High-dose vitamin D₃ reduces deficiency caused by low UVB exposure and limits HIV-1 replication in urban Southern Africans

Anna K. Coussens, Celeste E. Naude, Rene Goliath, George Chaplin, Robert J. Wilkinson, and Nina G. Jablonski

*Clinical Infectious Diseases Research Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Observatory 7925, South Africa; †Centre for Evidence-based Health Care, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa; ‡Department of Geography, The Pennsylvania State University, University Park, PA 16802; †Stellenbosch Institute for Advanced Studies, Stellenbosch 7600, South Africa; ‡The Francis Crick Institute, Mill Hill Laboratory, London NW7 1AA, United Kingdom; and ‡Department of Medicine, Imperial College London, London W2 1PG, United Kingdom

Cape Town, South Africa, has a seasonal pattern of UVB radiation and a predominantly dark-skinned urban population who suffer from vitamin D deficiency. This coexistent environmental and phenotypic scenario puts residents at risk for vitamin D deficiency, which may potentiate HIV-1 disease progression. We conducted a longitudinal study in two ethnically distinct groups of healthy young adults in Cape Town, supplemented with vitamin D₃ in winter, to determine whether vitamin D status modifies the response to HIV-1 infection and to identify the major determinants of vitamin D status (UVB exposure, diet, pigmentation, and genetics). Vitamin D deficiency was observed in the majority of subjects in winter and in a proportion of individuals in summer, was highly correlated with UVB exposure, and was associated with greater HIV-1 replication in peripheral blood cells. High-dosage oral vitamin D₃ supplementation attenuated HIV-1 replication, increased circulating leukocytes, and reversed winter-associated anemia. Vitamin D₃ therefore presents as a low-cost supplementation to improve HIV-associated immunity.

Significance

Vitamin D deficiency is associated with HIV/AIDS progression and mortality. Seasonal decline in UVB radiation, darkly pigmented skin, low nutritional vitamin D intake, and genetic variation can increase risk of deficiency. Cape Town, South Africa, has a seasonal UVB regime and one of the world’s highest rates of HIV-1 infection, peaking in young adults. In two ethnically distinct groups of young adults in Cape Town we found high prevalence of seasonal vitamin D deficiency resulting from inadequate UVB exposure. This deficiency was associated with increased permissiveness of blood cells to HIV-1 infection which was reversed by vitamin D₃ supplementation. Vitamin D may be a simple, cost-effective intervention, particularly in resource-poor settings, to reduce HIV-1 risk and disease progression.


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1To whom correspondence should be addressed. email: rgj2@psu.edu.

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Significance
Results

Highly Prevalent Seasonal Vitamin D Deficiency and Its Reversal by Winter Supplementation. One hundred healthy (asymptomatic, BMI <30) young (18- to 24-y-old) adults (Xhosa, n = 50; Cape Mixed, n = 50) were recruited from two neighboring districts of Cape Town in the summer and were reassessed in the winter (for loss to follow-up, see Fig. S1). In winter, all participants received cholecalciferol (50,000 IU) weekly for 6 wk, and 30 Xhosa participants were followed up for 6 wk after their winter visit (Fig. 1A). All enrolled participants were asymptomatic with no evidence of infection (Fig. S2A-C). The populations were well matched with similar female: male ratios and only a small difference in age (Xhosa 21 y vs. Cape Mixed 18.5 y; P < 0.0001) and smoking status (Xhosa 50% vs. 18%; P = 0.0014) (Table S1).

Xhosa participants had darker skin pigmentation as measured by upper inner arm and forehead melanin index (MI) and erythema index (EI, a measure of tanning) (P < 0.0001; Table S2).

Although their higher melanin content reduced the rate of skin vitamin D production, Xhosa participants actually had higher serum 25(OH)D levels in summer than Cape Mixed participants (median 72.6 vs. 65.5 nmol/L; P = 0.038, Table 1). Cape Mixed participants also had a trend toward greater vitamin D deficiency (<50 nmol/L) in summer (16 vs. 4%; P = 0.077). Conversely, there was no difference in 25(OH)D levels between population groups in winter, when a significant drop in 25(OH)D levels was observed in both populations (P < 0.0001) and the majority of participants became vitamin D deficient (Fig. 1B). Severe deficiency (<30 nmol/L) occurred in 18% of Xhosa participants and 12% of Cape Mixed participants in the winter, and overall 64% and 70%, respectively, had deficient serum 25(OH)D levels (Table 1). After winter supplementation, 77% of the Xhosa group gained optimal levels (≥75 nmol/L) [median 126.4 nmol/L, interquartile range (IQR) 74.631–57.1 nmol/L (Fig. 1C), and there was no change in corrected serum calcium (winter mean ± SD 2.31 ± 0.08 mmol/L vs. postsupplementation 2.34 ± 0.09 mmol/L) (Fig. S2D)]. Cape Mixed females had lower 25(OH)D levels in winter than Cape Mixed males (median 41.46 vs. 50.80 nmol/L; P = 0.0054), and Xhosa females had lower 25(OH)D levels after supplementation than Xhosa males (median 113.3 vs. 147.9 nmol/L, P = 0.047), indicating that females in both groups are at risk for lower 25(OH)D (Fig. 1D and E).

