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Abstract

Vitamin D deficiency is common in tuberculosis (TB) and this may modulate immune responses.

Objective—To determine vitamin D status in patients with TB and examine sources of vitamin D in Tbilisi, Georgia.

Research Methods and Procedures—We measured plasma 25-hydroxyvitamin D (25(OH)D) and dietary vitamin D intake in pulmonary TB patients (n=85) in Tbilisi, Georgia. To determine the impact of season on vitamin D status, we tested in vitro conversion of 7-dehydrocholesterol (7-DHC) to previtamin D3 after sunlight exposure.

Results—In TB subjects, mean plasma 25(OH)D concentrations were 14.5 ± 7.0 ng/mL, and vitamin D insufficiency (25(OH)D < 30 ng/mL) occurred in 97% of subjects. Dietary sources of
vitamin D were mainly fish, eggs, and butter. Daily intake was well below recommended daily intakes in TB subjects (172 IU + 196 IU). The conversion of 7-DHC to previtamin D$_3$ was undetectable between October to March, and highest in June and July between 11:00 and 14:00 h.

**Conclusion**—Insufficient vitamin D dietary intake and limited production of vitamin D from sunlight during the majority of the year, may explain the high prevalence of vitamin D insufficiency TB patients in Tbilisi.

**Keywords**
cholecalciferol; 7-dehydrocholesterol; dietary intake

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**INTRODUCTION**

Vitamin D is a seco-steroid hormone produced primarily in the skin upon exposure to UVB from the sun or obtained from limited dietary sources$^1$. Cutaneous production of vitamin D is the major determinant of an individual’s vitamin D status$^1$. Vitamin D is synthesized in skin when 7-dehydrocholesterol (7-DHC) is exposed to UVB radiation and then photolysed to previtamin D$_3$, which then undergoes a thermally induced isomerization to vitamin D$_3$. After entering the bloodstream, vitamin D$_3$ undergoes two sequential hydroxylations to form 25-hydroxyvitamin D (25(OH)D), the major circulating form of vitamin D, followed by the hormonal form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)$_2$D)$^2$. Along with cutaneous production, vitamin D can be obtained from dietary sources such as fish, mushrooms, or fortified foods$^1$. In addition to skeletal health and calcium homeostasis, vitamin D regulates many other biological systems including the immune system$^3$. For example, one function of vitamin D is to regulate production of cathelicidin/LL-37, an antimicrobial peptide involved in the innate immune response to *Mycobacterium tuberculosis* (TB) infection$^4$. Vitamin D may other effects on the immune system including effects on both adaptive and innate immunity$^3,5$.

There is a high prevalence of vitamin D deficiency worldwide. While the definition of vitamin D deficiency varies, most studies refer to deficiency as a plasma 25(OH)D concentration <20 ng/mL, and insufficiency as values <30 ng/mL$^1$. In the most recent NHANES, out of 13,369 non-institutionalized civilians in the United States from 2001-2004, 77% of the participants had vitamin D insufficiency$^6$. In North America, hypovitaminosis D has been attributed to obesity, decreased outdoor activity, inadequate dietary intake of vitamin D, and sunscreen usage$^6,7,8$. In other parts of the world, residents are deficient in vitamin D due to lack of food fortification, less sunlight exposure, decreased ambulatory capability and/or institutionalization of elderly adults$^9,10$.

Given the connection between poor vitamin D status and TB infection$^{11,12}$, we sought to assess circulating plasma 25(OH)D concentrations and dietary vitamin D intake in pulmonary TB patients residing in Tbilisi, Republic of Georgia. We also measured the effect of sunlight on *in vitro* conversion of 7-dehydrocholesterol (the vitamin D precursor in skin) to previtamin D$_3$ in the environment of TB patients living in Tbilisi, Republic of Georgia.

