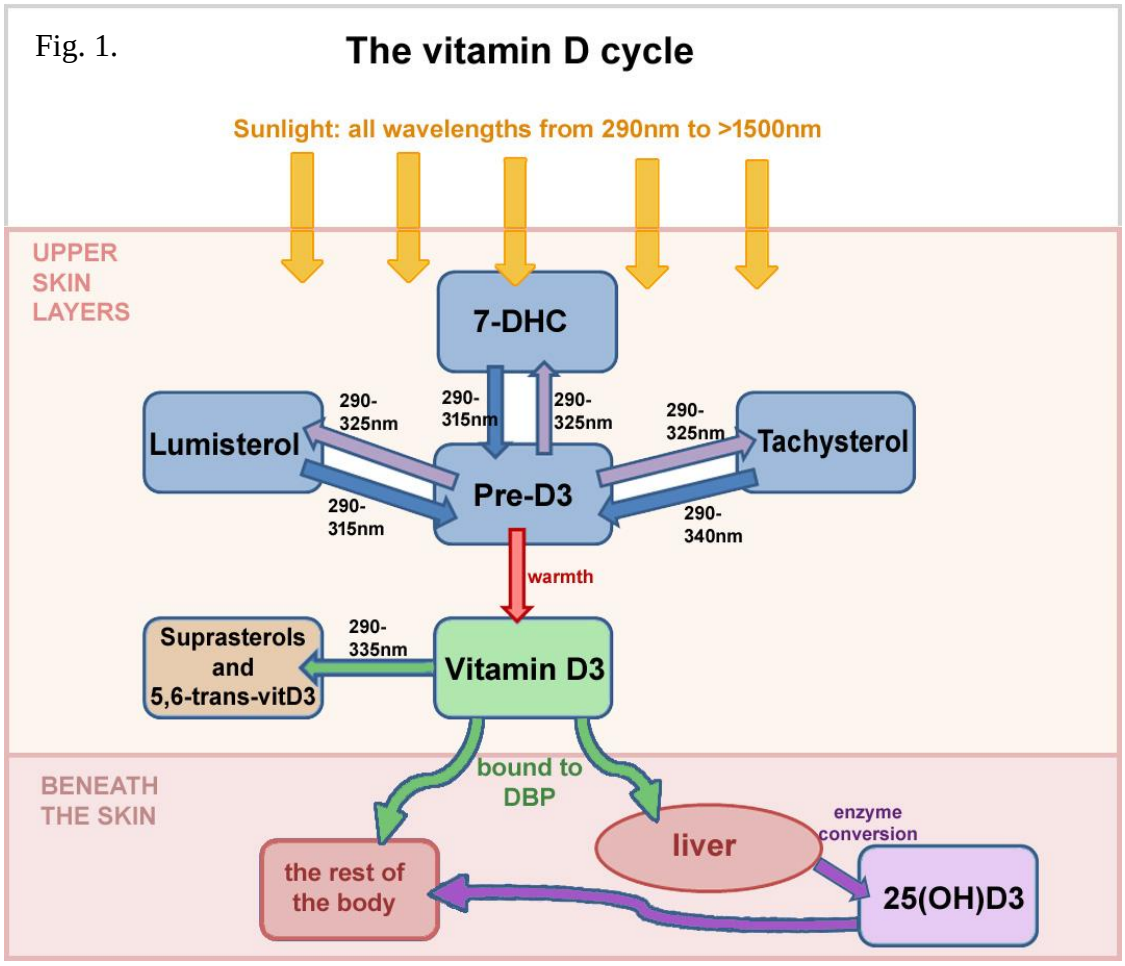
The well-known, simple version of the first part of the vitamin D3 pathway, first described by Holick *et al*. in 1980, goes like this: 7-Dehydrocholesterol (7DHC) is a cholesterol produced in the skin, which when exposed to UVB radiation, undergoes a photo-isomerisation to the precursor of vitamin D, pre-D3. Warmth causes pre-D3 to undergo thermal isomerization to vitamin D3, which then attaches to vitamin-D-binding protein and enters the bloodstream, to be carried away from the skin.

However, the researchers found that this could not be the whole story, because no matter how long the human skin samples were irradiated, the synthesis of previtamin D3 never used more than about 10 to 15 percent of the original 7-DHC present in the sample. Some sort of "feedback loop" was preventing overproduction of vitamin D3.

