CHAPTER 4

Photobiology of Vitamin-D Molecule and Its Seasonal Variation

Vitamin D is synthesized in the skin after exposure to solar UV-B radiation. Because only a few food items contain vitamin D most people gain the majority of their vitamin D intake from sunlight exposure (Committee, 1998). Regular replenishment of vitamin D is essential, although it can be stored in fatty tissue when in plenty (e.g. summer), thus providing some stores for periods when availability is reduced (e.g. winter), at latitudes with long winters this storage is insufficient and vitamin D status declines. Submariners, with no UV exposure, had vitamin D levels that declined by half in a period of 2 months (Dlugos DJ et al., 1995). Vitamin D levels are low in many populations and age groups. For example, in winter, one-third to one-half of preschool children in the United Kingdom have insufficient vitamin D status (Davies PS et al., 1999) and this childhood disease, Rickets (caused by lack of vitamin D) is being observed primarily but not exclusively, among children with pigmented skin (Kreiter SR et al., 2000; Pugliese MT et al., 1998). Adolescents in France exhibit vitamin D status that barely reaches sufficiency even in summertime (Guillemant J, 2001),
whereas vitamin D insufficiency has been shown among free living healthy adults in the United States (Tangpricha V et al., 2002). Other examples can be found in the Nordic countries (Lamberg-Allardt et al., 2001), European countries (Ovesen L et al., 2002), Canada and India (Arya V et al., 2004). In addition to its long-established role in calcium metabolism, vitamin D appears to have a number of other beneficial effects. A protective effect of vitamin D against cancer of the colon, prostate, and breast has been suggested on the basis of epidemiological studies (Garland C F et al., 1989; Grant W B, 2002; John E M et al., 1999) and experimentally for prostate and breast cancers (Banwell C M et al., 2003). The mechanisms for reduction in the risk of cancer incidence and death (Van den Bemd G J et al., 2002) are well documented, and sunlight exposure (suggesting vitamin D) has also been associated with improved cancer survival rates (Berwick et al., 2005, Garland C F et al., 2006; Gorham E D et al., 2005).

4.1. Chemical Structure of Vitamin-D

The term vitamin D as applied in this text refers to the secosteroids ergocalciferol (vitamin D$_2$) and cholecalciferol (vitamin D$_3$) which is irradiation products of the steroids ergosterol and 7-dehydrocholesterol respectively. Ergo sterol is the provitamin D$_2$ in plants, fungi and invertebrates and 7-dehydrocholesterol is the
provitaminD₃ in vertebrates. Irradiation of the parent steroid results in breakage of the B-ring at the 9,10-carbon bond, resulting in the conjugated trine system of double bonds. The numbering system of the carbon atoms of the vitamin D molecule is identical to that of the parent steroid. Vitamin D₂ (C₂₈H₄₄O; MW = 396.63) and vitamin D₃ (C₂₇H₄₄O; MW = 384.2) differ structurally only in the C-17 side chain, the former having an additional C-22 to C-23 double bond and a C-24 methyl group. The breakage of the B-ring frees the A-ring from the rigid C and D rings, giving the vitamin D molecule a high degree of conformational mobility. As the result of this mobility, the ring undergoes rapid inter conversion between two chair conformations so that the substituent’s alternate rapidly and continually between axial and equatorial positions. This is likely to present special problem for vitamin D receptors that are not encountered by the receptors for other steroid hormones.

![Figure 4.0. Numbering system of vitamin-D molecule.](image)
4.1.1 Side Chain Modification

The prefix 'er' is used table 4.0 to indicate the side chain (3) for the vitamin D₂ series, e.g. ercalciol. This prefix implies the 22E, 24R configuration shown in (3) unless otherwise specified. Ergocalciferol may still be used as an alternative trivial name for ercalciol but should not be used for naming metabolites.

\[
\text{Note. Because of the nature of the sequence rules it is not possible to transfer } R \text{ or } S \text{ from one compound to its derivatives.}
\]

4.1.2 Dihydro Derivatives

Dihydro tachy sterol is an important member of the vitamin D family. It should be called dihydrocalciol, although a more systematic name would be (5E)-(10S)-10, 19-dihydrocalciol [(4) which is the same as (5)].
Figure 4.1. Examples of stereochemistry at position 24 (and 25) in the vitamin D$_3$ series.