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**MATERIALS AND METHODS**

**Study Location, Participants, and Ethics**

Participants were recruited from the National Center for Tuberculosis and Lung Diseases (NCTBLD) and the Tbilisi Fitzio-Pulmonologic Center in Tbilisi as a subset of the initial subjects from a larger, ongoing double blind, randomized, controlled, prospective trial of high-dose vitamin D$_3$ treatment (clinicaltrials.gov identifier NCT00918086) for patients
with pulmonary TB infection. The inclusion criteria for TB patients were age above 18 years, documented new case of smear-positive pulmonary TB, ≤ 7 days of anti-TB therapy, agreement to receive anti-TB therapy in Tbilisi, and signed informed consent. The exclusion criteria for TB patients was greater than 30 days of lifetime TB therapy, current pregnancy or lactation, history of organ transplant, cancer in past 5 years (excluding non-melanoma skin cancer), seizures, hypercalcemia, hyperparathyroidism, sarcoidosis, nephrolithiasis, liver cirrhosis, serum creatinine >250 mmol/L, requirement of hemodialysis, corticosteroid use in the past 30 days, current use of cytotoxic or immunosuppressive drugs, current incarceration, inability to complete all study visits in Tbilisi.

The Institutional Review Boards from Emory University in Atlanta, USA and the NCTBLD in Tbilisi approved the study protocol. All subjects provided written informed consent for participation in the study. All data for this study was collected between November 1, 2009 and October 31, 2010.

Study Design

Evaluation of Seasonal Production of Vitamin D from Sunlight

Glass ampules containing 5 mL of synthetic 7-DHC in methanol were placed in direct sunlight for 1 hour each from 10 AM to 5 PM, monthly from November 2009 to October 2010. The investigators recorded the corresponding weather conditions. The ampule studies were completed during days where it was forecasted to be at minimum 50% to 100% sunny in attempt to capture the peak amount of vitamin D conversion during each month (www.weather.com). Two dates were studied each month and the most representative sunny day from the month was used for the analysis. The ampules were protected from light and stored in a -20 degree freezer before and after the hour-long sunlight exposure. The amount of pre-vitamin D$_3$ converted from 7-DHC was determined by standard high-performance liquid chromatography (HPLC) methods using previously published methods at Boston University School of Medicine, Boston, MA.\textsuperscript{3}

Vitamin D status in Participants

Subjects provided demographic information on age, gender and mean daily time spent outdoors. Participants were recruited throughout the year, and each subject provided a blood specimen upon enrollment into the study. Vitamin D status was determined by plasma 25(OH)D concentrations, measured by ELISA (Immunodiagnostics, LTD, Fountain Hills, AZ) at the Vitamin D and Bone Research Laboratory, Emory University. The Laboratory participates in the international Vitamin D External Assessment Scheme (DEQAS) to ensure quality control of the plasma 25(OH)D determinations.

Assessment of Vitamin D Content in the Georgian Diet

Baseline nutritional status included body weight, measured on a digital scale (Tanita Co., Arlington Heights, Illinois), and height measured using a stadiometer, to calculate a body mass index (BMI; kg/m$^2$). To define habitual daily vitamin D intake, one-on-one interviews were conducted by trained physicians, using a 3-day food recall questionnaire designed to capture all food and beverage intake, including staple foods and recipes common in the Georgian diet. Food models were used to accurately determine portion size of reported items. Vitamin D intake from reported intakes was quantitated by a registered dietitian (JF) at the Atlanta Clinical and Translational Science Institute Bionutrition Research Unit, Atlanta, GA using Nutrition Data System for Research software (NDS-R, version 2009, University of Minnesota, Minneapolis, MN).
Statistical Analysis

Descriptive statistics were performed for demographic information. Student’s T-tests were used to determine the mean plasma 25(OH)D concentrations, daily vitamin D intake, and daily intake of specific foods containing natural vitamin D in TB subjects.