The molecular sun-dance

This was first described by Holick *et al*. in 1981, and the process elucidated by MacLaughlin *et al*., in the same laboratory, in 1982. The "feedback loop" effect is a photo-regulation, due to a fine balance between four photo-isomers of 7-DHC (pre-D3, tachysterol, lumisterol and 7-DHC itself) with which the different wavelengths of UV in sunlight interact, transforming one into another, forming an unstable equilibrium of all four isomers in the skin. The proportion of each of these 'photoproducts' depends upon the spectral power distribution of the sunlight, i.e., the proportions of each wavelength present – and this depends upon the height of the sun in the sky. Figure 1 is a flow-chart to illustrate the process.

Fig. 1.



When UVB photons between 290nm and 310nm reach a molecule of 7DHC, this absorbs the energy and this causes a photochemical change in the molecule, to pre-D3. In a warm environment, this additional heat energy can further change the configuration of the pre-D3 molecule to vitamin D3. Alternatively, the pre-D3 molecule can absorb more UVB and short-wavelength UVA photons (between 290nm and 335nm) and its configuration can then change again, either back to 7-DHC or forward into either Lumisterol or Tachysterol.
UVB between 290nm and 310nm can simultaneously re-configure the Lumisterol and 7-DHC back to pre-D3; UVB between 290nm and 335nm can likewise simultaneously re-configure the Tachysterol back to pre-D3. All these reactions happen together under sunlight; the molecules dance in perpetual motion, and after a while, under constant solar UVB illumination, an equilibrium forms. The amounts of each substance depend upon four things: the amount of 7-DHC present, the UV spectrum of the sunlight, the irradiance (intensity) of the UV that reaches the 7-DHC, and the warmth of the skin (which determines the rate of conversion of pre-D3 to vitamin D3, thus removing it from the 'dance'.).

The photochemical conversions to and from pre-D3, as shown in Fig. 1., have slightly different energy requirements. For example, although the shortest wavelengths found in sunlight, below 300nm, are able to transform all the molecules in either direction, the longer wavelengths, between 310nm and 335nm, cannot be absorbed by 7-DHC and Lumisterol, so these wavelengths cannot enable either of these to transition to pre-D3.
Figure 2 shows the UV absorption spectra of the four isomers, illustrating the ability of pre-D3 and Tachysterol to absorb photons up to 325nm and 340nm, and thus be transformed by these longer wavelengths as well.

Fig. 2.

