Low Vitamin D Status despite Abundant Sun Exposure

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Context: Lack of sun exposure is widely accepted as the primary cause of epidemic low vitamin D status worldwide. However, some individuals with seemingly adequate UV exposure have been reported to have low serum 25-hydroxyvitamin D [25(OH)D] concentration, results that might have been confounded by imprecision of the assays used.

Objective: The aim was to document the 25(OH)D status of healthy individuals with habitually high sun exposure.

Setting: This study was conducted in a convenience sample of adults in Honolulu, Hawaii (latitude 21°).

Participants: The study population consisted of 93 adults (30 women and 63 men) with a mean (SEM) age and body mass index of 24.0 yr (0.7) and 23.6 kg/m^2 (0.4), respectively. Their self-reported sun exposure was 28.9 (1.5) h/wk, yielding a calculated sun exposure index of 11.1 (0.7).

Main Outcome Measures: Serum 25(OH)D concentration was measured using a precise HPLC assay. Low vitamin D status was defined as a circulating 25(OH)D concentration less than 30 ng/ml.

Results: Mean serum 25(OH)D concentration was 31.6 ng/ml. Using a cutpoint of 30 ng/ml, 51% of this population had low vitamin D status. The highest 25(OH)D concentration was 62 ng/ml.

Conclusions: These data suggest that variable responsiveness to UVB radiation is evident among individuals, causing some to have low vitamin D status despite abundant sun exposure. In addition, because the maximal 25(OH)D concentration produced by natural UV exposure appears to be approximately 60 ng/ml, it seems prudent to use this value as an upper limit when prescribing vitamin D supplementation. (*J Clin Endocrinol Metab* **92: 2130–2135, 2007**)

L OW VITAMIN D status[1] is extremely common (1–4), and may contribute to the development of osteoporosis and osteomalacia/rickets, as well as increase the risk for falls (5, 6). Moreover, low vitamin D status may play a role in nonmusculoskeletal diseases, including a variety of cancers, multiple sclerosis, infection, hypertension, and diabetes mellitus (7, 8). Although it is widely accepted that vitamin D status is determined by the measurement of the circulating concentration of 25-hydroxyvitamin D [25(OH)D] (9), the cutoff value to define low vitamin D status and a definition for success of vitamin D repletion therapy remain controversial (10, 11). This is partially due to the variability of vitamin D concentration by geographical location and differences in assay methodology (12–16). Despite this controversy, clinicians often endeavor to correct vitamin D deficiency by prescribing high-dose vitamin D (17). However, the goal for such therapy is unclear and could include achieving a serum 25(OH)D level greater than an accepted cutpoint (*e.g.* 30 ng/ml) or, alternatively, the upper limit of normal, a value that varies between laboratories (18).

The high prevalence of low vitamin D status is assumed to result from inadequate sun exposure. Because highly sun-exposed individuals likely possess normal vitamin D status from an evolutionary standpoint, the use of such individuals to define normal 25(OH)D status has been proposed (19). This argument is based on the view that contemporary humans are genetically adapted to the environment of our ancestors and that the profound lifestyle changes that have occurred over the past approximately 10,000 yr (importantly including reduced sun exposure) have been much too rapid for the human genome to adjust (20, 21). The current study was designed to assess whether, in fact, people living at a low latitude with high amounts of sun exposure have adequate vitamin D status, as expected, and to identify a target value of 25(OH)D for use in vitamin D therapy.

Subjects and Methods

Subjects and study design

Subjects older than 18 yr were recruited approximately equally from the University of Hawaii at Manoa (UH) and from patrons of the A'ala Park Board Shop, Honolulu, Hawaii (latitude 21° north), in late March 2005. The A'ala Park Board Shop is a skateboard shop frequented by young adults. Recruitment was performed by posted notice at the Board Shop and on the UH campus; volunteers were reimbursed for study participation. Volunteers were required to have self-reported sun exposure of 3 or more hours per day on 5 or more days per week for at least the preceding 3 months, and not to be currently taking phenobarbital, phenytoin, or prednisone. A total of 93 subjects (63 male and 30 female) participated.

The University of Wisconsin Health Sciences Institutional Review Board and the Committee on Human Studies at the UH reviewed and

[1] We have chosen to use the terminology "low vitamin D status" for a syndrome that others have variously described as "vitamin D insufficiency," "vitamin D inadequacy," or "hypovitaminosis D."

approved this research. All subjects provided written informed consent before the conduct of any study procedure.