Personal UVB Exposure, but Not Diet, Varies by Season. Dietary intake of vitamin D was estimated using a 7-d quantitative food frequency questionnaire administered at each study visit. According to the estimated average requirement (EAR) cutoff-point method, 78–88% of participants had intakes below the EAR (400 IU) throughout the year, with median intakes ranging from 170 to 235 IU across groups and seasons (Fig. 1F and Table 1). There was no difference in intake between sexes in the Xhosa participants, but Cape Mixed females had lower intakes than Cape Mixed males in both seasons (P < 0.001), mirroring the sex patterns observed for 25(OH)D levels (Fig. 1E, G, and H).

To understand the extent to which UVB exposure contributes to vitamin D deficiency, solar UVB was monitored daily for the duration of the study, and participants completed sun-exposure questionnaires. Both groups spent significantly longer in the sun in summer than in winter (P ≤ 0.014), with personal net UVB (PNUVB) exposure more than 10-fold higher in summer (P < 0.0001, Fig. 1F). Xhosa participants spent ~4 h longer each week in the sun in both seasons than Cape Mixed participants (median: 1,335 vs. 1,086 minutes in summer and 795 vs. 540 minutes in winter) (Table S2), and they exposed larger areas of the body in the summer (median 30.0 vs. 22.6%; P = 0.0002). Both groups reduced their body exposure in winter to similar levels (12.5%), although the relative winter decrease was greater for Xhosa participants. There was limited sunscreen use in both groups (Table S2). Thus, Xhosa participants may partly compensate for their darker skin pigmentation by increasing their PNUVB in the summer (median 42,982 vs. 20,603 J; P < 0.0001, which is reflected in higher 25(OH)D levels in summer in Xhosa participants than in Cape Mixed participants (Table 1). However, Xhosa participants did not maintain higher UVB exposure in the winter (PNUVB 1,304 J vs. 1,450 J; P = 0.63), and they were at greater risk of deficiency, with 18% of participants exhibiting severe deficiency.

Genetic Variation Has a Larger Effect on the Response to Supplementation than on Seasonal Deficiency. To investigate the effects of genetic variation on serum 25(OH)D concentrations, 10 SNPs in six genes associated with vitamin D deficiency were genotyped in all participants: DBP (rs7041 and rs4588), DHCR7 (7-DCH reductase...
Table 1. Serum 25(OH)D, dietary vitamin D intake, and UVB exposure by season and population

<table>
<thead>
<tr>
<th>Measure</th>
<th>Summer</th>
<th>Winter</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 25(OH)D, median nmol/L (range): Xhosa</td>
<td>72.6 (62.1–80.4)†</td>
<td>45.4 (35.7–51.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum 25(OH)D, median nmol/L (range): Cape Mixed</td>
<td>65.5 (54.6–76.1)</td>
<td>43.8 (33.5–54.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D status, nmol/L (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xhosa: Severe deficiency, &lt;30 nmol/L</td>
<td>0</td>
<td>6 (18)</td>
<td></td>
</tr>
<tr>
<td>Xhosa: Deficiency, &lt;50 nmol/L</td>
<td>2 (4)</td>
<td>21 (64)</td>
<td></td>
</tr>
<tr>
<td>Xhosa: Insufficiency, 50–75 nmol/L</td>
<td>28 (56)</td>
<td>11 (33)</td>
<td></td>
</tr>
<tr>
<td>Xhosa: Sufficiency, &gt;75 nmol/L</td>
<td>20 (40)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Cape Mixed: Severe deficiency, &lt;30 nmol/L</td>
<td>0</td>
<td>6 (12)</td>
<td></td>
</tr>
<tr>
<td>Cape Mixed: Deficiency, &lt;50 nmol/L</td>
<td>8 (16)</td>
<td>35 (70)</td>
<td></td>
</tr>
<tr>
<td>Cape Mixed: Insufficiency, 50–75 nmol/L</td>
<td>27 (54)</td>
<td>15 (30)</td>
<td></td>
</tr>
<tr>
<td>Cape Mixed: Sufficiency, &gt;75 nmol/L</td>
<td>15 (30)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Dietary vitamin D intake, median IU/d (IQR): Xhosa</td>
<td>213 (94–335)</td>
<td>170 (68–266)</td>
<td>0.26</td>
</tr>
<tr>
<td>Dietary vitamin D intake, median IU/d (IQR): Cape Mixed</td>
<td>235 (120–395)</td>
<td>230 (99–369)</td>
<td>0.24</td>
</tr>
<tr>
<td>Personal weekly UVB, median J (IQR): Xhosa</td>
<td>42,982 (28,651–90,552)†</td>
<td>1,304 (875.5–2,878)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Personal weekly UVB, median J (IQR): Cape Mixed</td>
<td>20,603 (10,887–42,897)</td>
<td>1,450 (392.6–3,530)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Wilcoxon rank test or Fisher’s exact test between seasons, bold type indicates statistically significant difference between summer and winter values.
†Mann-Whitney test significant between populations, P < 0.04, bold type indicates statistically significant difference between summer and winter values.

