

# Vitamin D Deficiency, Metabolism and Routine Measurement of its Metabolites [25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub>]

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## Abstract

Studies around the world, irrespective of latitude, show that the majority of the world's population has insufficient vitamin D status. Vitamin D deficiency is common in the middle east where sun shines round the year. Vitamin D deficiency is linked to many serious diseases such as rickets, multiple sclerosis, osteoporosis, CVD, tuberculosis, diabetes, certain cancers, cognitive decline, depression, pregnancy complications, autoimmunity, allergy, and even frailty. The prevalence of hypovitaminosis D is significantly high among population of UAE, Saudi Arabia and many middle eastern countries, especially among women, despite abundant sunshine. It is clear that sufficient levels of serum 25(OH)D are essential for optimizing human health. There is a growing consensus that the optimal range for 25(OH)D values lies above 30 to 32 ng/mL (75-80 nmol/L) for most populations, and it seems prudent that persons at high risk of vitamin D deficiency and/or insufficiency have their serum 25(OH)D assessed. An effective strategy to prevent vitamin D deficiency and insufficiency is to obtain some sensible sun exposure, ingest foods that contain vitamin D, and take vitamin D supplement.

**Keywords:** Vitamin D; Deficiency; Metabolism; Measurement; Elecsys; 25(OH)D<sub>2</sub>; 25(OH)D<sub>3</sub>; Electrochemiluminescence; Roche Diagnostics; United Arab Emirates

## Introduction

There is an epidemic of vitamin D deficiency sweeping across our modern world, and it's an epidemic of such magnitude and seriousness that is not only alarmingly widespread but a root cause of many serious diseases such as rickets, multiple sclerosis, cancer, diabetes, osteoporosis, tuberculosis and heart disease. Public interest in vitamin D is rising because there is intense and growing activity in the research community on the functions and benefits of vitamin D. People living near the equator who are exposed to sunlight without sun protection have robust levels of 25-hydroxyvitamin D - above 30 ng per milliliter (75 nmol/L) [1,2]. In a recent retrospective study presented at the 18<sup>th</sup> vitamin D workshop at Delft, the Netherlands, we found that among 136 countries tested Columbians were having the highest levels (>70 nmol/L) [3]. Columbia being located at 4°N and therefore, sunshine throughout the year. However, even in the sunniest areas, vitamin D deficiency is common when most of the skin is shielded from the sun. In studies in the United Arab Emirates, Saudi Arabia, Australia, Turkey, India, and Lebanon, 30 to 50% of children and adults had 25-hydroxyvitamin D levels under 20 ng per milliliter (50 nmol/L) [4-7]. Despite the important role of vitamin D in maintaining bone health, as well as a variety of other physiologic functions [8], many clinicians are reluctant to treat vitamin D deficiency or insufficiency in kidney stone formers because of the theoretical risk of increasing urinary calcium excretion. Hypervitaminosis D is a well-known cause of hypercalcemia and hypercalciuria, we are unaware of any prospective study in which the effects of standard replacement doses of vitamin D on urinary calcium excretion have been investigated among stone formers [9]. Calcium and vitamin D have been posed as one of the most important micronutrients affecting the prevention of low bone mass at all ages [10]. Calcium metabolism and the control of urinary calcium excretion are regulated by many factors, of which vitamin D is only one. Indeed, evidence suggests that various nutrient-gene interactions involving vitamin D might influence urinary calcium excretion. High calcium excretion is associated with a decreased trabecular BMD in elderly men and may predispose men to trabecular bone loss [11].

As the results of new studies become public, people who have been quietly and desperately suffering with health issues are finding vitamin D to be a remarkable solution for them. Vitamin D is actually a steroidal hormone like estrogen or testosterone. It stands alone as the only 'vitamin' the body can produce on its own. Vitamin D is needed by the body to properly use other substances like calcium, magnesium, phosphate, zinc and boron to build and maintain healthy tissue, skin, bone, teeth, and nerves. Vitamin D is called the "Sunshine Vitamin" because the body naturally produces it through exposure of your skin to the sun. A healthy young light-skinned person can generate up to 12,000 IU of vitamin D with just 20 minutes of sufficient bare skin exposure to a strong noon day sun under good conditions. So, it seems highly unlikely that consumption of more than 400 IU per day, the current suggested daily allowance, would be toxic.