Data acquisition

Blood was collected for serum chemistry, 25(OH)D, and PTH measurement. Participants were not required to fast for blood collection, which was performed by routine venipuncture. Samples were allowed to clot for 30–45 min at room temperature, centrifuged, and serum was promptly frozen on dry ice. All specimens were shipped and received frozen on dry ice, then stored at −80 C until thawed for analyses. The 25(OH)D analyses were performed in batches of nine to 16 samples (a total of eight HPLC runs were performed over 14 d) from 10–26 d after specimen acquisition. Three internal controls were run with each HPLC batch; the results of each control were consistently within previously established acceptable ranges. The PTH samples were performed 66–71 d after specimen acquisition in a single run. All participants completed a nonvalidated, self-administered questionnaire that included questions about ethnicity, sun exposure, sunscreen use, and dietary vitamin D intake.

To document sun exposure, skin color was measured by reflectance colorimetry (IMS SmartProbe, Millford, CT). The Commission Internationale de l'Eclairage L scale was used, which ranges from 0 (black) to 100 (white) and represents a system created by the International Commission on Illumination to represent accurately human color perception. A measurement was taken on the back of the hand and front of the distal thigh for the darkest measurement. In addition, skin color was measured under the arm and at the self-reported least sun-exposed area, often the breast or buttock, to determine the lightest or natural skin color. The lowest and highest L scale measurements were subtracted to determine the change in skin color or the delta skin color. A previously developed sun exposure index (22) was used to estimate the amount and duration of skin sun exposure. These data were obtained by asking the subjects to depict their usual amount of skin exposed on a diagram with and without sunscreen use. Subsequently, the rule of nines (23) was used to calculate skin sun exposure in which the front and back torso and each leg were counted as 18%, the arms and head as 9%, and the face only as 5%. This number was then multiplied by the reported average sun exposure per week without sunscreen to calculate the sun exposure index for each subject.

Serum analyses

Serum chemistries were measured using a Roche Integra autoanalyzer at General Medical Laboratories (Madison, WI). Serum 25(OH)D was determined by reverse-phase HPLC (24). The intraassay percent coefficient of variation (CV) for this assay ranges from 1.9% at a 25(OH)D concentration of 61.5 ng/ml to 6.3% at a 25(OH)D concentration of 14.3 ng/ml. The interassay percent CV is 3.2% at a 25(OH)D concentration of 59.8 ng/ml and 3.9% at a 25(OH)D concentration of 14.3 ng/ml. In assay proficiency evaluation, correlation with liquid chromatography

mass spectroscopy performed at the Mayo Medical Laboratories (Rochester, MN) revealed essentially identical results, with r^2 values of 0.99 and 0.97 for 25(OH)D$_2$ and 25(OH)D$_3$, respectively. In addition, 25(OH)D was also determined by RIA (Diasorin RIA, Stillwater, MN) in the laboratory of B.W.H. who also performed serum vitamin D measurement using HPLC (25) in a subset of 19 individuals. The intraassay and interassay percent CVs for these assays are less than 10%. Scantibodies Clinical Laboratory (Santee, CA) using the Scantibodies Laboratory whole PTH or (CAP; cyclase activating PTH) assay measured specific 1–84 PTH or whole PTH ("CAP PTH") (26). For this assay, the intraassay percent CV is 5% at 30.2 pg/ml, and the interassay percent CV is 7.4% at 31.9 pg/ml.

Statistical analyses

Normal data distribution was documented using the Shapiro-Wilk test. Subsequently, relationships between 25(OH)D and sun index, hours of sun exposure, PTH, *etc.*, were evaluated by linear regression. Differences between the lowest quartile and remainder of the cohort were evaluated by the unpaired *t* test. 25(OH)D assay comparison (HPLC to RIA) was evaluated by linear regression and Bland-Altman analysis. All analyses were performed using Statview software (Abacus, Cary, NC) or Analyze-it software (Leeds, UK).

