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Vitamin D status and sun exposure in India

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Background: Little if any cutaneous production of vitamin D_3 occurs at latitudes above and below 35° N and 35° S during the winter months. It was postulated that those residing in tropics synthesize enough vitamin D_3 year round. Several studies have documented the effect of latitude, season and time of the day on the cutaneous production of vitamin D_3 in an ampoule model. Studies from India have shown high prevalence of vitamin D deficiency despite abundant sunshine.

Results: The percent conversion of 7-DHC to previtamin D_3 and its photoproducts and formation of previtamin D_3 and vitamin D_3 was maximal between 11 a.m. to 2 p.m. of the day during the entire year (median 11.5% and 10.2% respectively at 12.30 p.m.).

Methods: We studied the influence of season and time of the day on synthesis of previtamin D_3 in an ampoule model in Tirupati, (latitude 13.40° N and longitude 77.2° E) south India, between May 2007 to August 2008. Sealed borosilicate glass ampoules containing 50 μg of 7-DHC in 1 ml of methanol were exposed to sunlight hourly from 8 a.m. until 4 p.m. The percent conversion of 7-DHC to previtamin D_3 and its photoproducts and the percent of previtamin D_3 and vitamin D_3 formed was estimated and related to solar zenith angle.

Conclusions: Therefore at this latitude exposure to sunlight between the hours of 11 a.m. and 2 p.m. will promote vitamin D production in the skin year round.

Introduction

Casual exposure to solar radiation wavelengths 290–315 nm results in the cutaneous production of previtamin D_3.[1] During sun exposure the UVB photons (290–315 nm) that enter the epidermis cause a photochemical transformation of 7-dehydrocholesterol (7-DHC) (provitamin D_3) to previtamin D_3. Previtamin D_3 exists in two conformeric forms cis,cis (CZC) and cis,trans (CZT). The S-cis,S-cis-previtamin D_3 (CZC) rapidly undergoes conformational change to the thermodynamically more stable S-trans,S-cis-previtamin D_3 (CZT).[2] However the thermodynamically less stable CZC conformer is the only form of previtamin D_3 that can convert to vitamin D_3. The 7-DHC is incorporated into lipid bilayer. During exposure to solar UV radiation (wavelengths 290–315 nm) 7-DHC in the triglyceride portion of the plasma membrane is converted only to czc-previtamin D_3, which then rapidly converts into vitamin D_3 at body temperature. Once formed the more stable vitamin D_3 is sterically altered and is ejected from plasma membrane into extracellular space. At body temperature it takes 12 h for ~99% of previtamin D_3 to be converted to vitamin D_3. The previtamin D_3 formed is also photolabile and excessive sunlight exposure results in its photoisomerization to at least two biologically inert products, lumisterol and tachysterol.[2]

The ability to synthesize previtamin D_3 is affected by latitude, rotation of earth about the sun (season) and its own axis (day and night)—time of day. Atmospheric pollution attenuates solar radiation. Dress code, skin pigmentation, and application of sun protection factor (SPF) of 15 reduces the UVB penetration into epidermis by > 95%, thereby limiting the production of previtamin D_3 by the skin. With age the cutaneous 7-DHC levels decline, reducing the skin's capacity to produce vitamin D_3. With the increase in solar zenith angle in winter (sun angle becomes more oblique), more UVB photons are absorbed by the stratospheric zone, and therefore very few of the UVB photons can penetrate to earth's surface to produce cutaneous previtamin D_3. Thus the amount of UVB radiation reaching earth's surface is a function of solar zenith angle, time of day, season of the year, amount of ozone, cloud, aerosols, and latitude and altitude, which all influence the cutaneous production of vitamin D_3.[3] Chen et al.[4] reported little if any cutaneous production of previtamin D_3 at latitudes above and below 35° N and 35° S during the winter months. For latitude above 51° (north and south of equator) UV index is less than 0.5 in winter months. Any casual exposure to sunlight will not result in any appreciable vitamin D_3 synthesis during these periods and is called "vitamin D winter."[5] Several studies have documented the effect of latitude, season time of the day and altitude on the cutaneous production of vitamin D_3.[5] It has been assumed that those residing in tropics can produce enough vitamin D_3 in the skin throughout the year.[6] Recent studies from India have shown high prevalence of vitamin D deficiency both in rural and urban

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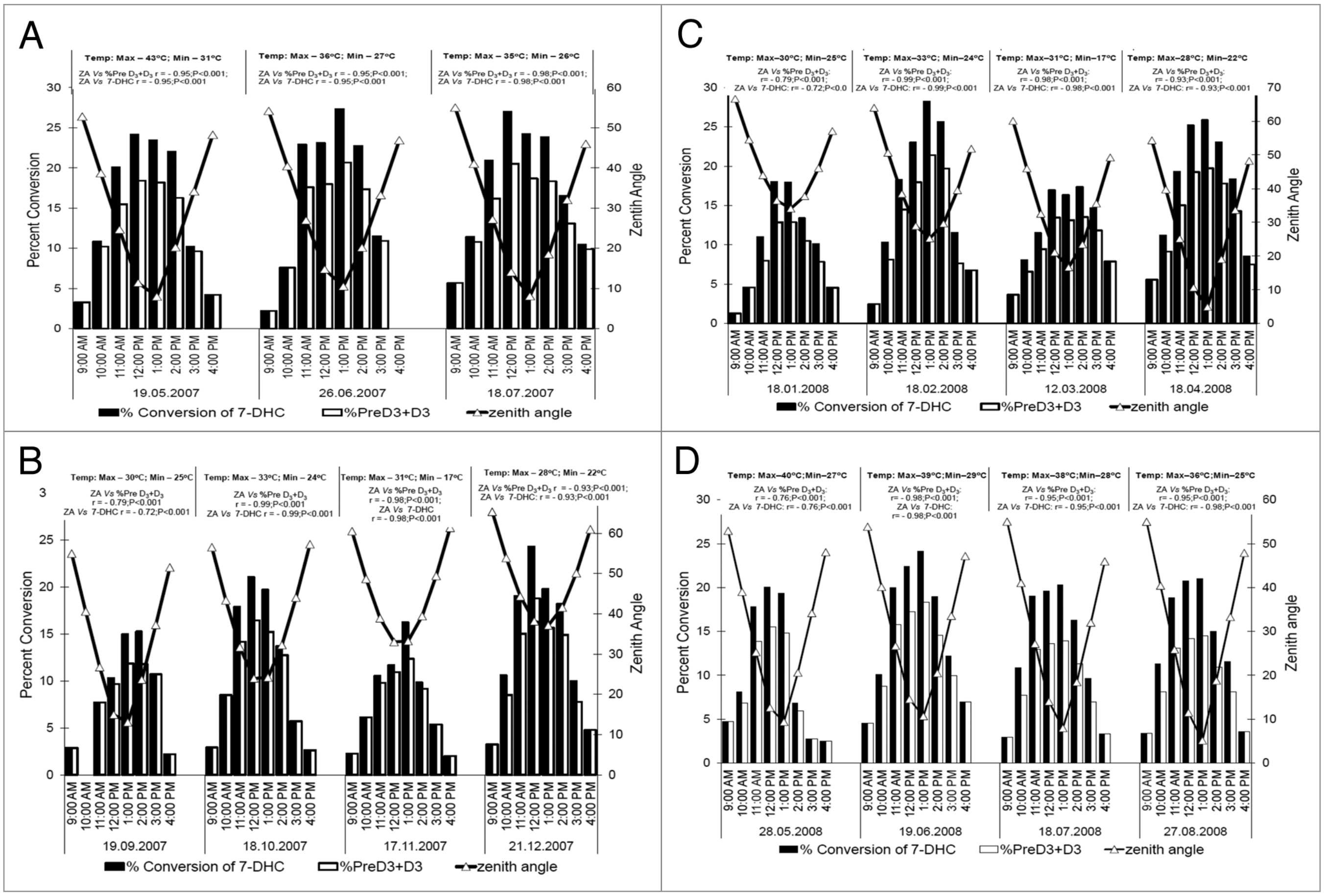