Note that the presence or absence of a 22(23) double bond in the bottom three examples does not change in this series the designation at position 24 (or 25)

(5E)- (10S)-10, 19-Dihydrocalcicol

**Note1.** Although this compound is derived from calciferol by hydrogenation of the 10(19) double bond it may also be considered as a derivative of tacalciol formed by 1, 6-addition of hydrogen to the 5(10), 6, 8-triene system, i.e. positions 9 and 10. **Note2.** A new chiral centre is present at position 10. If a synthetic sample contains a mixture of both
isomers, not necessarily in equimolar proportions, the affix *ambo* may be, used IUPAC-IUB Commission on Biochemical Nomenclature (CBN). Nomenclature of tocopherols and related compounds, Recommendations 1973, Arch. Biochem. Biophys. (1974), Biochem. J. (1975), Eur. J. Biochem., (1974). To indicate the presence of such a mixture, e.g. (5E)-10-*ambo*-10, 19-dihydrocalciol. If only one isomer is present, but with unknown stereochemistry, then this may be indicated by the use of *xi*, e.g. (5E)-(10 S)-10, 19-dihydrocalciol. When the absolute stereochemistry at C-10 is know this is shown in the normal way, e.g. (5E)-(10S)-10, 19-dihydrocalciol.

Table 4.0 Nomenclature for vitamin D compounds

<table>
<thead>
<tr>
<th>Current trivial name</th>
<th>Recommended trivial name</th>
<th>Systematic steroid name a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholecalciferol</td>
<td>calciol or cholecalciferol</td>
<td>(5Z,7E)-(3S)-9,10-seco-5,7,10(19)-cholestatrien-3-ol</td>
</tr>
<tr>
<td>25-Hydroxycholecalciferol</td>
<td>calcidiol</td>
<td>(5Z,7E)-(3S)-9,10-seco-5,7,10(19)-cholestatrien-3,25-diol</td>
</tr>
<tr>
<td>1,25-Dihydroxycholecalciferol</td>
<td>calcitriol</td>
<td>(5Z,7E)-(1S,3R)-9,10-seco-5,7,10(19)-cholestatrien-1,3,25-triol</td>
</tr>
<tr>
<td>Ergocalciferol</td>
<td>ercalcioi or ergocalciferol</td>
<td>(5Z,7E,22E)-(3S)-9,10-seco-5,7,10(19),22-ergostataetraen-3-ol b</td>
</tr>
<tr>
<td>1,25-Dihydroxyergocalciferol</td>
<td>ercalcitriol</td>
<td>(5Z,7E,22E)-(1S,3R)-9,10-seco-5,7,10(19),22-ergostatoctraen-1,3,25-triol c</td>
</tr>
<tr>
<td>22,23-Dihydroergocalciferol</td>
<td>(24S)-methylcalciol or 22,23-dihydroercalciol</td>
<td>(5Z,7E)-(3S)-9,10-seco-5,7,10(19)-ergostatrien-3-ol c</td>
</tr>
</tbody>
</table>
1,24R,25-Trihydroxycholecalciferol calcitriol
(5Z,7E)-(1S,3R,24R)-9,10-seco-5,7,10(19)-cholestratriene-1,3,24,25-tetrol

Previtamin D$_3$ (6Z)-tacalcioi
(6Z)-(3S)-9,10-seco-5(10),6,8-cholestratrien-3-ol

Tachysterol$_3$ tacalcioi
(6E)-(3S)-9,10-seco-5(10),6,8-cholestratrien-3-ol

Isovitamin D$_3$ (5E)-isocalciol
(5E,7E)-(3S)-9,10-seco-1(10),5,7-cholestratrien-3-ol

Dihydrotachysterol$_3$ dihydroercalcioi
(5E,7E)-(3S,10S)-9,10-seco-5,7-cholestadien-3-ol

$^a$ To confirm with the convention used in the Steroid Rules (Rule 2S-4.1.1 and IUPAC Nomenclature of Organic Chemistry double bond locants are cited before the stem name.

$^b$ 24R-configuration.

$^c$ 24S-configuration.

4.2. Photobiology of vitamin-D

The absorption of a quantum of ultraviolet radiation by conjugated double bond system of ring B of vitamin-D that converts it into an excited unstable form causing the C$_9$-C$_{10}$ bond to break forming previtamin-D as shown in fig.4.2 (Holick., 2002). The precursor of cholesterol to provitaminD$_3$ which is turning in rapidly converted to vitamin D$_3$ under favourable condition of temperature. Seasons, latitude, time of day, skin pigmentation, obesity, ageing, use of sunscreen and glass all influence the cutaneous synthesis of vitaminD$_3$. The cholesterol in the adipose tissues of the epidermis and absorbs UV-B radiation and gets converted to vitamin D$_3$. 
Figure 4.2. Synthesis of Vitamin D by Sunlight

A quantum of ultraviolet radiation is derived from the solar spectrum. The energy of ultraviolet radiation is reduced by the gaseous molecule in the atmosphere and particularly by the ozone layer. Ozone is preset between 10,000 to 40,000 feet. The maximum thickness of the ozone layer is found to be 20,000 feet but is usually expressed as an ozone layer of about 3 mm at NTP because the thickness of the ozone layer is not constant (which is discussed in next chapter). As with other source of radiation, ultraviolet radiation power is reduced if the incident light reaches a surface at an angle other then 90°, the power being spread
over a greater area. Power decline is proportional to the curve of the angle from normal ($\theta$), (illumination of angled surface and $\cos\theta$). As a consequence of these factors, the power of ultraviolet radiation reaching the earth's surface is much reduced as compared to that emitted by the Sun, and there is further substantial reduction in power on moving from the equator, where the incident radiation is perpendicular to the earth's surface, to the pole. Interestingly, complete cloud cover reduces ultraviolet energy by 50% and an important consequence of the scattering of ultraviolet energy is that shade does not reduce the energy by more than 40%.