The dietary sources of vitamin D are: cod liver oil, 1 tablespoon 1360 IU, salmon fish (sockeye), cooked, 3 ounces 794 IU, mackerel, cooked, 3 ounces 388 IU, tuna fish, canned in water, drained, 3 ounces 154 IU, milk, vitamin D-fortified, 1 cup 115-124 IU, sardines, canned in oil, drained, 2 sardines 46 IU, liver, beef, cooked, 3.5 ounces 46 IU and egg, 1 whole (vitamin D is found in yolk) 25 IU [12]. Vitamin D (which includes both D<sub>2</sub> and D<sub>3</sub>) carries out essential biologic functions through both endocrine and autocrine/paracrine mechanism. Vitamin D<sub>3</sub> is derived from a cholesterol precursor in the skin, 7-dehydrocholesterol (7-DHC). When the skin absorbs UV-B radiation, the precursor is converted to pre-vitamin D<sub>3</sub>, which undergoes thermally induced transformation to vitamin D<sub>3</sub>

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(cholecalciferol). Vitamin D2 (ergocalciferol) is a synthetic product produced by irradiation of plant sterols - Asclepius, mushrooms etc. Asclepius is a plant grown widely over the mountains of Colorado, USA and believed as God of medicine and healing in ancient Greek religion.

## Metabolism

Vitamin D, whether from the diet or the skin, is metabolized in the liver to 25(OH)D by 25-hydroxylase enzyme. Since 25(OH)D is the most plentiful and stable metabolite of vitamin D in the human bloodstream, it has been accepted as the functional indicator of vitamin D status [13]. 25(OH)D is a prohormone that serves as an immediate precursor to the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D; calcitriol). A single enzyme, 25(OH)D-1- $\alpha$ -hydroxylase (encoded by CYP27B1), is responsible for production of 1,25(OH)2D, in the kidneys which serves as a high-affinity ligand for the vitamin D receptor (VDR). In its endocrine action, 25(OH)D is converted by hydroxylation in the kidney to 1,25(OH)2D, which circulates in the blood as a hormone to regulate mineral and skeletal homeostasis. The primary target of 1,25(OH)2D is the intestinal mucosa in which it directs the calcium transport system to adapt to varying calcium intakes (Figure 1). Blood 1,25(OH)2D (calcitriol) levels are not good indicators of the amount of vitamin D and should not be used to determine sufficiency or deficiency. Therefore, description of the measurement of calcitriol in this review is out of scope. Sun is the key source of vitamin D3 as 90% of total vitamin D3 comes from the sun. Lanolin which is full of cholesterol and fatty acids is often used as a raw material from the skin of furry animals like sheep to extract vitamin D3.

Vitamin D was originally discovered for its ability to prevent the childhood bone disease. Rickets which is characterized by softening of the bones leading to deformity. The major physiological function of vitamin D is to maintain intracellular and extra cellular calcium concentrations within a physiologically acceptable range. Vitamin D plays a key role in maintaining the skeleton by regulating calcium and phosphate homeostasis. Vitamin D-deficiency leads to the bone disease rickets in children and osteomalacia in adults. However, vitamin D can also influence other components of the skeleton, notably the cells that control bone turnover [8,14].

Vitamin D receptors (VDR) have been found in almost every type of human cell, from brain to our bones. Vitamin D controls (directly or indirectly) more than 3000 genes that regulate calcium and bone metabolism, modulate innate immunity affect cell growth and maturation, regulate the production of insulin and renin, induces apoptosis and inhibit angiogenesis. Nearly every tissue in the body has receptors for the active form of vitamin D, 1,25 dihydroxyvitamin D3 [1,25(OH)2D3] or calcitriol. The immunomodulatory role for 1,25(OH)2D3 was proposed more than 25 years ago. This latest function was essentially based on the finding that monocytes/macrophages from patients affected by the granulomatous disease sarcoidosis constitutively synthesize the active form of vitamin D3 [1,25(OH)2D3] from the precursor 25-hydroxyvitamin D (25-OHD), as well as on the data indicating that the receptor for vitamin D (VDR) is detectable in activated, proliferating lymphocytes [15]. Nevertheless, only recently has a clearer picture of the function of 1,25(OH)2D3 as a determinant of immune responsiveness been obtained. The crucial role of 1,25(OH)2D3 in the immune system was confirmed by other evidence. Understanding the vitamin D metabolism is of fundamental importance to know the mechanisms involved in the maintenance of calcium homeostasis. The vitamin D hydroxylases have an important role in providing a tightly regulated supply of 1,25(OH)2D3 [16]. Certain specific factors involved in regulating the hydroxylases may lead