RESULTS

Seasonal Production of Vitamin D From Sunlight

The in vitro ampule studies demonstrated that the highest amount of previtamin D$_3$ production from 7-DHC occurred between May through August, and that no detectable previtamin D$_3$ was produced from October through March (Figure 1). The peak production of previtamin D$_3$ occurred between 11:00 and 14:00 h (Figure 2).

Demographics and vitamin D status in TB subjects

Demographic information of the subjects with TB is presented in Table 1. The majority of the subjects were Ethnic Georgian (91%). Mean plasma 25(OH)D concentrations were in the vitamin D deficient range (25(OH)D < 20 ng/mL) in 83% of TB subjects. Vitamin D insufficiency (25(OH)D < 30 ng/mL) was present in 97% of participants (Table 2). There were no differences in plasma 25(OH)D in the Ethnic Georgian subjects compared to the non-Ethnic Georgian subjects, (14.4 ± 7.0 vs 14.8 ± 7.0, respectively). We did not observe higher vitamin D status in those reporting greater than 4 hours of outdoor activity daily compared to those reporting less than 1 hour of outdoor activity daily.

Dietary Vitamin D Intake

Data from the 3-day food records revealed that the most frequently ingested foods with naturally occurring vitamin D content were fish, eggs, and butter the participants. Few foods in Georgia are fortified with vitamin D. The only fortified item identified after inspection of foods at over 12 grocery stores in Tbilisi was one commercial brand of low-fat milk. The daily intake of foods containing the most vitamin D was fish (188 ± 137 g), eggs (109 ± 83 g), and butter (81 ± 54). The daily habitual intake of total vitamin D from all food sources in TB was 172 ± 196 IU (Table 2). This level of dietary vitamin D intake is considerably lower than the new 2010 Recommended Dietary Allowance (RDA) recommendations from the US Institute of Medicine (600 IU/day for adults up to age 70 and 800 IU/d for those 71 years and older).13

DISCUSSION

This pilot study showed that adults with newly diagnosed pulmonary TB in Tbilisi, Georgia exhibit a very high prevalence of vitamin D deficiency and insufficiency. Dietary sources of vitamin D were quite inadequate compared to the current dietary RDA in this cohort. In addition to the limited intake of vitamin D foods, patients with pulmonary TB residing in Tbilisi, Georgia (42 ° N) had limited potential to produce vitamin D in skin during most of the year. To our knowledge, this is the first study to explore the major sources of vitamin D nutriture (sunlight and diet) in a population with pulmonary TB infection.

Several studies, including ours, have reported a high prevalence of vitamin D insufficiency in patients with TB infection11,14,15,16,17. Sita-Lumsden et al reported that patients with TB had lower vitamin D status compared to healthy controls. Furthermore, they reported that patients did not experience a seasonal increase in serum 25(OH)D in the summertime as did controls, suggesting that patients with TB do not get adequate sunlight exposure either to environmental factors or due to the infection with TB itself. In addition, a majority (79%) of participants did not have an appropriate increase in serum 25(OH)D concentrations despite 3
months of vitamin D repletion. This suggests that TB patients had alterations in the vitamin D metabolism. Given the high prevalence of vitamin D deficiency in this population, there has been interest to examine the impact of correction of vitamin D deficiency on TB outcomes; however, the data to date have yielded mixed results. This is in part due to variability in the doses, patient populations, and methodology in previously conducted trials. Historically, dating back to the 1800’s, vitamin D in the form of cod liver oil was used to treat pulmonary TB. More recently, two prospective studies have demonstrated a potential benefit of vitamin D supplementation on TB outcomes. Nursyam et al administered daily vitamin D as adjunctive therapy and found 23% greater sputum conversion compared to placebo. In a randomized control trial of 126 tuberculosis patients, Martineau et al demonstrated that vitamin D as an adjunct to anti-TB drugs decreased time to sputum conversion, though only in patients with the tt genotype of the taqI vitamin D receptor polymorphism. TB is a serious public health concern in Georgia due to poverty, lack of access to affordable healthcare, smoking, and less monitoring of drug therapy. Our current ongoing trial in Tbilisi, Georgia, in addition to other ongoing trials, of high dose vitamin D therapy in TB patients will shed further light on this issue.