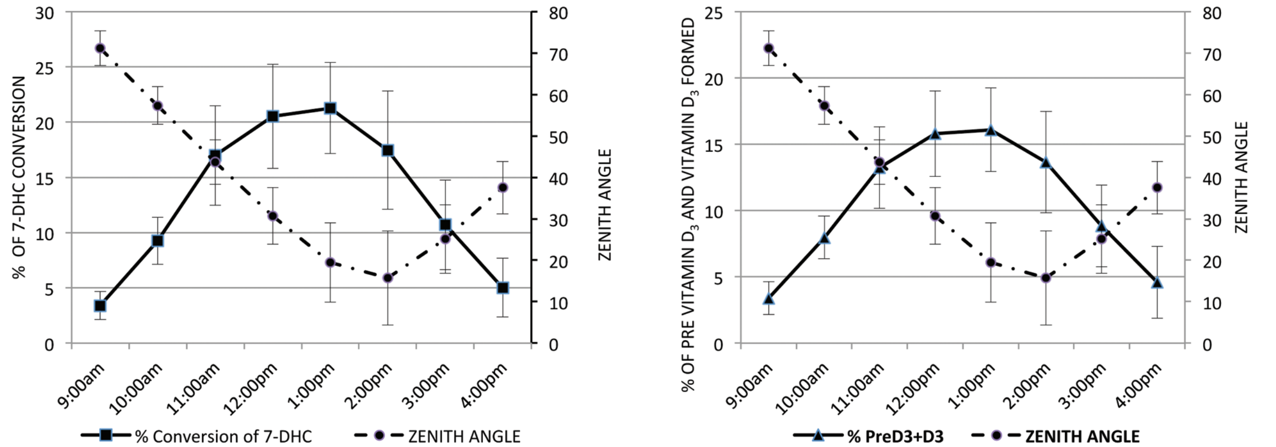
Figure 1. Influence of time of day and season on synthesis of previtamin D_3 at Tirupati located at 13.4° N and 79.2° E. Figure shows the relation between zenith angles (ZA), percent conversion of 7-Dehydrocholesterol (7-DHC) to previtamin D_3 and photoproducts, and the percentage of previtamin D_3 and vitamin D_3 (%Pre D_3+D_3) formed from May 2007 to August 2008. The correlations between them are shown individually for each day. The maximum (max) and minimum (min) temperature (Temp) on the day of the study is given above each day studied. August 2007 is not depicted. r, correlation coefficient; P, significance.

populations, in north and south India.[7] It has been shown in population surveys from south India (Tirupati latitude 13.40° N and longitude 77.2° E) that rural populations, who are agricultural laborers exposed to sunlight for more than 4 h with at least 35% of their body surface area exposed to sunlight, also have vitamin D deficiency.[8] There is no study that has determined the effect of time of day and season in India on previtamin D_3 synthesis. Hence we evaluated the influence of season and time of the day on synthesis of previtamin D_3 in an ampoule model in Tirupati, south India.

Results

The relationship between solar zenith angle, the percent conversion of 7-DHC to previtamin D_3 and its photoproducts, lumisterol and tachysterol and the percentage of previtamin D_3 and vitamin D_3 formed, along with the correlation coefficient r and the p values of significance are given against each day studied, is shown in **Figure 1A–D**. The maximum and minimum temperatures on the day of the study are depicted against each day. There was a strong negative correlation between zenith angle and percent conversion of 7-DHC to previtamin D_3 and vitamin D_3 for the whole duration of the study.

The hourly data obtained in a time dependent fashion from morning to evening for the whole duration of the study were analyzed together. The mean ± SD of hourly data and percent conversion of 7-DHC to previtamin D_3 and its photoproducts and vitamin D_3 is shown in **Figure 2**. There was negative correlation between the zenith angle and percent conversion of 7-DHC to previtamin D_3 and its photoproducts ($r = -0.84$; $p < 0.0001$) and zenith angle and percent of previtamin D_3 and vitamin D_3 formed ($r = -0.83$; $p < 0.0001$). With decreasing zenith angle, the percent conversion of 7-DHC to previtamin D_3 and vitamin D_3 was higher. At noon and 1 p.m. there was 7-fold higher conversion of 7-DHC to previtamin D_3 and its photoproducts, lumisterol and tachysterol, and five times more previtamin D_3 and vitamin D_3 formed compared 9 a.m. to 10 a.m. The data was analyzed together with zenith angle and the percent of previtamin D_3 and vitamin D_3 formed for the whole duration of the study (**Fig. 3**). A linear regression model was made to estimate the influence of angle on the production of previtamin D_3 and vitamin D_3 (product). The regression model gave the following results:



TIME	ZENITH ANGLE °				% CONVERSION OF 7-DHC				% PREVITAMIN D_3 AND VITAMIN D_3 FORMED			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
9:00am	57	5	53	67	3.40	1.25	1.27	5.68	3.40	1.25	1.27	5.68
10:00am	44	5	39	55	9.25	2.13	4.57	11.44	7.97	1.61	4.57	10.80
11:00am	31	7	25	44	16.98	4.50	7.69	22.91	13.24	3.08	7.69	17.59
12:00pm	20	10	11	38	20.50	4.70	10.30	26.97	15.78	3.21	9.68	20.50
1:00pm	17	11	5	37	21.26	4.11	14.96	28.22	16.08	3.16	11.86	21.40
2:00pm	26	8	18	42	17.46	5.37	6.82	25.62	13.65	3.83	5.97	19.71
3:00pm	38	6	32	50	10.71	4.06	2.76	18.34	8.83	3.08	2.76	14.29
4:00pm	51	5	46	61	5.02	2.65	2.02	10.44	4.91	2.47	2.02	9.86

Figure 2. Showing the mean ± SD of the zenith angles, percent conversion of 7-Dehydrocholesterol (7-DHC) to previtamin D_3 and photoproducts, and the percentage of previtamin D_3 and vitamin D_3 against time (for the study duration). The table below gives the individual values, minimum and maximum of the variables.

Previtamin D_3 and vitamin D_3 formed (Y) = 20.466 - 0.285*Zenith angle (X).

Keeping other parameters constant, a decrease in the angle by one degree, led to an increase in the product of 0.285 units. This model was also found to be significant; the p-value was almost zero in the ANOVA ($p < 0.0001$). In the above model the correlation between the percent of previtamin D_3 and vitamin D_3 formed and solar zenith angle was 0.813 and the R-square value was 0.66 and $p < 0.0001$, which denotes that about 67% of variation in previtamin D_3 and vitamin D_3 formed can be attributed to the zenith angle. The regression coefficient was also significant ($p < 0.0001$). It can also be observed from **Figure 3** that the percent of previtamin D_3 and vitamin D_3 formed is maximum when the zenith angle is minimum. Since the zenith angle derived, also incorporates the year, month and time of the day, the model is integrated with these parameters. We substituted the know zenith angles and derived the percent of previtamin D_3 and vitamin D_3 formed using the above equation. The results were not different with a p = 0.76 (NS).