to the design of drugs that can selectively modulate the hydroxylases. The ability to alter levels of these enzymes would have therapeutic

potential for the treatment of various diseases including bone loss disorders and certain immune diseases [16]. First, the intracrine induction of antimicrobial activity by 1,25(OH)2D3 is a pivotal function of the monocyte/macrophage response to infection. Second, sub-optimal vitamin D status is a common peculiarity of many populations throughout the world, with the possible support of monocyte/macrophage metabolism of 25OHD and subsequent synthesis and action of 1,25(OH)2D3 [17]. Its ability to bind to transporter protein, namely, vitamin D binding protein, enables it to reach other districts that will be its target. These effects create an environment suitable for gene transcription [18].

## Measurement of D2 and D3 Metabolites as Total Vitamin D

According to Mayo Clinic, the following patients should be screened for vitamin D deficiency [19]:

- Individuals receiving therapy to prevent or treat osteoporosis.
- Elderly people, especially those with minimal exposure to sunlight.
- Patients with signs and symptoms of hypocalcemia or hypercalcemia.
- Children and adults with suspected rickets and osteomalacia, respectively.
- Patients receiving vitamin D therapy who do not demonstrate clinical improvement.

Vitamin D testing has significantly increased in recent years, most likely due to emerging evidence linking D-deficiency to the development of cancer, diabetes, autoimmune and cardiovascular diseases. There are several FDA approved methods available for the measurement of vitamin D in blood. Here we found a list of FDA approved methods for vitamin D testing: DiaSorin (LIAISON); ESA Bioscience (HPLC); Siemens Health care Diagnostics (ADVIA); Abbott Diagnostics (ARECHITECT); Ortho-Clinical Diagnostics (VITROS®); Roche Diagnostics (ELECSYS); Tosoh Bioscience (AIA-Pack). However, three methods are popularly used in the labs/hospitals around the United Arab Emirates namely Roche Diagnostics (ELECSYS), DiaSorin (LIAISON®) and Abbott Diagnostics (ARECHITECT). We are routinely using ELECSYS Vitamin D Testing Method (Roche Diagnostics) in our lab for the last more than three years and getting consistent results. The ELECSYS Vitamin D Total assay is a competitive protein-binding assay using a recombinant VDBP (recVDBP) that captures both 25-OHD3 and 25-OHD2 metabolites, allowing the quantitative determination of total 25(OH)D. Signal generation is based on the electro-chemiluminescence technology, generating high sensitivity [20,21]. Most of the total 25(OH)D in plasma or serum is represented by 25(OH) D3, whereas 25(OH)D2 is present in significant amounts only in subjects taking vitamin D2 supplements [22-24]. The required sample volume is 15  $\mu$ l and the overall duration of the assay is 27 min. External quality controls are routinely used provided by the College of American Pathologists (CAP), Chicago, USA.

Dr. Ravinder Singh [25] of Department of Laboratory Medicine and Pathology, Mayo Clinic and Foundation, Rochester (MN) 55905 USA, has published about the use of liquid chromatography-mass spectrometry (LC-MS/MS) technique for vitamin D measurement. This method measures vitamin D2, vitamin D3, and the D3 epimer