Results

Subject demographics

A total of 93 subjects (63 male and 30 female) participated in this study. Overall, their mean (SEM) age was 24.0 yr (0.7), body mass index (BMI) was 23.6 kg/m^2 (0.4), and supplemental vitamin D intake was 107 IU (18) daily. In addition, their mean (SEM) creatinine, albumin, and calcium concentrations were 0.9 mg/dl (0.02), 4.5 g/dl (0.02), and 9.8 mg/dl (0.04), respectively. The mean (SEM) lightest skin color L scale value was 63.7 (0.5) and the darkest 50.5 (0.5), with a difference of 13.2 (0.4) (data not shown). On average, the 93 subjects reported being outside for 22.4 h/wk (1.6) with no sunscreen, and 28.9 h/wk (1.5) with and without sunscreen (Fig. 1). Of subjects, 40% (37 of 93) reported never using sunscreen. The resultant mean sun exposure index score, indicating hours per week of total body skin exposure with no sunscreen used, was 11.1 ± 0.7 (range 1.0–38.4). Only two subjects reported use of tanning booths; as such, the skin darkening noted previously reflects natural sunlight exposure.
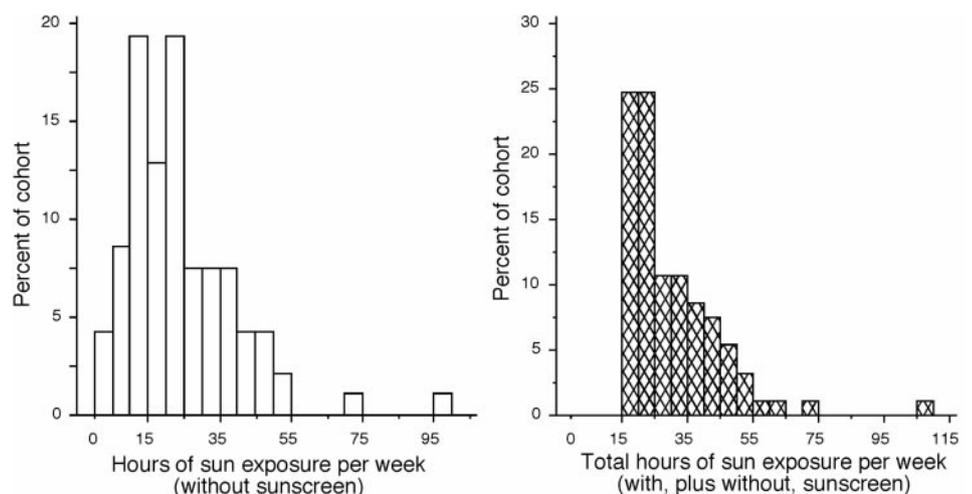

FIG. 1. Amount of sun exposure without and with sunscreen. The mean self-reported sun exposure without sunscreen use was 22.4 h (range 2–96), with a mean total of 28.9 ± 1.5 h/wk. Of this cohort, 40% reported no sunscreen use.

Serum 25(OH)D and PTH

Using the HPLC assay for serum 25(OH)D and applying a widely recommended cutpoint of 30 ng/ml (10), 51% (47 of 93) of these subjects had low vitamin D status (Fig. 2). The highest serum 25(OH)D concentration observed was 62 ng/ml. No correlation between serum whole PTH and 25(OH)D concentration was observed (Fig. 3). Moreover, there was no correlation between serum 25(OH)D measured by HPLC and age, lightest or darkest skin color, delta skin color, hours/week of sun exposure without sunscreen, sun index, total hours of sun exposure/week, or BMI (data not shown). Specifically, delta skin color was not correlated with either PTH ($P = 0.10$; $r^2 = 0.03$) or serum 25(OH)D ($P = 0.18$; $r^2 = 0.02$).

In an effort to evaluate determinants of serum 25(OH)D status, the quartile of individuals (n = 23) with the lowest circulating levels of 25(OH)D was compared with the remaining cohort. The serum 25(OH)D in the lowest quartile (20.7 ± 0.7 ng/ml) was significantly lower ($P < 0.0001$) than in the rest of the population (35.2 ± 1.1 ng/ml). In accord, PTH was higher ($P < 0.01$) in the lowest quartile (15.9 ± 1.4 pg/ml) compared with the remainder of the subjects (12.7 ± 0.5 pg/ml). In addition, the lowest quartile compared with the remaining population demonstrated a significantly lower ($P < 0.05$) sun exposure score (7.2 ± 0.8 *vs.* 12.3 ± 0.9) and delta skin color (11.6 ± 0.7 *vs.* 13.8 ± 0.4) than the remainder of the subjects. Age, BMI, vitamin D supplement intake, serum calcium, alkaline phosphatase, and creatinine did not differ between groups.