The recognition of the seasonal variations in 25(OH) D is related to the changes in sunlight that has stimulated interest in the photobiology of vitamin D. When human skin is exposed to UV-B radiation (290-320 nm), high-energy UV photons enter the epidermis and cause photochemical transformations of 7-dehydrocholesterol (7-DHC) to previtamin D. Once formed, previtamin D undergoes temperature-dependent isomerization to vitamin D (cholecalciferol) which at body temperature takes about 2-3d to reach completion. Vitamin D is then transported in the blood bound to DBP (Holick et al., 1981). Endogenous synthesis of cholecalciferol from 7-DHC is related to the amount of biologically effective UV radiation, the duration of exposure
of UV radiation and to the degree of skin pigmentation. Recently research on how sunlight and diet bring about their effects on vitamin D metabolism suggest a complexity previously unthought-of and partially explain how risk factors operates. Isomerisation over 2-3d in the skin is also rapidly degraded by sunlight. Because DBP has little affinity to lumisterol or tachysterol, translocation of these photo isomers into the circulation is negligible and they are probably sloughed off in natural turnover of skin. Thus, photochemical conversion of previtamin D to lumisterol appears to limit previtamin D formation during excessive exposure to the sun, and prevents vitamin D intoxication after prolonged exposure to the sun.

As the melanin pigment concentrations in skin increase, time of exposure necessary to maximize previtamin D formation, but not content, increases from 30min to between 1 and 3h (depending on degree of skin pigmentation). However, regardless of skin type, previtamin D reaches a maximum and at a plateau of about 15% of original 7-DHC concentration (Lo CW et al., 1986). Although the cutaneous level of 7-DHC and the efficiency of its conversion both fall with age, these factors do not appear to constrain the production of hydroxylated derivatives of vitamin D in healthy elderly. Differences in seasonal vitamin D noted between institutionalized and healthy fully ambulant elderly subjects are largely explained by marked differences in
solar exposure between groups. Experimental studies have confirmed that clothing also affects the vitamin D response to UV-B radiation (Matsuoka et al., 1987). The practical result of these complex effects of sunlight on vitamin D metabolism is that a short initial summer exposure can provide the body with enough vitamin D for the next few days, irrespective of further exposure. Thus, although there is an important relationship between sunlight and circulating concentrations of 25(OH) D, measurement of summertime hours of sunlight exposure cannot be expected to bear a simple relationship to the amount of vitamin D produced.

The amount of UV-B light incident on the earth's surface depends on the amount of ozone in the stratosphere and varies with season and latitude. Winter sunlight in high latitudes is ineffective for production of vitamin D, since when the sun is low in the sky its zenith angle increases and UV-B radiation is subject to more scattering and absorption by ozone than when the sun is directly overhead.

Sunlight was recommended as a therapeutic method to prevent rickets in infants, and a detailed description was published in the United States Children's Bureau Folder in 1931 (Hess A., 1936; Eliot MM., and Park EA., 1938). It was recognized that in the temperate zone, sunlight was feeble in its antirachitic properties in the winter, and thus, it was recommended that children be exposed to UV radiation from a
mercury arc or carbon arc lamp in the winter fig.4.3. During exposure to sunlight, the ultraviolet B (UV-B) radiation (290-315nm) is absorbed by 7-dehydrocholesterol in the skin to form previtamin D₃. Previtamin D₃ is inherently unstable and rapidly converts by a temperature-dependent process to vitamin D₃ (MacLaughlin JA et al., 1982) fig.4.4. Once formed, it is ejected out of the skin cell into the extracellular space, where it is drawn into the dermal capillary bed by the vitamin D binding protein (DBP).

The efficiency of vitamin D₃ synthesis in the skin is dependent on the number of UV-B photons that penetrate into the epidermis. An increase in skin melanin pigmentation and the topical application of a sunscreen both of which efficiently absorb UV-B photons can markedly diminish by more than 90% the production of vitamin D₃. Excessive exposure to sunlight cannot cause vitamin D intoxication because sunlight destroys any excess vitamin D₃ produced in the skin. Most UV-B photons from the sun are absorbed by stratospheric ozone. An increase in the sun’s zenith angle results in an increased path length for the UV-B photons to travel, and this explains why at higher latitudes (above 35° latitude), very little, if any, vitamin D₃ is produced in the skin from November through March. The preosteoclast to become a mature osteoclast, the mature osteoclast releases hydrochloric acid and collagens to dissolve bone and release its precious calcium and phosphorus stores.
into the circulation. Thus, the major physiologic function of vitamin D is to maintain serum calcium and phosphorus levels within the normal physiologic range to support most metabolic functions, neuromuscular transmission, and bone mineralization (Mellanby ET., 1919; Steenbock H., 1924).