The percent conversion of 7-DHC to previtamin D_3 and its photoproducts and percent of previtamin D_3 and vitamin D_3 formed was maximal between 11 a.m. to 2 p.m. when the zenith angle was at its minimum (**Fig. 3**). The minimum zenith angle observed was on the April 18, 2008 at 1 p.m. with an angle of 5°. At this time 28.9% of 7-DHC was converted to previtamin D_3 and vitamin D_3 and an additional 19.7% was converted to lumisterol and tachysterol. At a maximum zenith angle of 67° on the January 18, 2008 at 9 a.m. only 1.3% of 7-DHC was converted to previtamin D_3. At 1 p.m. on the same day (January 18, 2008) the Zenith angle was 34° with 18% 7-DHC conversion and 13% being previtamin D_3 and vitamin D_3 formed. The median percent conversion of 7-DHC to previtamin D_3 and its photoproducts was 11.5% with 10.2% being previtamin D_3 and vitamin D_3 formed at a zenith angle of 37° at 12.30 p.m. The maximum temperature on the day of study during the whole period is depicted along with zenith angle, percent conversion of 7-DHC to previtamin D_3 and its photoproducts and percent of previtamin D_3 and vitamin D_3 formed in the three-dimensional **Figure 4**.

During the study time of September 2007 and March 2008 when there were thick overcast clouds the zenith angle was less than 25°. The percent conversion of 7-DHC to previtamin D_3 and its photoproducts and previtamin D_3 was significantly reduced (**Figs. 4 and 5**). The maximum percent conversion of 7-DHC (> 20%) and percentage formation of previtamin D_3 (> 18%) occurred from December to July with exception of the month of January (7-DHC-18% and previtamin D_3 -12.9%) and March (7-DHC-17.3% and previtamin D_3-13.5%) (**Figs. 3 and 4**). The study was repeated for a similar season in the following year. The percent conversion of 7-DHC to previtamin D_3 and its photoproducts and formation of previtamin D_3 and vitamin D_3 was no different for the same three months in May, June and July in 2007 and 2008 (**Fig. 6**).

Discussion

The synthesis of vitamin D in the skin occurs in a two-stage process. As soon as the skin is exposed to solar UVB radiation

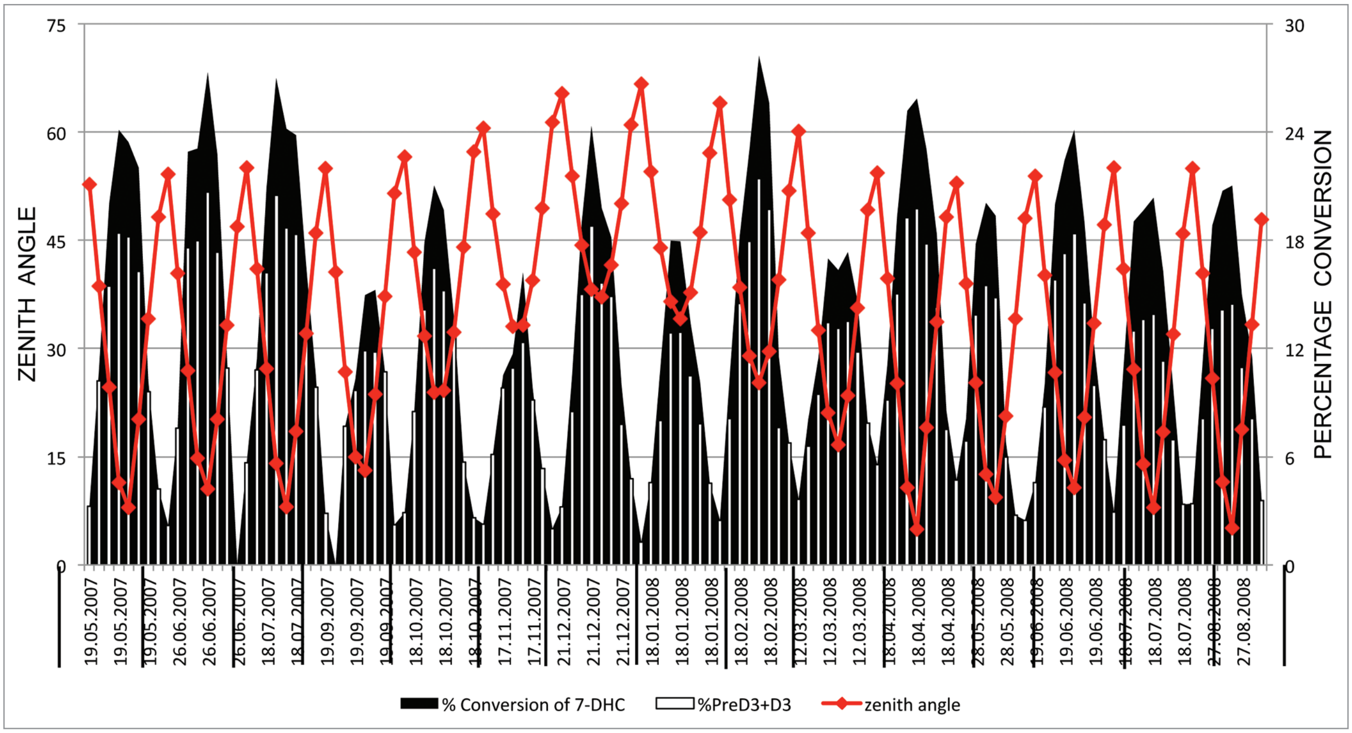


Figure 3. Figure depicting the percent conversion of 7-Dehydrocholesterol (7-DHC) to previtamin D_3 and photoproducts, and the percentage of previtamin D_3 and vitamin D_3 formed from May 2007 to August 2008 along with zenith angles of various time of the day during the whole study period.

7-DHC is photolyzed to previtamin D_3. After this initial photolysis the previtamin D_3 undergoes a temperature and membrane dependent isomerization in the skin to vitamin D_3. Latitude, altitude, season, time of the day, ozone amount, cloud amount, aerosol and reflectivity of the earth's surface (albedo) are the factors that control the number of UVB photons that reach the earth's surface. In Tirupati (latitude 13.40° N), the average duration of cloud-free sunshine is 8–10 h/day throughout the year. The UV index at this latitude during the study period is 7–9.

Among the factors that control the ambient UVB radiation is the cyclical and predictable solar zenith angle. With increasing solar zenith angle the UVB radiation traverses a long path through the ozone in the atmosphere resulting in the decreasing number of UVB photons reaching the earth's surface. When the sun is low in the sky, there is attenuation of UVB photons reaching the earth's surface. Motion of Earth rotating around the sun and about its own axis combined with season, time of the day and latitude all affects the solar zenith angle and thus cutaneous production of previtamin D_3.[9] This was observed in this study (**Figs. 1–4**). In our study location with the UV index of 7–9 the percent conversion of 7-DHC to previtamin D_3 and its photoproducts and formation of previtamin D_3 and vitamin D_3 was between 12 to 18% throughout the year with maximum production between 11 a.m. to 2 p.m. (**Figs. 1–3**). The amount of UVB radiation capable of producing previtamin D_3 in the skin is 70 times more effective at 25° than at 75° due to the influence of the zenith angle of the sun.[10] The present study demonstrated that the percent conversion of 7-DHC to previtamin D_3 is maximum at solar zenith angle of 15° or lower with maximum formation of previtamin D_3 and vitamin D_3 between 11 a.m. to 2 p.m. (**Fig. 2**).