Serum 25(OH)D as measured by reverse-phase HPLC and RIA was highly correlated ($r^2 = 0.76$; Fig. 4). However, a systematic bias was present with 25(OH)D values determined by RIA being approximately 6.8 ng/ml higher than by HPLC. Thus, if the Diasorin RIA had been used to determine the prevalence of low vitamin D status (using a cutpoint of 30 ng/ml), fewer individuals would have been classified as "low." However, even using the RIA, 25% of this population would be classified as having low vitamin D status. Finally,

FIG. 3. PTH and 25(OH)D. Specific 1–84 PTH or whole PTH ("CAP PTH") was not related to serum 25(OH)D status measured by HPLC in this cohort.

although these assays were highly correlated, greater scatter at higher 25(OH)D values was observed with the RIA, as has been previously reported (18). This greater scatter at higher values slightly increases the mean bias noted previously; however, even when limiting the analysis to the 47 individ-
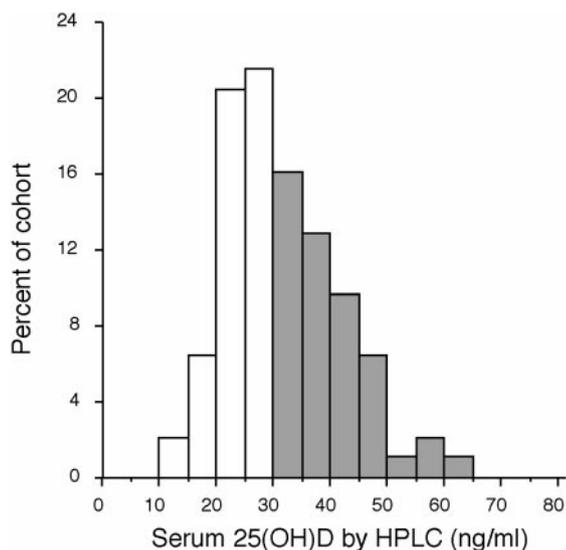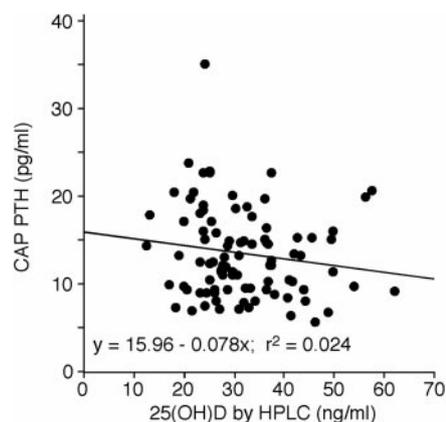

FIG. 2. Low vitamin D status in highly sun-exposed subjects. When an accepted cutpoint of 30 ng/ml is used to define low vitamin D status, 51% of these subjects (*open bars*) are low.
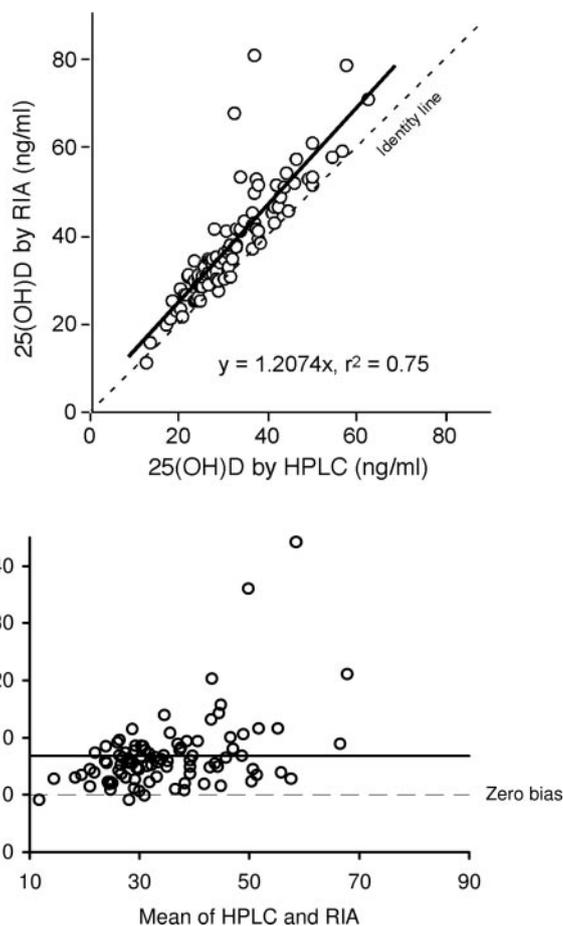
FIG. 4. Comparison of 25(OH)D as measured by HPLC and RIA. Although the correlation between these assays is good, a positive bias of 6.8 ng/ml is present using the Diasorin RIA in comparison to the HPLC assay used in this study.