We attempted to determine the causes of the high prevalence of vitamin D deficiency in our TB patients in Tbilisi. We found a high prevalence of vitamin D insufficiency in patients with pulmonary TB consistent with other studies at similar latitudes as Tbilisi (42 degrees N). In London (51 degrees N), Sita-Lumsden showed that only 6% TB patients had optimal serum 25(OH)D concentrations (>30 ng/mL) in contrast to 27% of healthy controls. A few studies have been conducted to examine vitamin D status of subjects without TB infection in the proximity of Tbilisi, Georgia. In a study of 391 adults above the age of 20 in Manisa, Turkey (38 degrees N), 75% of subjects had 25(OH)D levels < 20ng/mL and 14% had levels between 20-29 ng/mL at the end of winter. In Yekaterinburg, Russia (56 degrees N), Bakhtiyarova et al reported that 161 elderly patients above the age of 65 and found that 100% of the participants exhibited vitamin D deficiency (25(OH)D < 20 ng/ml). In a similar study conducted in Lleida, Spain (43 degrees N), 100% of institutionalized elderly women had 25(OH)D < 30 ng/mL. Therefore, patients with TB residing in Tbilisi, Georgia, are at risk for vitamin D deficiency in part due to the high latitude in which they reside.

In addition to latitude, the amount of UVB exposure from sunlight depends on environmental and personal factors. The altitude, time of day and year, cloud cover, pollution, and solar zenith angle (the angle at which sunlight hits the Earth’s surface) all affect UVB levels. Personal factors such as age, sunscreen usage, and clothing also affect UVB absorption. Most importantly, skin tone decreases the amount of vitamin D produced by sunlight exposure. Webb et al demonstrated that the previtamin D₃ synthesis in human skin samples in vitro was 40-70% lower than in ampules of 7-DHC, depending on the skin sample and color. Furthermore, Chen et al demonstrated that our in vitro technique best approximates vitamin D production in skin and is influenced by differences in skin tone. Black patients with TB often have lower vitamin D status compared to non-Black patients. Therefore, the differences in vitamin D status in Blacks and Whites may help explain racial disparities in TB infection.

Given the high prevalence of vitamin D deficiency in our TB patients, we examined the contribution of sunlight to vitamin D status in the region of Tbilisi, Georgia using a method that best approximates cutaneous vitamin D production in skin. Webb et al showed that in Boston, the same latitude as Tbilisi, production of previtamin D₃ was highest in June and July, and no vitamin D was detectable from the months of November to February. Lu et al confirmed that more previtamin D₃ was synthesized in the summer months and that it was produced in higher amounts during the day around 12:00. They also found that cities farther...
from the equator had decreased previtamin D₃ synthesis overall. Though cutaneous synthesis is the main source of vitamin D, our research confirms that UVB exposure adequate for vitamin D production is not available for most of the day and in winter months in Tbilisi. In our study, we did not find that vitamin D status was higher in individuals spending more time outside. This data helps explain the high rates of vitamin D deficiency in persons infected with TB living in this region and other regions that share the same latitude or higher.

In addition to sunlight, food may provide another source of vitamin D for individuals with TB infection. We found that pulmonary TB subjects had little vitamin D content in their diet, which may be due to lack of food fortification in this region or due to limited intake of foods naturally containing vitamin D. In a review of vitamin D status throughout Europe, Oveson et al showed that despite higher latitudes, participants in Sweden, Denmark, and the UK had had higher vitamin D status than in France and Italy, possibly because there was more vitamin D fortification of food in those areas. Given the low levels of vitamin D intake and the high prevalence of vitamin D deficiency, Georgians would likely benefit from increased availability of vitamin D-fortified foods. A systematic review by O’Donnel in 2008 demonstrated that fortification of various foods including milk, cheese, milk powder, and orange juice, did significantly raise plasma vitamin D levels.