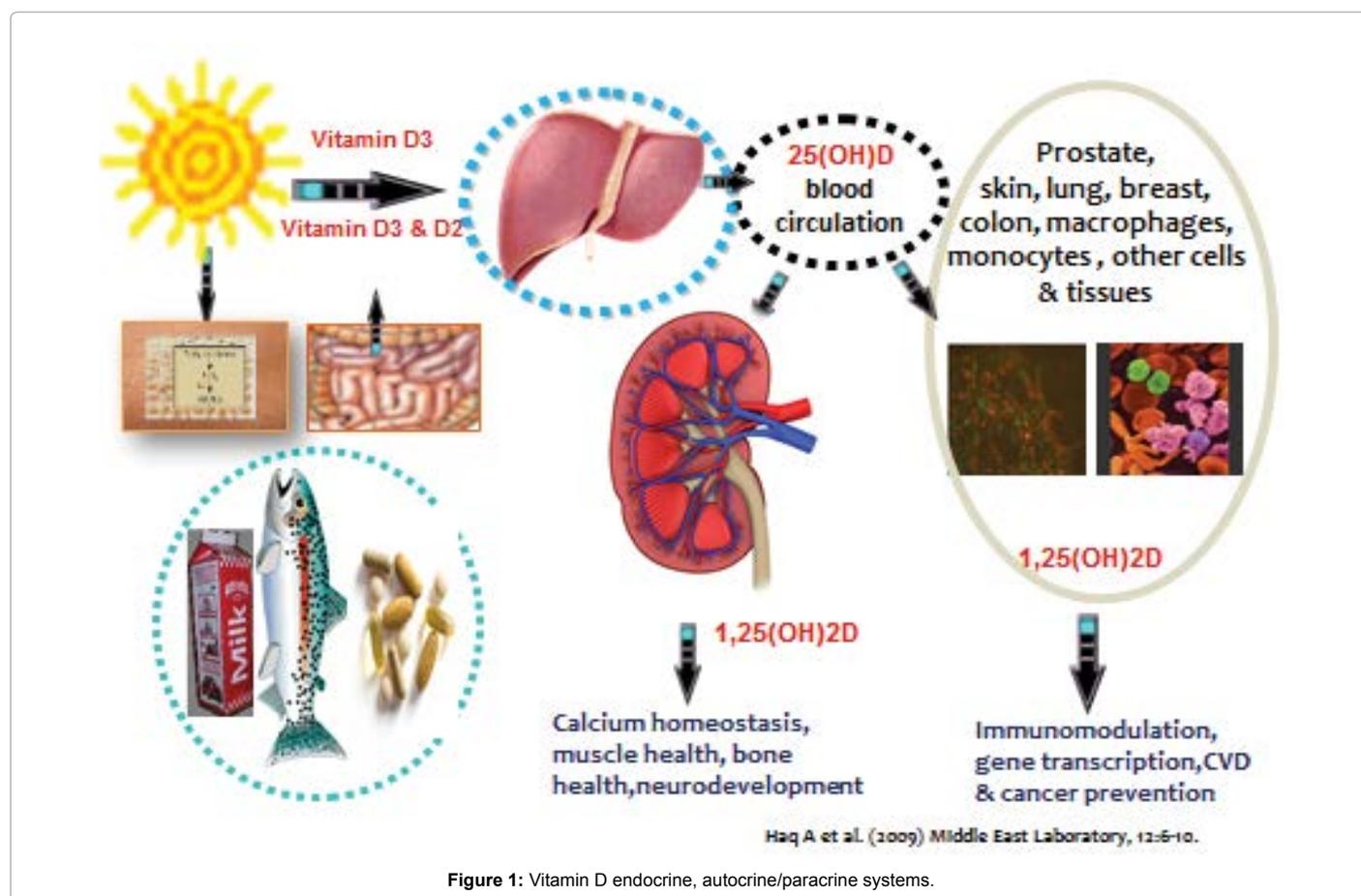


Figure 1: Vitamin D endocrine, autocrine/paracrine systems.

separately; through calculation, total vitamin D is reported. This method was chosen by the Nutritional Laboratory at the Centers for Disease Control and Prevention (CDC) and the National Laboratory in the United Kingdom for analysis of vitamin D for health and nutrition surveys, partly due to its ability to distinguish the various forms of vitamin D in plasma that may be found in people of all ages [26-28]. LC-MS/MS is a sensitive and specific method that is referred to as a 'gold standard' test. Can be slow and requires expensive equipment, routine maintenance and skilled staff. Laboratories performing 25(OH)D testing by LC-MS/MS technology have differences in their standard operating procedures, and thus inter-laboratory CVs are in the range of 20%. Multiplexed immunoassays may have the potential of achieving accuracy and precision for multiple vitamin D metabolites. For better patient care, the goal should be not only to have an accurate 25(OH)D value but also precision for 25(OH)D testing, with a CV <1% [25]. The SI units for vitamin D concentration are converted to traditional units by the equation  $25(\text{OH})\text{D nmol/l} = 25(\text{OH})\text{D ng/ml} \times 2.5$ .