Indian meteorologists classify seasons as follows: Winter is January to February; pre-monsoon is March to May; south east monsoon is June to September and winter monsoon or north east monsoon is October to December. It was observed by three dimensional aerosol assimilation models during Indian Ocean Experiment (INDOEX)[11] a layer of air pollution covers north Indian Ocean, India, Pakistan and parts of south Asia, south East Asia and China.[12] The vertical extent of this cloud extends to 3 km above sea level over Asia (Cloud Aerosol Lidar and Infrared pathfinder satellite–CALISPO data).[13] Termed as "Asian Brown Cloud," it occurs every year and extends over a period from November to April and possibly longer.[14] The Asian Brown Cloud is the result of biomass burning (rural) and fossil fuel consumption (urban). The fossil fuel consumption and biomass burning contribute to particulate (aerosol) pollution, while biomass burning plays a major role in gaseous pollution (as carbon monoxide). The most direct effect of Asian Brown Cloud documented in INDOEX is the 10% reduction of average radiative heat of the ocean and 50–100% increase in solar heating of lower atmosphere. In the present study (from September 2007 to March 2008) the percent conversion of previtamin D_3 and its photo products were significantly reduced (**Figs. 2 and 3**), indicating the Asian Brown Cloud effect.

The results obtained in the in vitro study using ampoule model represent the maximal conversion of 7-DHC to previtamin D_3 and vitamin D_3. There are studies that correlated the synthesis of previtamin D_3 and vitamin D_3 in the ampoule model to human

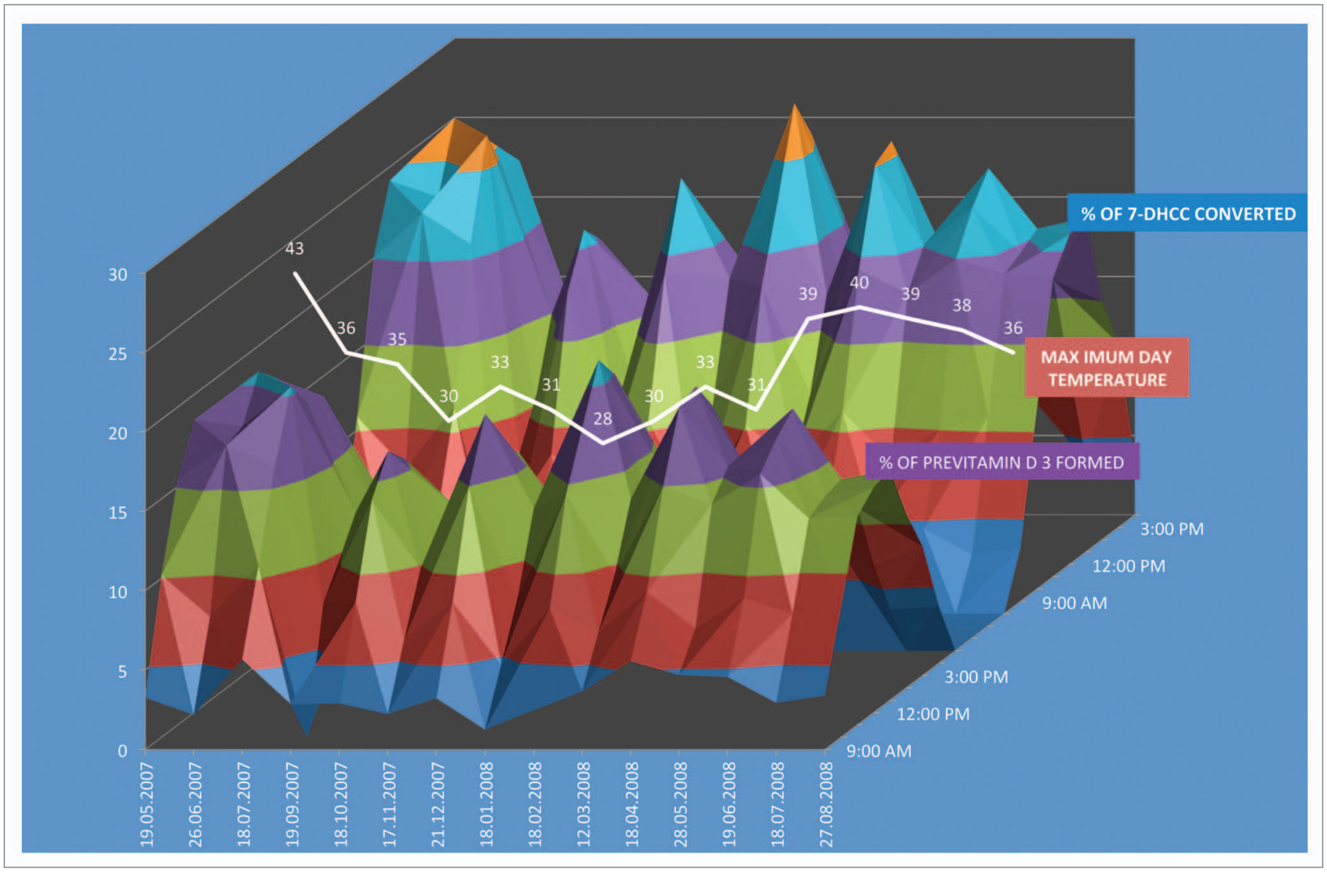


Figure 4. Three-dimensional depictions of the percent conversion of 7-DHC to previtamin D_3 and photoproducts and percentage of previtamin D_3 and vitamin D_3 along with zenith angles of various time of the day during the whole study period. Maximum temperature on the day of the study is also depicted in the same graph.

skin. Caucasian skin with skin type III (which sometimes burns and always tans) and skin type V (which never burns or tans)[5,15-17] demonstrated that 0.8% of 7-DHC converted to previtamin D_3 in the ampoules before any previtamin D_3 appeared in the skin samples in type III skin and 1.8% of 7-DHC converted to previtamin D_3 in the ampoules before any previtamin D_3 appeared in the skin samples in type V skin. Indians belong to skin category V. Subjects residing in the northern India have lighter skin pigmentation as an adaption to UV radiation compared with the subjects in southern India who have darker skin pigmentation and exposed to higher UV radiation.[18] The 25 (OH) D levels in South Indian subjects are relatively higher compared with the subjects from North India from the various studies published in literature. There is a strong inverse correlation between the 25 (OH) D levels and latitude ($r = -0.48$; $p < 0.0001$) from various studies conducted in the country (**Fig. 7A**; **Table 1**). The 25 (OH) D levels of various studies from India along with latitude and location are shown in **Figure 7B**.