Figure 4.3. UV radiation therapy for rickets. (A) Photograph from the 1920s of a child with rickets being exposed to UV radiation. (B) Radiographs demonstrating florid rickets of the hand and wrist (left) and the same wrist and hand taken after treatment with 1 hour UV radiation 2 times a week for 8 weeks. Note mineralization of the carpal bones and epiphyseal plates (right). Reproduced from (Ganmee, KML., 1927)
Figure 4.4. The photo production and metabolism of vitamin D and the various biologic effects of 1,25(OH)_2D on calcium, phosphorus, and bone metabolism. Vitamin D is either produced in the skin by exposure to UV-B radiation or is ingested in the diet. Vitamin D (represents vitamin D_2 or vitamin D_3) is converted by the vitamin D-25-hydroxylase (25-OHase) in the liver to 25(OH) D. 25(OH) D is converted in the kidneys by 1-OHase to 1,25(OH)_2D. Once formed, 1,25(OH)_2D enhances intestinal calcium and phosphorus absorption and stimulates the expression of RANKL on the osteoblasts to interact with its receptor RANK on preosteoclasts to induce mature osteoclastic activity, which releases calcium and phosphorus (HPO4^2-). In addition, 1,25(OH)_2D inhibits the renal 1-OHase and stimulates the expression of the renal 25(OH) D-24-hydroxylase (24-OHase). The induction of the 24-OHase results in the destruction of 1,25(OH)_2D into a water-soluble inactive metabolite calcitriol acid, which is then excreted in the urine.
4.3. Metabolism of Vitamin-D

The active form of vitamin D is 1,25-dihydroxyvitamin D, 1,25(OH)₂D, which is produced after vitamin D₃ has formed in the skin, or after vitamin D₂ or vitamin D₃ taken orally has been hydroxylated first by the liver and then by the kidney. The first (liver) hydroxylation produces 25-hydroxy vitamin D 25(OH) D₃, and it is the plasma volume of 25(OH) D that is the usual measure of vitamin D status. 25 (OH)D is observed to respond to exposure to UV-B radiation, increasing in the summer months or with artificial radiation, and declining with lack of exposure; for example, in the winter time. There is a well documented seasonal cycle of 25(OH) D in people living at mid to high latitudes (Davies P S et al., 1999). The active form, 1,25(OH)₂ D is, however, very tightly regulated and has little response to sun exposure unless vitamin D status is low. Because the active form of the vitamin D does not increase with sun exposure which is show in fig 4.5.
Figure 4.5. Modified Structure of Vitamin D after circulation of Liver and Kidney.

It was hard to explain how vitamin D could protect against cancer, even though 1, 25(OH)$_2$D is known to be a potent inhibitor of abnormal cell growth (Verlinden L et al., 1998). This explanation came with the discovery that colon cancer cells have receptors for 25(OH)D (Cross HS et al., 1997) and can internally metabolize this into the active form of the vitamin, 1,25(OH)$_2$D. 1, 25(OH)$_2$D then exerts its anti proliferate action on the cell and so is a preventive measure against cancerous growth. Receptors for 25(OH)D have since been found in many other cells, including the breast and prostate (John EM et al., 1999;
Schwartz G G et al., 1998; Zehnder D et al., 2001; Ingles SA et al., 2000; Yee YK et al., 2005) whose cancers have been negatively correlated with sunlight exposure. Vitamin D has also been suggested as being effective against hypertension with receptors found in blood vessels and hypertension decreasing with increasing UV-B exposure (Krause R et al., 1998). It may also help prevent the development of some autoimmune diseases such as multiple sclerosis and Type I diabetes. There are clear benefits of UV exposure, and in the absence of careful dietary control UV exposure is necessary for the skeletal health and has potential benefits for a range of other diseases. It is also clear that whereas a moderate amount of UV exposure is beneficial, too much is detrimental. In this paper we examine moderate UV exposure by optimally balancing the beneficial and harmful effects of UV radiation. We have model the erythemal and vitamin D effective solar radiation for all seasons and latitudes to enable estimates of the optimum exposures at different latitudes and for different skin types. We are not aware of any other study that provides guidelines on UV exposure duration taking all major variables into account, including variable atmospheric and surface conditions, time of day, percent body exposure and dietary vitamin D intake case scenario are stated, when applicable. For results associated with the UV indices, the matching cloud liquid water column was applied. FastRT have used to compute erythema vitamin D₃ effective
UV doses. (MacKinley A F et al., 1987; Mac Laughlin JA et al., 1982.)