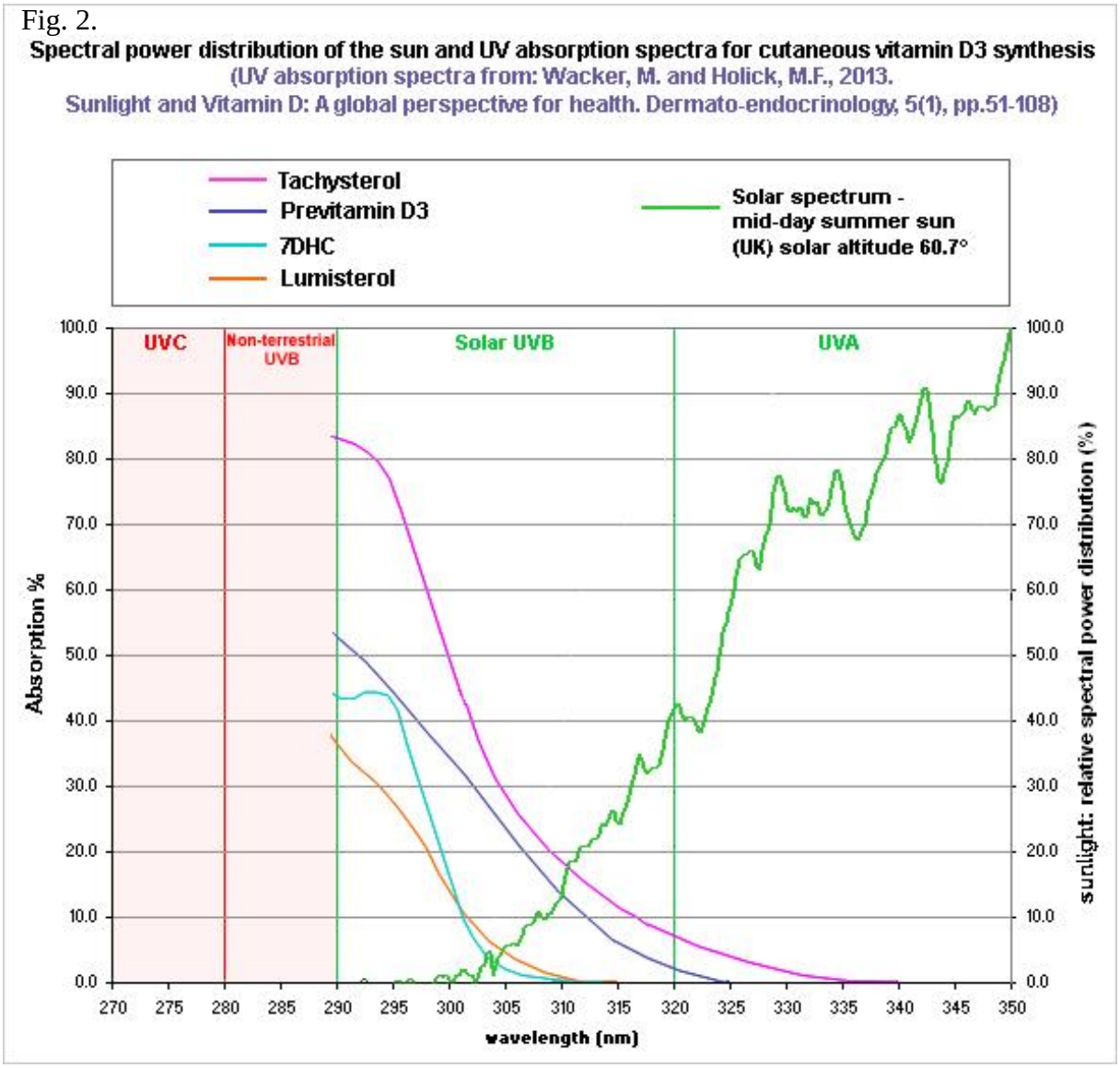


Figure 2 also shows the solar spectrum (overlaid, green trace) for sunlight taken at mid-day in June in the UK, with a solar elevation of just over 60 degrees; this is approximately the height of the sun in the sky around 10.00am on the equator. When this spectrum was collected, the UV Index was 6.4.

Considering the inter-relationship between the solar spectrum and the extinction co-efficients for the four isomers makes it possible to understand how overproduction of vitamin D3 is prevented. Holick *et al.* (1981) noted that "during prolonged exposure to the sun, the synthesis of preD3 reaches a plateau at about 10 to 15 percent of the original 7-DHC concentration … Tachysterol formation also plateaus at about 5 percent, whereas Lumisterol formation continues to increase."

First, let's consider the 60-degree sun providing fairly strong sunlight with a UVI of 6.4 and the spectrum shown in Fig. 2. The shortest wavelengths are around 295nm, and the irradiance increases steadily with increasing wavelength.

There is only a small amount of radiation below 310nm, but the irradiance is strong, so a significant amount of 7-DHC will be converted to pre-D3. If the skin is warm, some of this will undergo the temperature-dependent transformation to vitamin D3; however, this is not a rapid process, and before some of the molecules have been transformed, the UVB will further convert a proportion of them to Lumisterol and Tachysterol, and convert some of them back to 7-DHC. Since there is considerably more UVB radiation from the wavelengths above 310nm than from below it, and even the shortest wavelengths can also promote further conversion, the chances are high that any given pre-D3 molecule will undergo one of these secondary conversions before it can be transformed to vitamin D3.

However, the "dance" does not stop there.

If it was converted to Tachysterol, any radiation up to 340nm can convert it back again to pre-D3. Since there is plenty of this radiation in our example, molecules will likely switch between pre-D3 and Tachysterol easily, and little Tachysterol will accumulate.

However, if it was converted to Lumisterol, only wavelengths below 310nm can convert it back to pre-D3. Since there is more radiation above 310nm than below it, more will be produced than can be converted back to pre-D3; hence Lumisterol will accumulate in the skin.