There are other interfering factors of solar zenith angle like air pollutants and reflectivity of the surface. Most pollutants are emitted into the lowest kilometer or so of the atmosphere called the planetary boundary layer (BL). Aerosols emitted in this layer exert cooling and drying effects on the surface temperatures. Scattering aerosols tend to cool the BL and absorbing aerosols in the BL destabilize the atmosphere. Aerosol particles indirectly impact atmospheric stability and radiation by affecting cloud microphysics. Extensive studies have been conducted on the impact of aerosols on earth's radiative balance and on climate (aerosol radiative force). The geophysical parameters like normal zenith angle (θ), total ozone amount, surface albedo (the fraction of light reflected from earth's surface), absorption and scattering by gas particles affect vitamin D_3 production in the human body.[19] Ozone is the only important absorbing gas near UV spectrum. The present study was conducted on a flat surface of a terrace of the hospital. With natural surface and vegetation in the region of study the reflectivity was estimated to be approximately 5–10%. Even in clean and clear atmosphere a major attenuation process is Rayleigh scattering of air molecules a process that is inversely proportion to the fourth power of wavelength, λ (i.e., scattering $\alpha\ \lambda^{-4}$).[9] The short UVB wavelengths are scattered by this process. There is a report of high incidence of vitamin D deficiency rickets in toddlers living in areas of high atmospheric pollution in Delhi, India (28.35° N).[20]

The turning of the Earth, the weather or the state of atmosphere is a variable that controls the UV rays reaching the sun. The height, thickness and spatial distribution of the clouds attenuate the UVB radiation reaching the earth's surface. Fair weather cumulus clouds called as "cotton wool clouds" that do not cover

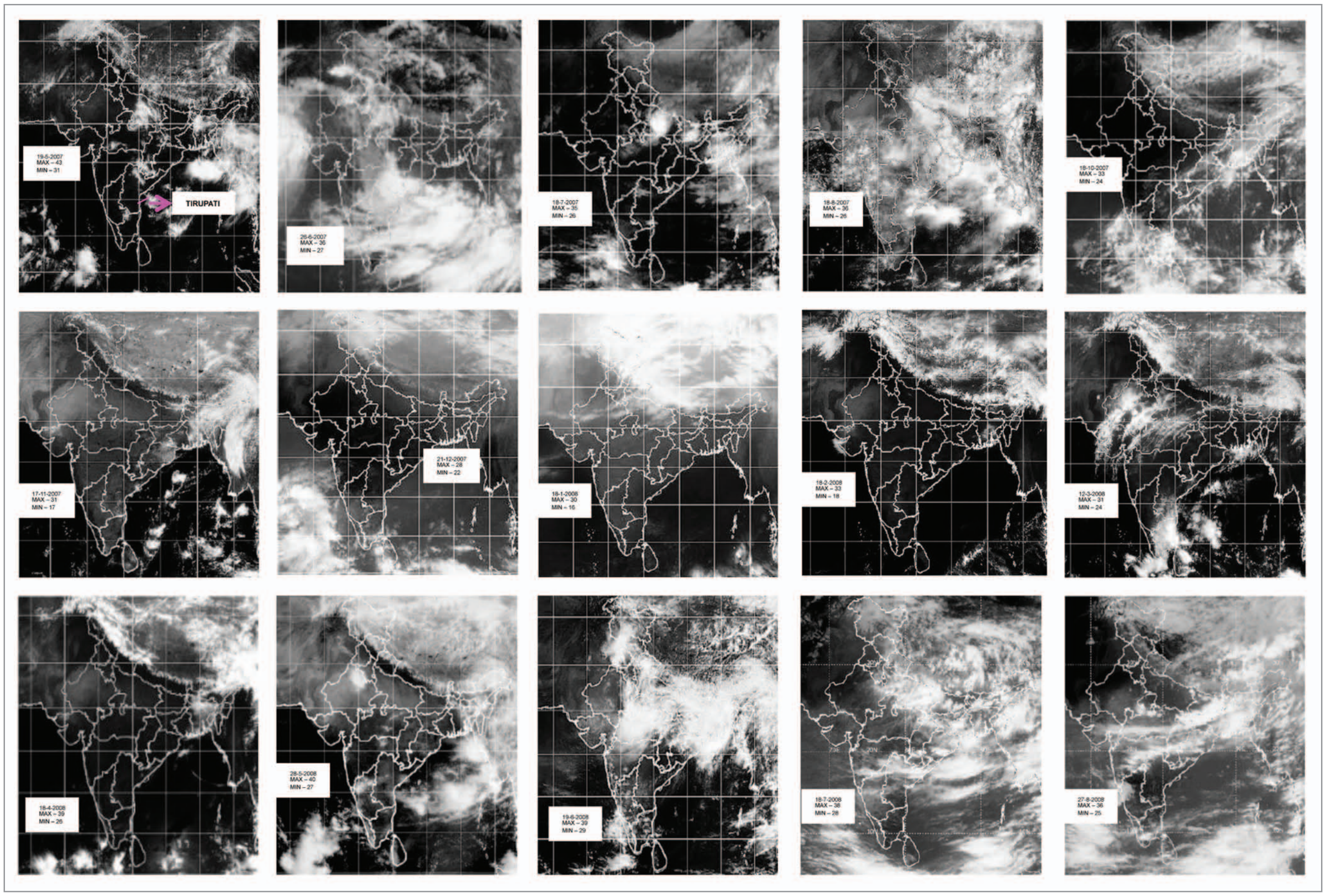


Figure 5. Satellite picture of the country on the day of study at 11.30 h. First picture on the left upper panel (row 1) shows the location of study site (TIRUPATI—latitude 13.40° N and longitude 77.2° E). The date and maximum and minimum temperature on the day of study is shown in each picture. The satellite picture is downloaded from www.hinduonnnet.com under section miscellaneous—weather chart.

the sun have little effect on the UV radiation reaching the surface. Indeed they may enhance the UV for a brief period due to reflection from cloud sides.[15,16] On the other hand a thick layer of stratus cloud covering the sky also called as "heavy overcast clouds" strongly attenuates the UV radiation reaching the surface of earth.[21-23] Also, clouds are very unpredictable in mid-high latitudes interspersed with regions of low and high pressure system. In the present study we tried to inter-relate the weather satellite picture of the country on the day of study and the data acquired (**Figs. 5 and 6**). The median percent conversion of 7-DHC to previtamin D_3 and its photoproducts and percent of previtamin D_3 and vitamin D_3 formed was 11.5% and 10.2% respectively at a solar zenith angle of 36.8° and at 12:30 p.m. Since the study period overlapped for three similar months in the years 2007 and 2008 we tried to compare the percent conversion of 7-DHC to previtamin D_3 and its photoproducts and previtamin D_3 formation for the months of May, June and July in 2007 and 2008 (**Fig. 6**) and correlated with weather changes in the atmosphere. In all three months in both years the solar zenith angle was less than 10° and the percent conversion was more than 20% of 7-DHC into previtamin D_3, and its photoproducts and the formation of previtamin D_3 was above 15%. Asian Brown Cloud had a significant reduction in the percent conversion of 7-DHC to previtamin D_3 and its photoproducts and previtamin D_3 formation from September 2007 to March 2008 (**Fig. 3**).