and rs12785878), CYP2R1 (vitamin D 25-hydroxylase and rs10741657), CYP24A1 (vitamin D 24-hydroxylase and rs6013897), CYP27B1 (vitamin D 1α-hydroxylase and rs10877012), and VDR (rs2544037, rs10783219, rs10735810, and rs731236) (9–11, 20). All were in Hardy–Weinberg equilibrium, and five SNPs (CYP27B1 rs10877012; VDR rs10783219 and rs731236 TaqI; and DBP rs7041 and rs4588) had significantly higher minor allele frequency (MAF) in the Cape Mixed participants (P < 0.03, Table 2). DBP rs7041 and rs4588 are combined to form the Group component (Gc) haplotype of which there are three major alleles, Gc1F, Gc1S, and Gc2, with the Gc1F protein having higher binding affinity for serum 25(OH)D (8). Xhosa participants had significantly higher minor allele frequency (MAF) of Gc1F/Gc1S carriers (38 vs. 18%; P = 0.0002) (Table 2). Serum DBP levels also were measured in all participants; there was no effect of season, vitamin D supplementation or Gc haplotype on serum DBP levels in either population, but Cape Mixed participants had lower median levels in both seasons (summer/winter: 106/107 vs. 125/127 μg/mL; P < 0.0092) (Fig. S2 E and F).

Although the powering was modest, genotypes were added to the stepwise regression and general linear models (GLM) for determinants of total 25(OH)D (Table 3 and Tables S3 and S4). In exploratory analyses of combined participants, a greater genotypic effect was observed after supplementation than with seasonal variation; those heterozygous for VDR FokI (rs10735810) had lower 25(OH)D in the winter and postsupplementation (P < 0.04); those heterozygous for CYP24A1 rs6013897 had lower

Table 2. SNP frequency in Xhosa and Cape Mixed participants

<table>
<thead>
<tr>
<th>Gene and SNP (also known as)</th>
<th>Location (function)</th>
<th>Allele*</th>
<th>Xh (Major)</th>
<th>Xh (Het)</th>
<th>Xh (Minor)</th>
<th>CM (Major)</th>
<th>CM (Het)</th>
<th>CM (Minor)</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2R1 rs10741657 (rs2060793)‡</td>
<td>Promoter</td>
<td>A/G</td>
<td>0.72</td>
<td>0.22</td>
<td>0.06</td>
<td>0.54</td>
<td>0.38</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>CYP27B1 rs10877012 (rs4646536)§</td>
<td>5′ UTR</td>
<td>C/A</td>
<td>0.86</td>
<td>0.12</td>
<td>0.02</td>
<td>0.54</td>
<td>0.42</td>
<td>0.04</td>
<td>0.002</td>
</tr>
<tr>
<td>CYP24A1 rs6013897</td>
<td>3′ downstream</td>
<td>A/T</td>
<td>0.52</td>
<td>0.42</td>
<td>0.06</td>
<td>0.66</td>
<td>0.26</td>
<td>0.08</td>
<td>0.24</td>
</tr>
<tr>
<td>DBP rs7041 (Asp416Glu)</td>
<td>Exon 11 (nonsyn)</td>
<td>C/A§</td>
<td>0.8</td>
<td>0.18</td>
<td>0.02</td>
<td>0.5</td>
<td>0.44</td>
<td>0.06</td>
<td>0.007</td>
</tr>
<tr>
<td>rs4588 (Thr420Lys)</td>
<td>Exon 11 (nonsyn)</td>
<td>T/G§</td>
<td>0.96</td>
<td>0.04</td>
<td>0.02</td>
<td>0.78</td>
<td>0.2</td>
<td>0.02</td>
<td>0.027</td>
</tr>
<tr>
<td>VDR rs2544037</td>
<td>Promoter</td>
<td>A/G</td>
<td>0.52</td>
<td>0.38</td>
<td>0.1</td>
<td>0.58</td>
<td>0.3</td>
<td>0.12</td>
<td>0.7</td>
</tr>
<tr>
<td>rs10783219</td>
<td>Intron 0</td>
<td>A/T</td>
<td>0.9</td>
<td>0.1</td>
<td>0</td>
<td>0.72</td>
<td>0.22</td>
<td>0.06</td>
<td>0.044</td>
</tr>
<tr>
<td>rs10735810 (FokI)</td>
<td>Exon 3 (nonsyn)</td>
<td>A/G</td>
<td>0.64</td>
<td>0.3</td>
<td>0.06</td>
<td>0.56</td>
<td>0.38</td>
<td>0.06</td>
<td>0.69</td>
</tr>
<tr>
<td>rs731236 (TaqI)</td>
<td>Intron 9</td>
<td>A/G</td>
<td>0.72</td>
<td>0.28</td>
<td>0</td>
<td>0.54</td>
<td>0.4</td>
<td>0.06</td>
<td>0.069</td>
</tr>
<tr>
<td>DHCR7 rs12785878 (rs7944926; rs3794060)†</td>
<td>Intron 2</td>
<td>G/T§</td>
<td>0.52</td>
<td>0.42</td>
<td>0.06</td>
<td>0.5</td>
<td>0.44</td>
<td>0.06</td>
<td>0.98</td>
</tr>
</tbody>
</table>