If the pre-D3 molecule was converted back to 7-DHC, more radiation below 310nm will likely convert it to pre-D3 again, and the "dance" will go on.

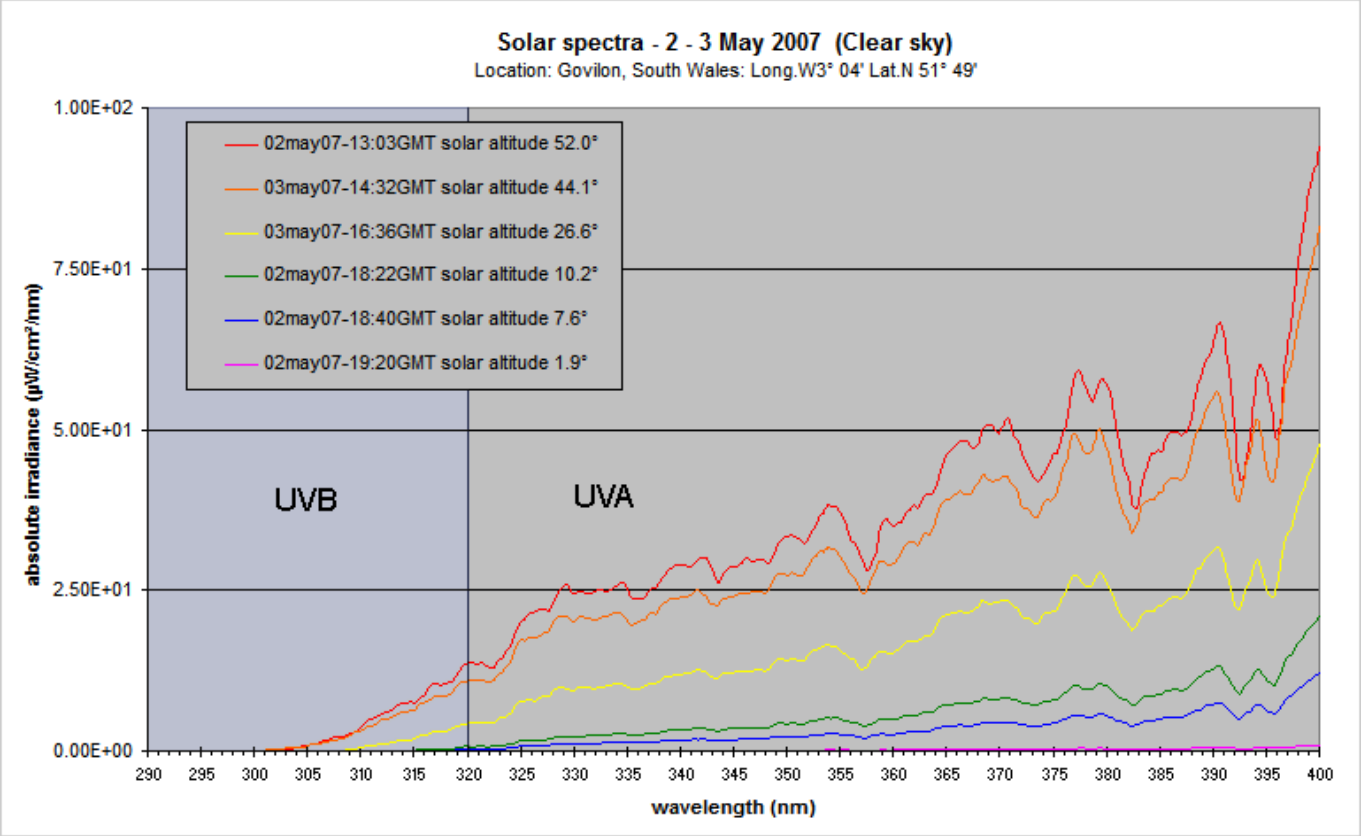
This explains why, at the end of a period of exposure to this irradiance, 7-DHC levels have not been greatly reduced – the only "losses" are from photoconversion to Lumisterol, which has accumulated in the skin, and from any pre-D3 which underwent thermal conversion to vitamin D3 and was carried away into the bloodstream.

Sunrise, sunset

Things get very interesting, however, when the spectrum – either of sunlight at different times of the day, or of UVB lamps – has different proportions of short-wavelength and long-wavelength UVB.