The estimated UV exposure for sufficient vitamin D status for North America is derived from total ozone mapping spectrophotometer (TOMS) satellite instrument ozone and cloud reflectivity measurements.[19] The time required to obtain the recommended UV dose for adequate vitamin D synthesis in human skin is "1 Standard vitamin D Dose" (SDD).[24] While UV index is instantaneous value, SDD is a time accumulated dose. One SDD is calculated for skin type II on assumption that ¼ MED (minimal erythemal dose) on ¼ skin area (hands, face and arms) to produce UV equivalents of oral dose of 1000 IU of vitamin D at 42° N in March.[25] The website ftp//es-ee.tor.ec.gc.ca/pub/vitamind/ gives 1 SDD for all six skin types at different latitudes. For a skin type V (Indian) 1 Med is 600 (Jm^{-2} erythemal).[25] The minimum recommended exposure to achieve 1SDD (111.4 Jm^{-2} effective) is 50 min at 42.5° N (at solar noon). At lower latitudes lesser time is required. At 11.5° N for skin type V it is 10–15 min throughout the year (at solar noon), at 29° N for skin type V it is 10–45 min throughout the year (at solar noon) with longer duration in winter.[25] India is located 8.4 and 37.6° N. Cloudy skies or pigmented skin will increase the times, low ozone or high altitude and highly reflective environment will decrease times.[25]

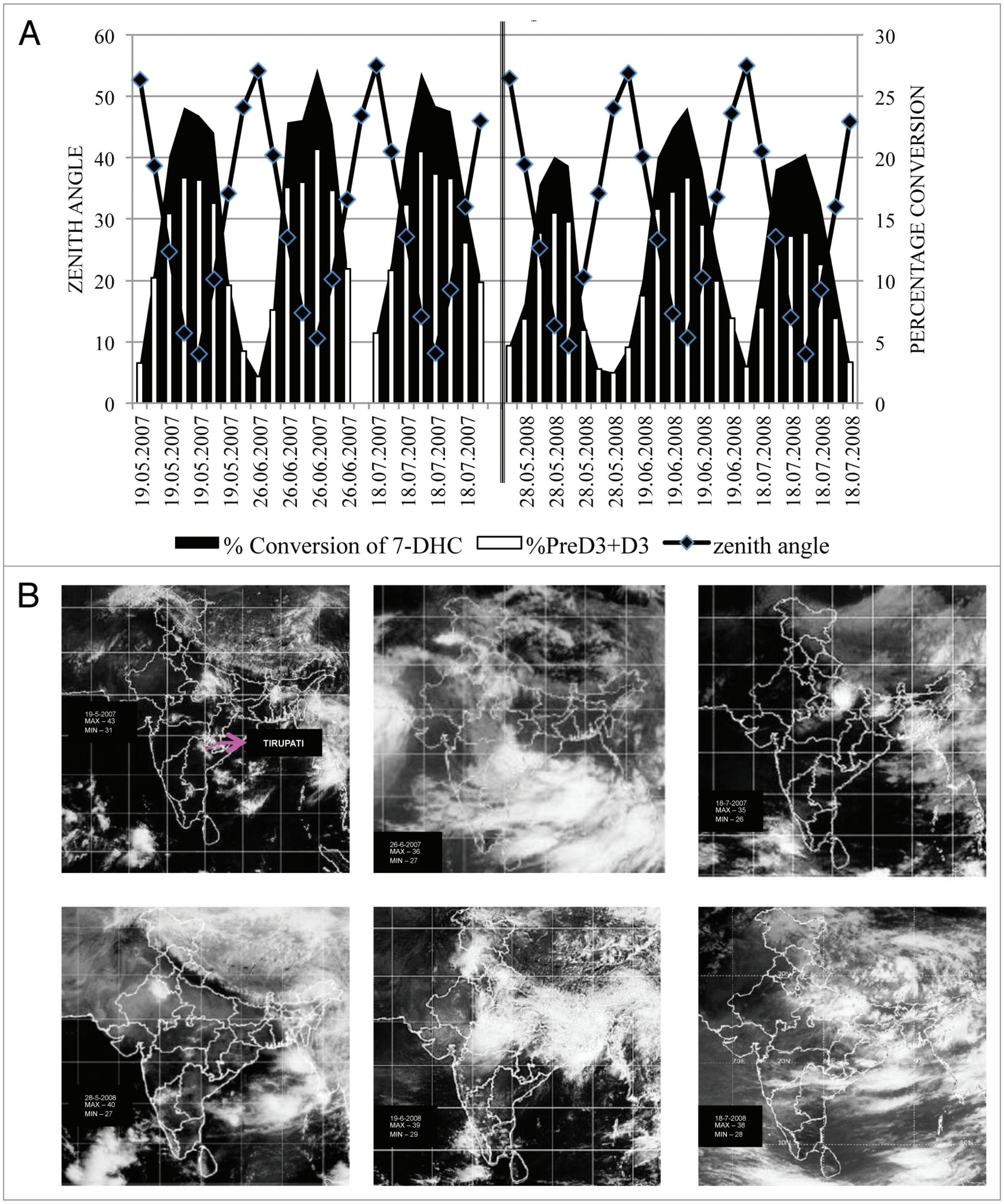


Figure 6. Percent conversion of 7-DHC to previtamin D_3 and photoproducts and formation of previtamin D_3 and vitamin D_3 for three similar months in consecutive years—May, June and July in 2007 and 2008.

More accurate computation can be done using FastRT web page which takes into consideration, the latitude, dietary consumption of vitamin D, duration and time of exposure, etc.[26] Clouds aerosols, thick ozone events, can reduce the vitamin D synthesis and force a "vitamin D winter" even near the equator.[27]

This study reports on the efficiency of sunlight at tropical latitude to photolyze 7-DHC to previtamin D_3 and its photoproducts and previtamin D_3 formation in a clean and clear atmosphere. The equation derived from the study gives a near approximate percentage of conversion of 7-DHC to previtamin D_3 and its photoproducts (FastRT web page gives the duration of exposure to sunlight required for 1 SDD). Attenuating factors like the clothing, duration of exposure to sunlight, type of skin, sunscreen usage and age of individual need to be taken into consideration for extrapolation to humans. Studies from the Indian subcontinent have shown wide prevalence of vitamin D deficiency despite