Reference ranges used are based upon the recommendations according to the Endocrine Society [29] and the Society for Adolescent Health and Medicine [30]. Thus hypovitaminosis D was defined in presence of 25(OH)D levels <75.0 nmol/L (30 ng/mL). Furthermore, severe vitamin D deficiency was defined as 25(OH)D levels <25.0 nmol/L (10 ng/mL) and were defined as follows: deficiency: <25 nmol/L, optimal/sufficiency: 75-200 nmol/L, insufficiency: 25-75 nmol/L and toxicity: >250 nmol/L. According to the latest Institute of Medicine (IOM) recommendations, 25(OH)D levels corresponding to a serum 25(OH)D status of at least 50 nmol/L indicates sufficiency [31]. However these levels might be true for rickets and other bone

related diseases but not for non-calcemic disorders where higher levels of vitamin D up to 200 nmol/L are recommended.

The historical underpinnings of contemporary perspectives on vitamin D toxicity are rarely appreciated, but the concept that vitamin D is one of the most toxic fat-soluble vitamins has been instilled in the psyche of health regulators and the medical community. Currently, there is great concern about the potential for the widespread increased use of vitamin D increasing the risk for kidney stones, cardiovascular calcifications, and even death [32-33]. Professor Michael Holick recently published an interesting editorial about the various aspects of vitamin D toxicity [33]. The Endocrine Society's practice guidelines suggest daily vitamin D supplementation of 400 to 1000, 600 to 1000, and 1500 to 2000 for ages 0 to 1 year, 1 to 18 years, and all adults, respectively [34] (obese adults require doses 2-3 times higher). However, there are clinical circumstances that can cause hypercalcemia when giving patients these recommended doses of vitamin D. These conditions include patients with granulomatous disorders including sarcoidosis, William syndrome, some lymphomas, and the rare genetic disorder of the absence of the 25-hydroxyvitamin D-24- hydroxylase [34-37]. The evidence is clear that vitamin D toxicity is one of the rarest medical conditions and is typically due to intentional or inadvertent intake of extremely high doses of vitamin D (usually in the range of >50,000-100,000 IU/day for months to years) [38]. Glucocorticoids have been routinely used to treat patients with vitamin D intoxication. However, the adverse effects of treatment can be considerable, including the increased risk for gastrointestinal bleeding, aseptic necrosis of the hip and infectious diseases. Simply reducing the calcium intake, wearing sun protection to prevent vitamin D production, and eliminating all

vitamin D from dietary sources will result in a gradual decrease in serum 25(OH)D levels, with no significant sequel from the toxicity [34,37].