The figure below (Fig.3.) shows a series of UV spectra for the sun at different solar altitudes. The greatest irradiance, including the shortest wavelengths, occurs at solar noon, when the sun is highest in the sky and the rays have the shortest distance to travel to the earth's surface. The closer the sun gets to the horizon, the thicker the atmosphere, which not only reduces the irradiance, but also filters out and scatters the shorter wavelengths, the very shortest, in the UVB range, being lost altogether. In the example shown in Fig. 3, the shortest wavelength at solar noon (12:15 GMT in Wales, UK) is around 300nm. By 16:30 GMT, it has fallen to 308nm; by 18:40h it is only 315nm, and a few minutes before sunset, at 19:20h it is 354nm. As the irradiance below 310nm is reduced, and finally lost altogether as sunset approaches, the rate of conversion of 7-DHC and Lumisterol falls and finally fails altogether, but pre-D3 can still be formed from any remaining Tachysterol, and this either reverts to 7-DHC, adds to the reservoir of Lumisterol, or becomes vitamin D3 via thermal isomerisation, which may continue for several hours after sundown if the skin is still warm (Andreo 2015; Cisneros *et al*. 2017). The 7-DHC and Lumisterol remaining in the skin are then ready for recycling into pre-D3 as soon as wavelengths below 310nm appear in the solar spectrum the next day

Fig. 3. The effect of solar altitude upon the spectral power distribution of sunlight.



This molecular dance results in much smaller quantities of pre-D3 achieving thermal conversion to vitamin D3 during UVB exposure than might occur if the buffering effects of Lumisterol and Tachysterol formation did not occur, and ensures that even under very high irradiances, the skin is not flooded with pre-D3.

Once formed, molecules of vitamin D3 are removed by vitamin-D-binding-protein and carried from the very superficial skin layers, where they form, deeper into the skin and into the bloodstream, out of range of all UV radiation. However, if there is so much vitamin D3 being produced that the capacity for removal by vitamin-D-binding protein is exceeded, another process also prevents overproduction. This is the destruction of the excess vitamin D3 molecules left behind in these very superficial skin layers by the very same wavelengths that are involved in its synthesis (290nm – 335nm). They are converted to inert substances, suprasterols and 5,6-transvitamin D3, which remain in the upper skin layers and are eventually shed with the dead skin cells. (See: Webb *et al.* 1989.)
Of course, cells in the skin also “consume” vitamin D3 for a wide range of functions, converting it intracellularly into the active hormone used in gene transcription and inter-cellular signalling before metabolising it to inactive waste products. Epidermal skin cells also use enzymes to break down vitamin D3 into several newly-recognised secosteroids which actively protect cells against oxidative damage from UVB (Slominski *et al*. 2015).

What happens next…
Once the vitamin D3 has been removed from the most superficial layers of the skin, some enters the living skin cells below and is metabolised within them to the active hormone. Most is carried further, into the bloodstream, and is distributed to all organs of the body. Some is taken up by their cells and is similarly metabolised; some is simply stored in fat cells; a large proportion reaching the liver is converted by enzymes in liver cells to the storage form, 25(OH)D3. This is released into the bloodstream, and although some of this also enters fat cells and is stored there too, most remains primarily in the blood and supplies all organs, in particular the kidneys, which have a constant requirement of very small amounts for enzymatic conversion to the active endocrine hormone 1,25(OH)D3, vital for maintaining calcium homeostasis.

Does weak sunlight "destroy vitamin D"?
Occasionally, concerns are raised about the possibility of the UV in sunlight – in particular the longer wavelengths in the range 310nm to 335nm – breaking down the body's vitamin D3 stores when skin is exposed only to these longer wavelengths, for example, in winter in the Northern Hemisphere when the shorter wavelengths creating pre-D3 are absent from the sunlight.
However, this is very unlikely to occur. Only excess vitamin D3 remaining in the most superficial skin layers is destroyed; anything taken into the circulation is very rapidly taken up by living cells throughout the body, converted to 25(OH)D3 or metabolised into waste products. The half-life of vitamin D3 in the body is only 24 hours. 25(OH)D3 is extremely stable, with a half-life of around two weeks (in humans, at least) and this storage form of the vitamin cannot be destroyed by UV radiation even if it is exposed to it when blood flows through fine capillaries near the surface of the skin.