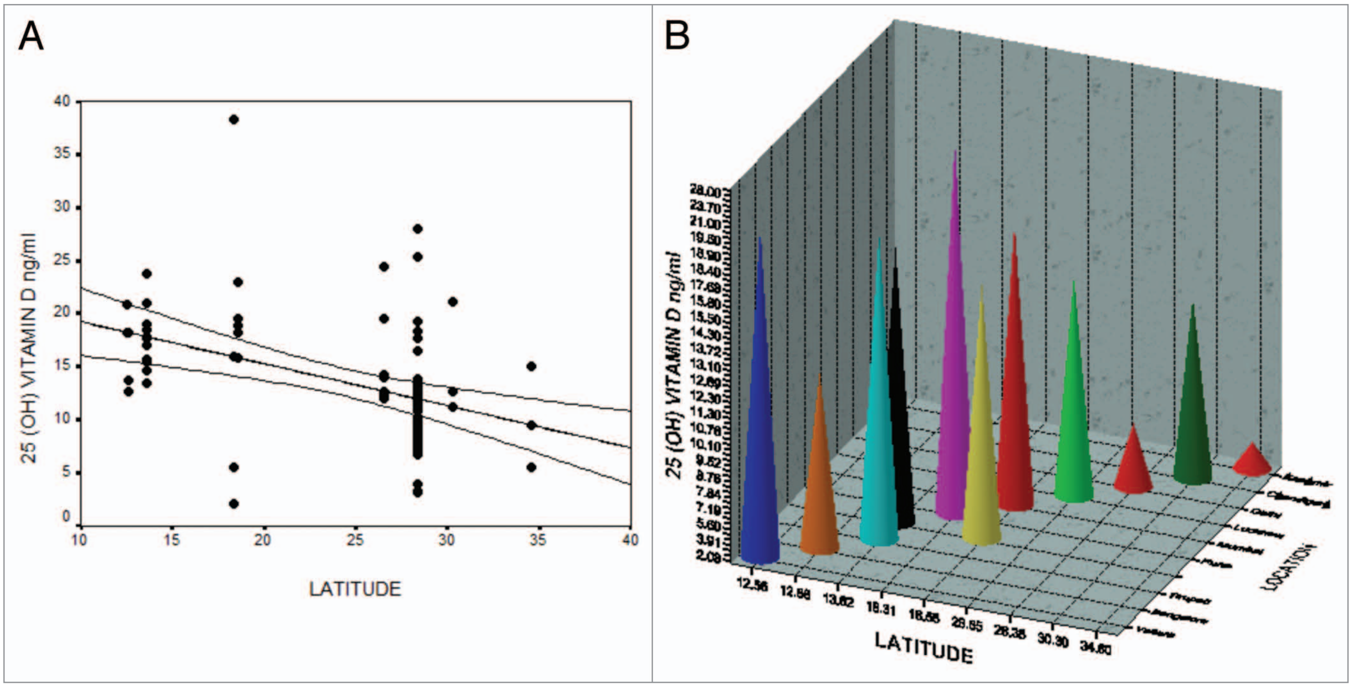


Figure 7. (**A**) Graph showing the inverse correlation between the 25 (OH) D levels and latitude ($r = -0.48$; $p < 0.0001$) from various studies conducted in the country (**Table 1**). (**B**) The 25 (OH) D levels of various studies from India along with latitude and location from various studies conducted in the country (**Table 1**).

plentiful sunlight (**Table 1**).[28-52] The studies from south India as well as from north India have shown that there is high prevalence of vitamin D deficiency [25(OH)D < 20 ng/ml] (**Table 1**). Studies from Pune (18.31° N) have shown that toddlers exposed to sunlight (playing outside) for more than 30 min exposing more than 40% of their body surface area have a normal vitamin D status compared with the toddlers who were indoors for most part of the day (in a crèche).[46] In the Middle East vitamin D status is very low in summer [97% have 25 (OH) D levels less than 30 ng/ml] [11.6% had vitamin D insufficiency (between 20 to 30 ng/ml) and 85.2% had vitamin D deficiency (< 20 ng/ml)]. This was attributed to conservative clothing and very hot weather in summer causing and the people to stay indoors.[53] In India also the summers are very hot and arid and many people stay indoors. This may be one of the contributing factors for low vitamin D status in the Indian population apart from other factors discussed above. The present study also demonstrates that previtamin D_3 production is highly variable depending upon the time of day, season, latitude, cloud cover, etc. The photo conversion of 7-DHC to previtamin D_3 and its photoproducts is maximal between 11 a.m. to 2 p.m. throughout the year. Therefore we as human can get vitamin D from abundant sunshine. Exposure of an adult in a bathing suit to an amount of sunlight that causes a slight pinkness to the skin (1 MED, known as a minimal erythemal dose) is equivalent to ingesting approximately 20,000 IU of vitamin D.[54] Thus exposure of arms (18% body surface) to sunlight between 11 a.m. and 2 p.m. for an amount of time to cause an MED would be equivalent to ingesting about 3600 IU vitamin D. Since the vitamin D produced in the skin lasts two times longer in the body, casual exposure to an amount of sunlight that is equivalent to 0.5 MED of arms and legs, three times a week can provide adequate amount of vitamin D. The amount of time it takes to get 0.5 MED between 10 a.m. to 3 p.m. depends on skin pigmentation, latitude, etc., described above. In populations where there is limited exposure to sunlight, vitamin D supplementation may also be required.

Materials and Methods

The study was conducted in Tirupati latitude 13.40° N and longitude 77.2° E from May 2007 to August 2008. Sealed borosilicate glass ampoules containing 50 μg of 7-DHC in 1 ml of methanol were exposed to sunlight hourly beginning from 8 a.m. until 4 p.m. The ampoules were placed in a Petri dish (precoated with black paint) which was placed on ice to maintain a temperature of 4°C to prevent temperature dependent changes. The ampoules were placed on an open terrace of the hospital exposed to sunlight on a cloudless day when possible with no interference from buildings or vegetation. An ampoule was placed outside each hour so that the photolysis of 7-DHC could be studied in a time dependent fashion for the whole day. From 12 p.m. to 1 p.m. a control ampoule was placed together with ampoule for study. The control ampoule was wrapped in a silver foil so that no UVB irradiation could enter it. At the end of the day of the study the hourly solar zenith angle of the study period was obtained from the website http://solardat.uoregon.edu/SolarPositionCalculator.html. The satellite picture of the country on the day of study at 11:30 a.m. was downloaded from the website www.hinduonnet.com under section miscellaneous—weather report from the archives of the newspaper. From the weather report published in

Table 1. Vitamin D status of India summarized based on latitude and longitude

LAT	LONG	Location	n	STUDY POPULATION	AGE (Yrs)	25 OH D	UNIT	Ref No
34.6° N	74.48° E	Kashmir	64	Men	28.8 ± 4.9	37.7 ± 30	nmol/l	Zargar et al.[28]
		Valley	28	Women	26.8 ± 4.8	13.8 ± 11	nmol/l	
30.3° N	76.47° E	Chandigarh	329	Males and females (summer)	19.4 ± 1.48	52.9 ± 33.7	nmol/l	Santosh et al.[29]
			237	Males and females (winter)	19.4 ± 1.43	31.8 ± 21.1	nmol/l	
28.35° N	77.12° E	Delhi	12	Controls (Resident Doctors)	25–35	8.3 ± 2.5	µg/ml	Harinarayan et al.[30]
		Delhi	29	Pregnant women (summer)	23 ± 3	21.9 + 10.73	nmol/l	Goswami et al.[31]
			29	Newborn (summer)	newborn	16.72 + 4.99	nmol/l	
			31	Soldiers males (winter)	21.2 ± 2	41.17 ± 11.73	nmol/l	
			19	Phys. and nurse (summer)	23 ± 5	7.89 ± 3.49	nmol/l	
			19	Phys. and nurse (winter)	24 ± 4	17.97 ± 7.98	nmol/l	
			15	Depigmented persons (winter)	43 + 16	18.2 ± 11.23	nmol/l	
		Delhi	26 (5)	Toddlers (Mori gate)	16 ± 4 mo	12.4 ± 7	ng/ml	Agarwal et al.[20]
			1	Infants (Gurgaon)	16 ± 4 mo	28 ± 7	ng/ml	
		Delhi Slums	47	Sunder Nagar Jan 2001	9–30 mo	96 ± 25.7	nmol/l	Tiwari et al.[32]
			49	Rajiv colony Feb 2001	9–30 mo	23.8 ± 27	nmol/l	
			48	Rajiv Colony Aug 2001	9–30 mo	17.8 ± 22.4	nmol/l	
			52	Gurgoan Aug 2001	9–30 mo	19 ± 20	nmol/l	
		Delhi	193	LSES School girls	12.4 ± 3.2	34.6 ± 17.43	nmol/l	Puri et al.[33]
			211	USES School girls	12.3 ± 3	29.4 ± 12.7	nmol/l	
		Delhi	42	LSES School boys	10–12	12.4 ± 5.5	ng/ml	Marwaha et al.[34]
			85		13–15	11.3 ± 5.8	ng/ml	
			40		16–18	11.3 ± 5.3	ng/ml	
			33	USES School boys	10–12	19.3 ± 8.8	ng/ml	
			70		13–15	13.1 ± 7	ng/ml	
			55		16–18	13.5 ± 7	ng/ml	
			78	LSES School girls	10–12	11 ± 6.5	ng/ml	
			123		13–15	10 ± 6.2	ng/ml	
			62		16–18	11 ± 5.7	ng/ml	
			47	USES School girls	10–12	12.5 ± 8.9	ng/ml	
			62		13–15	10.2 ± 5.7	ng/ml	
			63		16–18	12.9 ± 10.5	ng/ml	
		Delhi	40	Indian Paramilitary forces men	20–30	18.4 ± 5.3	ng/ml	Tandon N et al.[35]
			50	Indian Paramilitary forces women	20–30	25.3 ± 7.4	ng/ml	
		Delhi	32	Rural males	42.8 ± 16.6	44.2 ± 24.4	nmol/l	Goswami et al.[36]
				Rural females	43.4 ± 12.6	26.9 ± 15.9	nmol/l	