## Discussion

The prevalence of vitamin D deficiency due mostly to sun deprivation in a sun-blessed country among young-adult Emiratis. The results of all these studies emphasize the need for urgent measures in the Middle East to avoid long-term complications related to vitamin D deficiency; e.g. vitamin D supplementation, life style and fortification of some highly consumed food, milk and other dairy products. Educational endeavors about sensible sun exposure should be implemented to improve vitamin D status among this population. Women had a higher measure of sun avoidance attitude, suggesting that the sex differences in 25(OH) D concentrations are attributed to behavior toward sun avoidance or exposure. Emirati women and men usually cover most of the body for cultural and religious reasons; however, our data show that women tend to avoid the sun more than do men. The mean serum 25(OH)D concentration for females tested in winter was significantly higher than that in summer. This seasonal pattern is the opposite of what is reported for many countries. This finding could be attributed to the fact that winter in Abu Dhabi is cool yet sunny, hence residents tend to engage highly in outdoor activities in contrast to the summer, which is humid and extremely hot, a time when people significantly limit their outdoor activities. The observed differences in serum 25(OH) D by season are in concordance with the findings reported by Saadi et al. [37] who indicated that optimal levels were obtained in April compared with August and October. There was a seasonal variation in the blood status of 25(OH)D due to increased sun exposure. The exposure time was noted between 11 in the morning and 3 in the afternoon. Mean serum 25(OH)D concentrations were higher in April, which marks the end of the short, cool winter season and lower in October, which marks the end of the hot summer season. Although many studies have reported the inverse association between BMI and serum 25(OH)D concentrations, few have also demonstrated the absence of correlation in some populations. Further exploration of how to remedy vitamin D deficiency in Emiratis is needed. Staple foods are hence good targets for food fortification, provided that the right recommended intake is really attained. Jordan recently issued a food policy that mandates the fortification of bread with vitamin D [38,39]. However, the amount of vitamin D in food would have to be about 1,000-3,000 IU/day to be effective in increasing serum 25(OH) D status to the 100 nmol/L that optimal health requires [40-42]. This study documents the prevalence of vitamin D deficiency due mostly to sun deprivation in a sun-blessed country among Emiratis. The results of all these studies emphasize the need for urgent measures in our part of the world to avoid long-term complications related to vitamin D deficiency; these measures include vitamin D supplementation and fortification of some highly consumed food, milk and other dairy products. Educational endeavors about sensible sun exposure should be implemented to improve vitamin D status among this population. Whether Emiratis are predisposed to vitamin D deficiency by inability to maintain adequate vitamin D status (due mainly to sun avoidance) or the possible existence of polymorphism in the vitamin D receptor gene or other related genes is worth investigating [43]. Other recent publications based on the Elecsys assay have confirmed that the assay has good precision and accuracy, and shows close agreement to other well established methods for 25-OHD analysis, making it very suitable for routine assessment of vitamin D status [44-47]. In conclusion, the Elecsys assay demonstrated low imprecision, high sensitivity, good lot-to-lot consistency, as well as good overall agreement with

measurements obtained using LC-MS/MS and HPLC methods. One key element for establishing a reliable automated immunoassay for 25(OH)D is to ensure high consistency in the production of individual assay components. For a routine vitamin D assay, it has been calculated that the total imprecision (within-laboratory precision should be <10% and the data in earlier publication show that the Elecsys assay fulfills this requirement [48]. Recent publications based on the Elecsys assay have confirmed that the assay has good precision and accuracy, and shows close agreement to other well established methods for 25(OH) D analysis, making it very suitable for routine assessment of vitamin D status [49].

## Conclusion

Research during the last two decades in the field of vitamin D suggests that vitamin D is much more than a nutrient needed for bone health; it is an essential hormone required for regulation of a large number of physiologic functions. All studies, in virtually all nations, irrespective of latitude, show that the majority of the world's population has insufficient vitamin D status. It is clear that sufficient levels of serum 25(OH)D are essential for optimizing human health. However, many questions remain unanswered. For example, what levels of serum 25(OH)D are optimal? What amount of supplementation or sunlight exposure is needed to achieve and maintain these optimum levels? There is a growing consensus among the leading researchers in the field of vitamin D that the optimal range for 25(OH)D values lies above 30 to 32 ng/mL (75-80 nmol/L) for most populations, and it seems prudent that persons at high risk of vitamin D deficiency and/or insufficiency have their serum 25(OH)D levels assessed. Elecsys assay have confirmed that the assay has good precision and accuracy, and shows close agreement to other well established methods for 25(OH) D analysis, making it very suitable for routine assessment of vitamin D status. Successful completion of clinical trials is essential to establish the efficacy and safety of vitamin D supplementation on a population level. Public education should be provided about the safety of vitamin D supplementation and the value of sensible sunlight exposure. Last but not least, still there are questions to be answered: Should vitamin D, a natural product be subject to the same stringent methods used to test novel drugs? What is the optimal method of delivery of vitamin D, oral therapy or stops therapy, is it daily, monthly or weekly supplementation? Are there genetic variants in vitamin D metabolism in the Middle East responsible for endemic vitamin D deficiency regionally? What is the role of atmospheric pollution (overhead sand, carbon emissions, clouds) in inhibiting the sun's UVB rays reaching the ground?

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