Artificial sources of UVB – a risk of overproduction?
The molecular dance, and the resulting quasi-equilibrium of photoproducts, evolved under the solar spectrum and as we have seen, the amount of vitamin D synthesised depends upon the spectrum, and is limited by both the irradiance (the intensity of the sunlight) and the proportion of wavelengths above and below 310nm. As long ago as 1982, MacLaughlin *et al*. described a significant increase in vitamin D3 yield from 7-DHC in skin samples when irradiated with a narrow UVB waveband (restricted to between 290 and 300nm) rather than a spectrum similar to sunlight or sunlight itself, and demonstrated that this was due to the lack of longer wavelengths creating lumisterol and tachysterol and "buffering" the system. The clinical benefits of this effect are only now being utilised, however, with the development of narrow-band UVB lamps for the treatment of vitamin D3 deficiency in humans. These lamps have been designed to enable rapid, strong vitamin D3 synthesis, with the shortest exposure time possible, essential in a clinical setting. These lamps can produce high yields of vitamin D3 with brief exposures, owing to the loss of much of the normal "buffering". See: Barnkob *et al.* 2016; Morita *et al.* 2016; Kalajian *et al.* 2017; Veronikis *et al.* 2020 and Lin *et al.* 2021.

Of course, for treatment regimes, this is desirable. But reptile UVB lamps are used to create the effect of natural full spectrum sunlight in the vivarium, with modest levels of UV offered in a basking zone along with sources of visible light and infrared, provided for full daylight hours (typically 10 – 12 hours per day). Lamps with spectra in the UVB and short-wavelength UVA range which are similar to sunlight should not cause overproduction of vitamin D3, as the "buffering" will be maintained. The UV Index, a measure of the photoreactivity of sunlight on human skin, has been developed as a useful guide to suitable, "natural" UV ranges for creating safe basking zones. For example, UVI 4.0 in the basking zone is often suggested as appropriate for bearded dragons, following research on wild, free-living animals in Australia (Howard, 2019) demonstrating UVI 4.0 as a preferred exposure level.

UVB LEDs – a new problem?
The UV Index was designed to measure the effects of a natural solar spectrum, not one only containing wavelengths driving vitamin D synthesis with "no buffer". An early trial with UVB LEDs conducted using a ZooMed prototype (Cusack *et al.* 2017) demonstrated that exposure to UVI averaging 0.24 (extremely low) created high serum 25(OH)D3 levels in bearded dragons, whereas, unsurprisingly, a traditional compact UVB lamp with a UVI average 0.92 (nearly 4 times higher, but still very low) and a spectrum in the UV range similar to sunlight did not raise serum 25(OH)D3 levels at all, over the test period of 11 months. This study in particular is alarming, since a UVI of 0.24 would not be expected to enable any increase in serum 25(OH)D3 levels in bearded dragons at all! The risk of hazardous uncontrolled vitamin D3 synthesis from these lamps under apparently modest UVI cannot be ignored; and whether the UV Index can be used as a measure of vitamin D3 synthetic ability is in serious doubt with these very un-natural UVB spectra.

No long-term studies of blood levels of vitamin D3 have yet been conducted with these new LED lamps, either with animals maintained under UVI levels recommended by the manufacturers or as indicated by the Ferguson Zones (See: Baines *et al.* 2016). For sun-basking species these UVI recommendations are far higher than used in the trials conducted by Cusack *et al.* The question that remains unanswered is: will these lamps cause excessive synthesis and even hypervitaminosis D? In theory, they certainly should!
It is essential that properly controlled trials are set up, with significant numbers of animals and preferably including several different species. These must include blood tests for 25(OH)D3 measurements before, during and after long-term daily lamp use, using the LC-MS/MS chromatographic method, not an immunoassay as those appear to be very inaccurate with reptile bloods owing to cross reactivity with different vitamin D metabolites, DBP and other factors (See: Hurst *et al.* 2020).
Other measurements should include serum calcium, ionised calcium, vitamin D3, parathyroid hormone and 1,25(OH)D3 to assess calcium metabolism, since hypercalcaemia is the primary diagnostic feature of hypervitaminosis D as well as the cause of its toxicity. Unfortunately, the symptoms of hypercalcaemia are mild, insidious and non-specific, variously described as lethargy, anorexia, increased thirst, muscle weakness and lameness. In addition, high serum calcium levels are normal in female reptiles during folliculogenesis, which may confuse the issue. Mobilisation of calcium from bone results from increased oestrogen activity and calcium levels return to normal after egg laying.