*Mean ± SEM; **Values are median and inter-quartile range; For conversion from nmol to ng—multiply by 0.4; Age Adju, Age Adjusted; LAT, Latitude; LONG, Longitude.

Table 1. Vitamin D status of India summarized based on latitude and longitude

		Delhi		Mothers	NA	9.8	ng/ml	Jain et al.[37]
					14 weeks	10.1	ng/ml	
		Delhi	97	Mothers 1st Trimester (summer)	24.4 ± 2.67	23.4 ± 11.3	nmol/l	Marwah et al.[38]
			59	Mothers 1st Trimester (winter)		19.6 ± 9.2	nmol/l	
			125	Mothers 2nd Trimester (summer)	25 ± 2.94	25.7 ± 15.1	nmol/l	
			93	Mothers 2nd Trimester (winter)		20.2 ± 10.6	nmol/l	
			77	Mothers 3rd Trimester (summer)	24.26 ± 2.82	27.7 ± 9.2	nmol/l	
			90	Mothers 3rd Trimester (winter)		21.1 ± 12.4	nmol/l	
			sub-set	Mothers 6 wks postpartum		19.6 ± 8.3	nmol/l	
				Infants	6 weeks	22.3 ± 10.5	nmol/l	
		Delhi	703	Women	50 ± 9.5	9.78 ± 8.3	nmol/l	Marwah et al.[39]
			643	Males	50 ± 9.5	9.81 ± 6.79	nmol/l	
26.55° N	80.59° E	Lucknow	140	Pregnant women (urban)	24 ± 4.1	14 ± 9.5	ng/ml	Sachan et al.[40]
			67	Pregnant women (rural)	24.7 ± 5.1	14 ± 9	ng/ml	
			29	Cord Blod (OSM)	-	12 ± 8	ng/ml	
			178	Cord Blod (no OSM)	-	14.3 ± 9.5	ng/ml	
		Lucknow	139	Pregnant women (summer)	Age Adju	55.5 ± 19.8	nmol/l	Sahu et al.[41]
			139	Pregnant women (winter)	Age Adju	27.3 ± 12.3	nmol/l	
			28	Girls (winter)	Age Adju	31.3 ± 1.5	nmol/l	
			34	Boys (winter)	Age Adju	67.5 ± 29	nmol/l	
		Lucknow	53	Controls		61 ± 36	nmol/l	Balasubramanian et al.[42]
			40	Rickets/OSM		49 ± 38	nmol/l	
		Lucknow	92	Healthy volunteers	34.2 ± 6.7	12.3 ± 11	ng/ml	Arya et al.[43]
18.56° N	72.54° E	Mumbai	42	Mothers Suppl Ca 250–500 additnal	20 to 35	23 ± 11	ng/ml	Bhalala et al.[44]
			42	Cord Blood	-	19.5 ± 9.6	ng/ml	
			35	Infants	3 mo	18.2 ± 9.8	ng/ml	
		Mumbai	558	Males	30.11 ± 3.53	18.9 ± 8.9	ng/ml	Shivane et al.[45]
			579	Females	30.52 ± 3.57	15.8 ± 9.1	ng/ml	
18.31° N	73.55° E	Pune	25	Male toddlers (outdoor)	2.26 ± 0.8	95.86 (91.6) **	nmol/l	Ekbote et al.[46]
			25	Female toddlers (outdoor)	2.53 ± 0.8	130.2(67.7) **	nmol/l	
			31	Male toddlers (indoor)	2.94 ± 0.6	14.0 (32.0) **	nmol/l	
			29	Female toddlers (indoor)	2.70 ± 0.6	5.2 (21.1) **	nmol/l	
13.62° N	79.4° E	Tirupati	191	Tirupati rural*	44 ± 1.03	21 ± 0.46	ng/ml	Harinarayan et al.[47]

*Mean ± SEM; **Values are median and inter-quartile range; For conversion from nmol to ng—multiply by 0.4; Age Adju, Age Adjusted; LAT, Latitude; LONG, Longitude.

Table 1. Vitamin D status of India summarized based on latitude and longitude

			125	Tirupati urban*	45.5 ± 0.95	13.52 ± 0.59	ng/ml	
		Tirupati	134	Urban men*	47 ± 1.5	18.54 ± 0.8	ng/ml	Harinarayan et al.[8,48]
			109	Rural men*	45 ± 1.4	23.7 ± 0.8	ng/ml	
			807	Urban women*	46 ± 0.4	15.5 ± 0.3	ng/ml	
			96	Rural women*	41 ± 1.4	19 ± 0.9	ng/ml	
			30	Urban children male*	11 ± 1	15.57 ± 1.2	ng/ml	
			34	Rural children male*	12 ± 0.7	17 ± 1.3	ng/ml	
			39	Urban children female*	13.5 ± 0.6	18.5 ± 1.66	ng/ml	
			36	Rural children female*	12.6 ± 0.5	19 ± 1.6	ng/ml	
		Tirupati	164	Post menopausal	54 ± 8	14.6 ± 7	ng/ml	Harinarayan et al.[49]
		Tirupati	55	Women in reproductive age group*	37.5 ± 0.94	15.7 ± 1.38	ng/ml	Harinarayan et al.[50]
				Post menopausal*	53.3 ± 0.72	17.7 ± 0.94	ng/ml	
12.58° N	77.38° E	Bangalore	150	Males*	50 ± 1.44	12.69 ± 0.55	ng/ml	Harinarayan et al.[51]
			606	Females*	51 ± 0.6	13.72 ± 0.38	ng/ml	
12.55° N	79.08° E	Vellore	150	Post menopausal women	60.1 ± 5	20.85 ± 8.63	ng/ml	Paul et al.[52]

*Mean ± SEM; **Values are median and inter-quartile range; For conversion from nmol to ng—multiply by 0.4; Age Adju, Age Adjusted; LAT, Latitude; LONG, Longitude.

the newspaper the maximum temperature recorded in the place of study was also recorded. The study was repeated around the same day every month in the same location for the whole period of the study. The analysis of the ampoules for 7-DHC, previtamin D_3 and photoproducts including lumisterol and tachysterol were analyzed as previously described.[5]

Statistical analysis. Descriptive results are presented as mean ± SD. Pearson's coefficient was calculated for the correlation. *p* values < 0.05 were considered significant. Analysis of variance was used to estimate the main effects. Analysis was performed with the use of SPSS (version 11.5; SPSS Inc.).

Disclosure of Potential Conflicts of Interest

No conflicts of interest were disclosed.

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