4.4. Seasonal Variation of Vitamin-D Synthesis

Clinical observations in the past indicated that rickets was common in spring but rare in autumn, suggesting that summer sunshine was an important source of vitamin D. Using bioassay techniques, seasonal variation in plasma antirachitic activity was detected in southern latitudes of the USA, but effects of sunshine were thought to be minimal in the UK. The development of biochemical assays for vitamin D metabolites permitted an examination of the relative importance of diet and endogenous skin synthesis and lead to the finding of a seasonal variation in plasma 25(OH) D levels in healthy white subjects in the cross-sectional and longitudinal studies in Britain (McLaughlin et al., 1974; Stamp TCB., 1974). This was the first demonstration of the prominent seasonal variation of vitamin-D level in the plasma. Peak 25(OH) D levels were found in the autumn and were unrelated to dietary vitamin D intake. This indicated that a contrary to earlier views, summer sunshine was an important determinant of vitamin D synthesis in Britain. The finding of low dietary intakes in some healthy subjects, together with the fact that vitamin D intake did not correlate with peak autumn plasma levels, suggested that summer sunshine might indeed make a
greater contribution than diet to vitamin D stores. Subsequent studies from many parts of the world have confirmed a significant seasonal variation of the 25(OH) D in all groups examined, and sunlight exposure is now accepted as the major determinant of vitamin D stores (Haddad & Hahn., 1973; Fraser DR., 1983). Studies of the selected groups have provided additional information on the factors affecting seasonal variation in vitamin D levels in plasma and insights into causes of clinical vitamin D deficiency.

4.4.1. Age and sex

In a cross-sectional study of a selected healthy population in Baltimore aged from 20 to 94 years, significant seasonal fluctuation occurred in both men and women, and was unaffected by age. In the combined population the values for 25(OH) D increased from a nadir in April-May of 72.9nmol/l to a zenith in October of 95.8nmol/l. The mean amplitude of the cyclic sine wave was similar in men and women (11.9 (SD 3.02) v. 11.0 (SD 3.48) nmol/l). Since control of the circulating active hormonal form of vitamin D is tightly regulated, it was interesting that there was also a significant seasonal variation in 1,25(OH)₂D for both sexes, suggesting a substrate-product relationship (Sherman et al., 1990).
An inverse seasonal relationship has been reported between seasonal serum 25(OH) D and PTH concentrations. In a large cross-sectional study of healthy white postmenopausal American women in Massachusetts who had low median Ca (408 mg/d) and vitamin D (2.8 mg/d) intakes, highest PTH concentrations were found in the spring, when 25(OH) D levels were lowest (Krall EA et al., 1989). Postmenopausal white women (aged 52-77 years) from the mid-west of the USA found to have decreased serum 25(OH) D had low vertebral bone density compared with matched controls with normal circulating 25(OH) D. Significant seasonal variation in serum 25(OH) D was observed in the controls but not in the low-25(OH) D group. Since the women had similar dietary vitamin D intakes, the results indicated that in white postmenopausal women, vitamin D deficiency is primarily accounted for by diminished sunlight exposure (Villareal DT et al., 1991). However, serum total and ionized Ca levels, which are under tight homeostatic control, do not show seasonal changes and are relatively stable with age (Sherman et al., 1990).

4.4.2. Ageing and immobility

The lowest serum 25(OH) D concentrations have been found in institutionalized elderly people in countries (such as Britain) that do not routinely fortify foods with vitamin D. Three elderly populations in Boston, grouped according to disability, were investigated to assess the
relative seasonal contributions of diet and outdoor activity as sources of vitamin D. They comprised free-living elderly subjects (mean age 80 (SD 6) years); independently mobile people in a residential home (mean age 81 (SD 8) years); and limited mobility nursing home residents (mean age 82 (SD 9) years). Sunshine, UV-B exposure measured by polysulphide badges showed marked differences between groups in their solar UV-B exposure. This was greatest in free-living elderly, but even the most care-dependent residents gained some outdoor exposure in midsummer (Webb et al., 1990). Serum 25(OH) D concentrations and seasonal changes in healthy mobile independent elderly are similar to those in young adults. However, seasonal changes decline in magnitude in elderly subjects with decreasing mobility, resulting in reduced outdoor activity and sunshine exposure. This is accompanied by a decrease in the seasonal variation related to decrease UV exposure. Dependent institutionalized elderly individuals have much lower circulating concentrations of 25(OH) D at all times of the year, and are much more likely to have low levels associated with privation disease during both winter and summer months (Sherman et al., 1990).