UVB Overexposure
When any artificial source of UVB is used, especially when the lamps are new or the irradiance is unknown, animals should be monitored regularly for any sign of UVB damage to skin and eyes, since this is a symptom of acute over-exposure to high UVB. The cornea (or spectacle of snakes and geckos) is usually affected first. Photo-kerato-conjunctivitis presents as an opacity or lesion on one or both corneas, causing intense pain. Affected animals will become immobile and depressed. Those with eyelids will keep the eyes permanently closed to reduce the pain. The eyelids often become swollen and their skin may appear burned. In young animals, especially, death may result from shock and dehydration following the debilitating blindness. More severe overexposure causes UV radiation burns to the skin of the rest of the body as well. Milder burns resemble dysecdysis; more severe damage will form blisters and layers of dead skin which may slough. (See: Gardiner *et al.* 2009.)

What about Vitamin D3 supplements?
Although a discussion on the use of vitamin D3 supplements is outside the remit of this article, it should be noted that there is always said to be a risk of overdose with oral supplements, since there is no limiting pathway or buffering system preventing the absorption of pre-formed vitamin D3 from the gut. The vitamin D3 is largely absorbed in chylomicrons (tiny fat droplets) rather than D-binding protein, and these are processed by the liver or sequestered in fat, so most is quickly metabolised and has an even shorter half-life in the circulation. The content of vitamin D3 is very low in most of the powdered supplements used to dust feeder insects, so if used properly there is very little risk of toxicity. However, if supplements were to be used extensively alongside strong UVB lamps, in particular ones with narrowband UVB such as the new LEDs, could this become a problem?

Hypervitaminosis D
Toxic levels of vitamin D3 have proven hard to determine and almost certainly vary with species. In some cases spurious results may have arisen owing to the tests used, as mentioned above. Nevertheless, extraordinarily high serum 25(OH)D3 levels with no signs of toxicity have been reported in blood samples from both wild and captive animals from several species. For example, apparently healthy green iguanas living outdoors in Honolulu Zoo were reported to have serum 25(OH)D3 levels exceeding 400 ng/ml (Allen and Oftedal 2003), wild Ricords iguanas averaged 147 ng/ml (Ramer *et al.* 2002) and veiled chameleons in captivity under UV lighting given additional oral vitamin D, vitamin A and calcium supplements, with serum

25(OH)D3 exceeding 100 ng/ml, thrived best of all animals in a trial (Hoby *et al.* 2010). It may be difficult to overdose reptiles with vitamin D3, even if given orally. Confirmed cases of vitamin D toxicity in reptiles are almost non-existent. Hypercalcaemia is very rarely diagnosed and the few case reports (eg. Frye *et al.* 1991) suggest it follows treatment of reptiles for metabolic bone disease (MBD) which have pre-existing hyperparathyroidism resulting from prolonged *hypo*calcaemia as a result of calcium or vitamin D deficiency or renal failure. The hyperactive parathyroid gland is refractory to negative feedback from serum calcium and vitamin D levels, and continues secreting parathyroid hormone, stimulating continued removal of calcium from bone. Treatment for MBD with calcium and vitamin D then results in worsening hypercalcaemia, which can result in renal failure, metastatic calcification and death. However, contrary to popular belief, metastatic calcification does not necessarily indicate vitamin D toxicity. Allen and Oftedal (2003) described extremely low circulating concentrations of 25(OH)D3 in green iguanas with extensive calcification of soft tissues, pathologic fractures of long bones and demineralized bone with no indication of high PTH levels. They suggested that soft tissue calcification might reflect vitamin D deficiency rather than toxicity. They wrote, "Given this evidence, it is not appropriate to diagnose vitamin D toxicity when soft tissue calcification is seen in reptiles unless supporting evidence such as very high dietary vitamin D and circulating calcidiol (25(OH)D3) levels are available."

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