uals with a 25(OH)D less than 30 ng/ml by HPLC, a positive bias of 5.2 ng/ml persisted.

This study population was of mixed race, with 37 reporting their race as white, 27 as Asian, 18 as multiracial, and 7 as Hawaiian/Pacific Islander. Given the small number, individuals reporting their race/ethnicity as Black or Hispanic (1 and 2, respectively) were not included in this analysis. Serum 25(OH)D was higher ($P < 0.01$) among whites (mean 37.1 ± 1.6 ng/ml) than among Asians (mean 24.7 ± 1.3 ng/ml) or in multiracial individuals (mean 28.9 ± 1.7 ng/ml). In addition, the maximum L score, indicating whiter skin, was higher ($P < 0.01$) in those reporting their race as white (mean 66.3 ± 0.6) than among Asians (mean 62.2 ± 0.8) and multiracial individuals (mean 62.7 ± 1.0).

Serum cholecalciferol (D_3)

In the subset of 19 subjects in whom circulating D_3 was measured, a logarithmic relationship ($r^2 = 0.67$) between serum D_3 and 25(OH)D was observed. It was not until serum D_3 exceeded approximately 15–20 ng/ml that serum 25(OH)D was definitively higher than 30 ng/ml (Fig. 5). Serum D_3 concentration was not correlated with sun index, delta skin color, or BMI (data not shown).

Discussion

In this cohort of young adults, substantial variability in serum 25(OH)D concentration exists despite abundant sun exposure. Surprisingly, a 25(OH)D concentration that many would argue to be too low (10), is common in this highly sun-exposed population. Furthermore, regardless of the amount of sun exposure, the serum 25(OH)D concentration does not increase to more than approximately 60 ng/ml.

Although the presence of "low" 25(OH)D concentration in this population seems counterintuitive, this might be anticipated from an evolutionary standpoint because the high calcium intake of early humans (27) may have allowed maintenance of calcium homeostasis despite low vitamin D status. Moreover, it is certainly plausible that genetic differences exist in the amount of vitamin D necessary to maintain optimal physiological function. Such differences could contribute to the lack of a direct relationship between serum PTH

FIG. 5. Circulating vitamin D_3 in relationship to 25(OH)D concentration. In this population of 19 individuals with a mean sun exposure without sunscreen of 20.2 h/wk and a mean sun exposure index of 10.9, low circulating vitamin D_3 values exist.

and 25(OH)D on an individual basis that is observed in many studies (28, 29). This absence of a direct relationship between PTH and 25(OH)D emphasizes that PTH measurement cannot be used clinically as a surrogate marker of vitamin D deficiency, as exemplified by enhanced calcium absorption at higher vitamin D levels despite normal PTH status (30). In addition, it is possible that genetic differences in the cytochrome P450 enzymes activating and degrading vitamin D exist. Finally, the data reported here are consistent with prior reports of highly sun-exposed individuals that also demonstrate substantial variability in 25(OH)D status. For example, in 18 Puerto Rican farmers with self-reported sun exposure from 32–70 h/wk, two individuals had a 25(OH)D level less than 30 ng/ml (31). Similarly, low 25(OH)D values were reported in some subjects who used a tanning bed at least once a week for 6 wk (32) and among outdoor workers with a sun index of 11.5 (22). Thus, even substantial sunlight or UV exposure does not ensure maintenance of vitamin D adequacy for all individuals, according to currently accepted standards. This implies that the common clinical recommendation to allow sun exposure to the hands and face for 15 min may not ensure vitamin D sufficiency.