4.4.3. Dietary and supplemental vitamin-D

The diet is thought to contribute very little to vitamin D stores, and dietary intake of vitamin D fails to provide an adequate amount for institutionalized elderly with minimal outdoor activity, even in the USA
where milk is fortified. However, vitamin supplements can modulate the normal seasonal variation in serum 25(OH) D, and use of a vitamin D supplement providing 10μg (400 IU) vitamin D maintained serum 25(OH) D concentrations at satisfactory levels in institutionalized elderly in Boston throughout the year. In contrast, fit independent elderly, who enjoy the increased sunlight exposure their increased mobility had seasonal serum 25(OH) D levels comparable with those achieved by institutionalized elderly taking vitamin D supplements. Seasonal change in vitamin D status was apparent in nursing home residents as a whole, but was small compared with differences between elderly with and without vitamin D supplements. Seasonal variation was smaller in those who took a daily multivitamin tablet. In the healthy free-living elderly, dietary intake of vitamin D is less important (Webb et al., 1990).

The modulation of seasonal changes by vitamin supplements has also been shown in healthy white postmenopausal American women (mean age 58 (range 43-71) years). In this group the overall inverse relationship between serum 25(OH) D and PTH levels was found to be dependent on vitamin D intake. In women whose estimated intake of vitamin D was under 5.5 mg/d the mean PTH levels were lowest between August and October, when peak values for 25(OH) D were obtained, and highest between March and May, when 25(OH) D trough levels were observed.
However, for those women with vitamin D intakes of more than 5.5 mg/d, serum PTH and 25(OH) D levels did not vary with the season. Seasonal and reciprocal changes in PTH were no longer apparent at 25(OH) D levels>95nmol/l which is well above what, is considered 'normal' (Krall et al., 1989).

4.4.4. Skin pigmentation and vegetarian diet

As a result of immigration, about 2.5% of United Kingdom residents are now of south Asian or Indian subcontinent origin. Clinically significant vitamin D deficiency was first recognized among Glasgow's immigrant Asian community in the 1960s and is now known to be endemic in the British Asian population (Clements MR., 1989; Henderson JB et al., 1990). Latitude and sunshine exposure have been considered important determinants of Asian rickets in the UK, but even in the south of Britain subclinical vitamin D deficiency is prevalent among apparently healthy non-hospitalized Asians, and bone biopsy has shown that a high proportion of Asian outpatients attending medical clinics have histological osteomalacia (Finch PJ et al., 1992b). Although some have implicated dietary factors in the aetiology, others have considered inadequate exposure to naturally occurring UV light to be an important factor. The British Asian population constitute the largest reservoir of endemic vitamin D deficiency in the UK but dark skinned people in the Britain originating from Africa or the West Indies
do not appear to be at risk of vitamin D deficiency. Studies of seasonal changes in serum 25 (OH) D levels have provided useful insights in explaining the paradox of vitamin D deficiency among Asian immigrants. If inadequate solar exposure is to be considered an aetiological factor in vitamin D deficiency in Asian immigrants, there should be an evidence of reduced seasonal variation in 25(OH) D among vitamin D-deficient Asians. In Asians living in Rochdale a significant seasonal rise in serum 25(OH) D was confirmed (Stephens WP et al., 1982). The seasonal increase in adult males was greater than that in adult females, and it was found that the vitamin D-replete subgroup (spring 25(OH) D >12.5nmol/l) achieved a mean autumn level (39.0 (SD 19.0) nmol/l) which was higher than in the vitamin D-deficient individuals (16.5 (SD 8.5) nmol/l). A highly significant relationship was observed between the seasonal change in serum 25(OH) D concentrations and the prevailing (mean of spring and autumn) level. These results indicated that vitamin D-deficient Asians, who had an attenuated seasonal rise, responded to summer sunshine in a different way to vitamin D-replete Asians. The authors suggested that the most likely explanation for these findings was that vitamin D-deficient Asians were exposed to less summer sunshine, despite the fact that their assessment of mean solar exposure showed no significant difference between the groups (Stephens WP et al., 1982). Asian immigrants in
Britain are heterogeneous but the Rochdale study did not distinguish between ethnic subgroups. A more recent report has suggested that an attenuated seasonal rise in vitamin D in some of Asians may be due to culturally determined differences in the diet. In a large cross-sectional study in south London serum 25(OH) D levels were measured in 297 adult Asian and sixty-eight white subjects.

Seasonal variation was compared between subjects grouped according to ethnic origin, religion and dietary habit. A subgroup of Asians with symptoms and biochemical changes suggestive of osteomalacia underwent bone biopsy. Histological osteomalacia was detected in fifteen subjects (and borderline changes in a further thirteen subjects). The majority of these cases were among vegetarian Hindus. Significant seasonal variation was observed among all groups, but with lower peak and trough levels among Asians compared with white subjects. However, an examining subgroups of Asians it was found that non-vegetarian Asians had similar seasonal rise and peak values to those of white subjects, while vegetarian Asians (who were overwhelmingly Hindus) had significantly lower peak values and an attenuated rise compared with white subjects and non-vegetarian Asians (Finch PJ et al., 1992a).
Figure 4.6. Seasonal changes in serum 25-hydroxycolecalciferol 25(OH) D for white subjects (---), non-vegetarian Asians (-----). Values are means with their standard errors represented by vertical bars. From Finch et al., (1992a). Reproduced by permission of editor of European Journal of Nutrition.