Limitations of our study include the lack of a control group of healthy patients. Due to the relatively small sample size of TB patients, we were not able to adequately compare plasma 25(OH)D concentrations throughout seasons of the year. Lastly, the 3-day food records may have missed intake of certain foods that participants eat regularly, as they may have not ingested it in the time period assessed.

CONCLUSIONS

Vitamin D deficiency and insufficiency are highly prevalent in patients with pulmonary TB in Tbilisi, Republic of Georgia. An evaluation of environmental and dietary factors demonstrate that UVB light between October and March is inadequate for cutaneous production of vitamin D and also that vitamin D is limited in the typical Georgian diet, respectively. Georgians, especially those with TB infection, may benefit from supplementation of vitamin D through oral preparations and fortification of food, especially during the winter months. Trials to evaluate whether vitamin D supplementation will improve health outcomes in patients with TB in this population are warranted in light of the high prevalence of vitamin D insufficiency and the potential role of vitamin D in the regulation of antimicrobial peptides involved in the innate immune response to Mycobacterium tuberculosis infection.

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Figure 1. Percent conversion of 7-dehydrocholesterol (7-DHC) to previtamin D₃ by month of year in Tbilisi, Georgia

Ampules containing 7-DHC were placed in direct sunlight hourly from 10:00-17:00 h once a month for one year in Tbilisi, Georgia. The percent conversion to previtamin D₃ was analyzed using HPLC. The mid-day conversion rates (12:00-13:00 h) are presented for each month. The most efficient conversion of 7-DHC to previtamin D₃ occurred between May to August. There was no detectable production of previtamin D₃ from September to March.
Figure 2. Percent conversion of 7-dehydrocholesterol (7-DHC) to previtamin D₃ by time of day in Tbilisi, Republic of Georgia

Ampules containing 7-DHC were placed in direct sunlight hourly from 10:00-17:00 h on one sunny day each month for one year in Tbilisi, Republic of Georgia. The percent conversion to previtamin D₃ was analyzed using HPLC. The most previtamin D₃ was produced from 12:00 to 13:00h with no conversion seen from September through March.
### Table 1

Demographic information of TB cases

<table>
<thead>
<tr>
<th></th>
<th>TB cases N=85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>33 (12)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>53 (62)</td>
</tr>
<tr>
<td>BMI (kg/m²), mean (SD)</td>
<td>21.0 (3.7)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
</tr>
<tr>
<td>Ethnic Georgian</td>
<td>77 (91)</td>
</tr>
<tr>
<td>Azeri</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Armenian</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Russian</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Ossetian</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Daily Time Outdoors, n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;30 min</td>
<td>3 (4)</td>
</tr>
<tr>
<td>30 min-1 hour</td>
<td>17 (20)</td>
</tr>
<tr>
<td>1-2 hours</td>
<td>20 (24)</td>
</tr>
<tr>
<td>2-4 hours</td>
<td>18 (21)</td>
</tr>
<tr>
<td>&gt; 4 hours</td>
<td>27 (32)</td>
</tr>
</tbody>
</table>

*Nutrition. Author manuscript; available in PMC 2013 April 1.*
Table 2

Vitamin D Intake and Vitamin D Status of TB cases

<table>
<thead>
<tr>
<th></th>
<th>TB cases N=85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Vitamin D Intake, IU (SD)</td>
<td>172 (196)</td>
</tr>
<tr>
<td>Plasma 25(OH)D, ng/mL (SD)</td>
<td>14.4 (7.0)</td>
</tr>
<tr>
<td>Vitamin D deficiency (25(OH)D &lt; 20 ng/mL) (%)</td>
<td>83%</td>
</tr>
<tr>
<td>Vitamin D insufficiency (25(OH)D &lt; 30 ng/mL) (%)</td>
<td>97%</td>
</tr>
</tbody>
</table>