A probable explanation for the "low" 25(OH)D status of some individuals is found in their failure to obtain high circulating D_3 concentrations. Possible explanations for this include inadequate cutaneous production of D_3, enhanced cutaneous destruction of previtamin D_3 or vitamin D_3, downregulation of cutaneous synthesis by sun-induced melanin production, or abnormalities of transport from the skin to the circulation. In this regard, Holick et al. (33) documented that human skin has the intrinsic ability to limit vitamin D production. Moreover, a reduction in cutaneous concentration of 7-dehydrocholesterol and a concomitant declining capacity of the skin to make vitamin D occur with advancing age (34, 35). However, in our study the population was predominantly young, which should have obviated such reduced capability for vitamin D synthesis. Importantly, lizards with behaviorally high sun exposure have a lower capacity to produce vitamin D than closely related species with habitually less sun exposure (36). Thus, it appears likely that factors exist, which are not yet well understood, that can restrict skin production of vitamin D in response to UV radiation. In any case, it is crucial that we do not wantonly accept the concept that vitamin D deficiency is due exclusively to inadequate UV exposure. Rather, it seems self-evident from this study that low vitamin D status, as it is currently defined, may occur despite "more than adequate" sun exposure.

An alternate explanation for the "low" values in these highly sun-exposed adults and the corresponding high prevalence of low vitamin D status might reflect 25(OH)D assay variability. However, the prevalence of low vitamin D status in this population is substantial whether 25(OH)D is measured using HPLC or RIA. The systematic bias between HPLC and RIA observed in this study emphasizes the difficulty with setting a single cutpoint value, e.g. 30 ng/ml, below which individuals are classified as having low vitamin D status. The widespread availability of 25(OH)D assay calibrators currently being developed by the National Institute
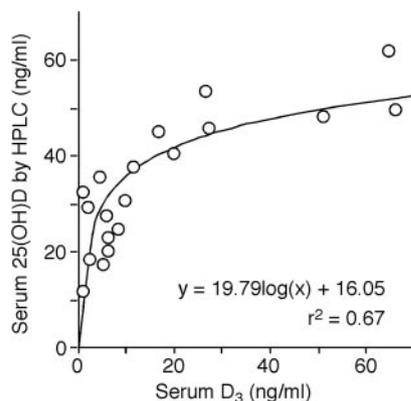
of Standards and Technology could be expected to reduce the magnitude of systematic bias noted here.

These results may allow for rational provision of guidance for clinicians prescribing vitamin D treatment, in that the highest 25(OH)D concentration achievable by sun exposure is approximately 60 ng/ml. An apparent physiological ceiling does not support attempts to achieve higher values by pharmacological intervention. It is of interest that the highest 25(OH)D values observed in this study are quite similar to that reported in other highly sun-exposed populations. For example, the individuals in this study with the three highest levels had serum 25(OH)D concentrations of approximately 60 ng/ml. Similarly, among Nebraska outdoor workers, the three highest reported values were between 81 and 84 ng/ml (22). However, it should be noted that these values were obtained using a competitive protein binding assay for 25(OH)D that measures other vitamin D metabolites in addition to 25(OH)D (37) and, therefore, results in a higher value than that obtained with the HPLC system used in this study.

Limitations of this report include the cross-sectional design and self-report of sun exposure. It is possible that some individuals incorrectly reported their sun exposure and/or body surface exposed. Despite this limitation, this population was clearly highly sun exposed as documented by darkening of exposed skin. In addition, because this study included only highly sun exposed individuals, these observations may not be generalizable to those with less sun exposure. Additionally, it may be argued that the use of 30 ng/ml as a cutpoint is inappropriately high. However, even if a more conservative cutpoint of 20 ng/ml, as suggested by some (10, 29), is used, a substantial minority (~10%) of these individuals would still be "low." Moreover, as noted previously, it is possible that racial and/or genetic differences underlay differences in vitamin D status. However, the racial groups in this study are of insufficient size to define such potential differences. Further investigation of this possibility is appropriate. Finally, this study was conducted following the Hawaiian equivalent of winter during which time there is reduced capability for cutaneous vitamin D production. Despite this limitation, given the low latitude of Hawaii, substantial UV exposure and, therefore, vitamin D production are possible year round (38, 39).

In conclusion, high amounts of sun exposure do not ensure what is currently accepted as vitamin D adequacy. Thus, clinicians should not assume that individuals with abundant sun exposure have adequate vitamin D status. In the event of vitamin D deficiency, the goal of vitamin D replacement therapy should be no greater than the maximum that appears attainable, a serum 25(OH)D concentration of approximately 60 ng/ml.

Acknowledgments

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