These findings are paradoxical since in vivo studies have shown that the activity of hepatic 25-hydroxylase is greatest at low levels of vitamin D supply, and in white subjects the 25(OH) D response to vitamin D administration is inversely related to the initial 25(OH) D level. However, the findings in south London Asians are consistent with the earlier observation in Rochdale that vitamin D-deficient Asians responded to summer sunshine with an attenuated rise in 25(OH) D, compared with vitamin D-replete Asians. Analysis of the findings on seasonal changes in 25(OH) D in Asians resident in the south of England by religious and cultural groups indicate that Asians have the potential for achieving the same peak 25(OH) D levels as the indigenous white population. However, in lacto vegetarians (largely Hindus) seasonal responses are blunted, resulting in significantly lower peak values than for white subjects or non-vegetarian Asians. Furthermore,
vegetarian Asians who show this attenuated seasonal variation in vitamin D levels have a substantially greater risk of osteomalacia than non-vegetarian Asians. Whether the effect of being vegetarian is due to accelerated catabolism of endogenous 25(OH) D as a result of hyperparathyroidism consequent on a low-Ca, high-cereal diet (Clements MR., 1989), or other factors (such as impaired hepatic hydroxylation) remains obscure. In the south London study multivariate analysis failed to show an effect of vegetarian diet on PTH independent of osteomalacia.

4.5. Possible Biological and Clinical Significance of Seasonal Variation in Vitamin-D

First, the possible effects of seasonal variation of vitamin D changes on mineral metabolism will be examined, and next, possible consequences for wider cellular functions of vitamin D of particular interest are biological phenomena or clinical conditions which have a seasonal or latitude gradient.

4.5.1. 1,25-dihydroxycholecalciferol and calcium absorption

Serum Ca is tightly controlled, but a number of studies have reported that urinary Ca excretion appears to peak in summer months. This could be explained by higher intestinal Ca absorption in summer compared with winter, which was first reported in early balance studies.
(McCance RA., 1943; Malm OJ., 1958). Recently, seasonal changes in Ca absorption have been demonstrated with radioisotope methods for assessing Ca absorption and retention. Fractional Ca absorption was significantly higher in postmenopausal women evaluated in the months from August to October than that from March to May (Krall & Dawson-Hughes B., 1991). Interestingly, an increased level of Ca retention was associated with a lower rate of bone loss from the radius. These changes in Ca absorption and excretion could be accounted for by the small seasonal changes in hormonal metabolite of vitamin D $1,25(OH)_2D$ found in some, but not all studies.

4.5.2. Parathyroid hormone and bone mineral density

As mentioned previously, serum PTH levels have been found to have a significant inverse relationship with $25(OH)D$ in postmenopausal women and elderly subjects. These findings have suggested that a seasonal increase in PTH levels results from a seasonal decrease in $1,25(OH)_2D$ stimulated intestinal absorption of Ca. The seasonal increase in PTH may have adverse effects on bone. It is well recognized that accelerated bone loss occurs in hyperparathyroidism, but it is not known whether slight increases in PTH also affect bone loss. However, seasonal variations in bone mineral content of metacarpal and lumbar spine bone have been reported in healthy postmenopausal women (Krolner B., 1983). Postmenopausal women with low $25(OH)D$
concentrations (and absent seasonal variation) were found to have significantly reduced vertebral bone density compared with controls. Increased PTH activity was the major determinant of the vertebral osteoporosis. Serum concentrations of 25(OH) D of >95nmol/l, which are considerably above the lower end of the normal range, prevent a seasonal increase in PTH (Krall ER et al., 1989). Although even small increases in PTH may have a potentially harmful effect on the skeleton by accelerating bone loss in winter, such an effect, and its prevention by an increased intake of vitamin D, should be documented before specific recommendations are made.

4.5.3. Metabolic bone diseases

In the late 19th and early 20th century childhood rickets was known to have a peak seasonal incidence in late winter and early spring. This is not surprising in view of the subsequent demonstration of seasonal changes in vitamin D. No such seasonal presentation has been described for adult osteomalacia but histological studies have shown that the major determinant of osteomalacia in Asian immigrants is a vegetarian diet. Vegetarian Asians have an abnormally attenuated seasonal rise in 25(OH) D. Increasing severity of osteomalacia is associated with increasingly strict vegetarian practice, which accounts for the excess risk in females, Hindus and Asians originating from East Africa (Finch PJ et al., 1992a, 1992b).
Seasonal changes in hip fractures, with a winter peak and summer nadir similar to seasonal vitamin D variation, have been recognized in both Britain and the USA. Analysis of data on 33000 Scottish hospital admissions for fractured neck of the femur over a 5-year period (1983-87) showed significant seasonality, with seasonal amplitude about 10%, which becomes greater with age (Douglas AS., 1993). The role of vitamin D deficiency in the pathogenesis of osteoporotic fractures is controversial and any association may be coincidental. However, reports of seasonal changes in bone mineral density the recognition of inverse seasonal changes in PTH which may be responsible for accelerated bone loss (Webb AR et al., 1990), association of low 25(OH) D levels with osteoporosis in postmenopausal women and the role of vitamin D in regulating the synthesis of the abundant bone-matrix protein osteocalcin (Reichel H et al., 1989) make the possibility of a causal association with osteoporotic fractures plausible.

4.5.4. Behaviour

Specific vitamin D receptors are found in a number of parts of brain and spinal cord. From their anatomical distribution functional implications have been postulated. It has been suggested that seasonal changes in 25(OH) D and 1,25(OH)$_2$D could underlay seasonal changes in hormonal function, mood and behaviour, cell proliferation, differentiation and reproductive functions. Interestingly, vitamin D
receptors have been detected in a number of brain sites in vertebrate seasonal breeding animals but were undetectable in the same tissues in non-seasonal breeders. Biologists have proposed that all components of sunlight, UV, visible and long wavelengths, co-operate towards effective seasonal and diurnal adaptation to assure development, growth and reproduction for the survival of the species. These suggestions are speculative but it would be of interest to determine what changes in seasonal activity are attributable to visual light input via retinal and extra ocular photoreceptors, and those which might be due to genomic target effects of vitamin D on brain vitamin D-receptor regions that parallel seasonal changes in UV-B induced blood levels of the steroid hormone of sunlight (Stumpf WE., 1991). The relevance to human behaviour, if any, is unknown. However, affective disorders with cyclic seasonal onset also appear to have a striking latitude gradient. Mood changes are thought to be due to reduction in daylight hours and altered circadian secretion of melatonin. Whether seasonal changes in UV light and vitamin D contribute is not known.

4.5.5. Cellular growth and proliferation

The notion that vitamin D may protect against colon cancer is based on epidemiological observations, animal experiments and intervention studies. Mortality rates are highest in areas of the USA and rest of the world that receive the least amounts of sunlight. Vitamin D
metabolites suppress in vitro growth and increase differentiation of human colon cancer cells, and reduce incidence of experimental colon tumours in rats. In a prospective study moderate amounts of dietary vitamin D reduced the incidence of colon cancer in men in USA. Additional evidence has come from a prospective study of about 26000 volunteers which investigated the relationship between 25(OH) D and risk of colon cancer in cases and controls. The risk of getting colon cancer was decreased threefold in people with a 25(OH) D concentration of 20ng/ml or more. The study did not take account of possible confounding factors such as consumption of milk, meat and fat in diet. However, these findings, in conjunction with previous epidemiological and laboratory studies, suggested that vitamin D metabolites, possibly working in conjunction with Ca, reduce colon cancer risk (Garland CF et al., 1989).

4.5.6. Immunoregulation

There is animal experimental evidence both in vitro and in vivo for an immune regulatory role for 1,25(OH)₂D in both lymphocytes and monocytes (Yang S et al., 1990), but the path physiological implications of these properties have yet to be established in a clinical context. However, some clinical observations are of interest. Provisional rickets is associated with increased frequency of infections and impaired neutrophil phagocytoses (Reichel H et al., 1989). A significant excess
of tuberculosis (TB) in Hindu compared with Muslim Asians has been reported, and more recently a case control study of Indian subcontinent Asians in south London has shown a significant trend of increasing risk of TB with decreasing frequency of meat or fish consumption. Strict lacto vegetarians (never eat meat or fish) had an 8.5-fold risk compared with those who took meat or fish daily. These results suggest that a vegetarian diet may be an independent risk factor for TB in immigrant Asians. Since vitamin D deficiency is more common among vegetarian Asians, who have an attenuated seasonal response, and it is known to have effects on immunological function in animals, vitamin D deficiency may be the factor in a vegetarian diet responsible for reduced immune competence and subsequent reactivation of TB. A number of other incompletely understood disorders are known which have a prominent seasonal component and/or whose prevalence is affected by latitude; they include multiple sclerosis, where mortality rates show a clear north-south gradient, and ischemic heart disease, where mortality rates are affected by both season and by latitude. Disorders of cellular proliferation and differentiation such as psoriasis, and various common malignancies (colon and prostate cancer) also show prevalence rates which vary with latitude.