DBP Gc Haplotype

(alleles rs7041–rs4588)‡

| Gc1F/Gc1F | Exon 11 (nonsyn) | CC-TT | 0.76 | 0.34 |
| Gc1F/Gc2 | Exon 11 (nonsyn) | CC-TG | 0.04 | 0.14 |
| Gc2/Gc2 | Exon 11 (nonsyn) | CC-GG | 0 | 0.02 |
| Gc1F/Gc1S | Exon 11 (nonsyn) | CA-TT | 0.18 | 0.38 |
| Gc2/Gc1S | Exon 11 (nonsyn) | CA-GT | 0 | 0.06 |
| Gc1S/Gc1S | Exon 11 (nonsyn) | AA-TT | 0.02 | 0.06 |

CM, Cape Mixed; Het, heterozygous; Xh, Xhosa.
*Major/minor alleles and frequencies.
†P values for differences between sites tested by χ² test for trend, bold type indicates statistically significant difference in SNP frequencies.
‡In linkage disequilibrium, r² = 1.00 (9, 10).
§On reverse strand.
†Predicted according to ref. 21.
25(OH)D levels postsupplementation ($P = 0.006$); and those heterozygous for CYP2R1 rs10741657 and DBP rs7041 had higher 25(OH)D levels postsupplementation ($P = 0.002$) (Fig. S3).

**Personal UVB Exposure Is the Major Determinant of Vitamin D Status.** We next used regression models applied to all variables (SI Materials and Methods) to identify the determinants of serum 25(OH)D in both groups. Stepwise regression (Table S3) identified PNUVB as the dominant determinant ($F = 123.2, P < 0.0001$), followed by area of skin exposed ($F = 10.7, P = 0.0013$) and arm MI ($F = 7.40, P = 0.0072$). DBP haplotype Gc1F/Gc1S and two VDR SNPs, Tagl rs731236 and rs2544037, also contributed to the model, as did duration of UVB exposure and skin redness (arm EI), to a lesser extent. To determine the directionality of effect a GLM approach was applied adjusting for age and smoking status (Table 3). Again PNUVB was the dominant determinant, followed by area of skin exposed and weekly duration of exposure, all positively contributing to serum 25(OH)D, whereas VDR FokI rs10735810 AG and Cape Mixed ancestry where negatively correlated with 25(OH)D. The two measures of skin redness (arm and forehead EI), as well as Gc1F/Gc1S, VDR FokI rs10735810 AA, and DBP rs7041 CA, positively contributed to serum 25(OH)D.

The determinants of severe deficiency in winter and sufficiency in the summer also were examined using a GLM approach (Table S4). Sunlight exposure as a component of PNUVB did not prevent serious deficiency in the winter, but the area exposed and duration were important. UV light content of winter sunlight is weak, but individuals who were in the sun for longer periods and who had the most surface area exposed produced more vitamin D. Darker skin (higher arm and forehead MI) also was associated with lower serum 25(OH)D status. Sex had an effect but correlated with area exposed and duration of exposure. Sufficiency in the summer was affected most strongly by duration of exposure and PNUVB and to a lesser extent by the degree of skin redness (arm EI). Possession of the DHCR7 rs12785878 GG genotype had a small effect on optimal serum 25(OH)D, but no other genes had a significant effect. Smoking status and sex also were influential in summer but only through correlation with patterns of sun exposure and skin pigmentation, respectively.

**Winter Vitamin D$_{3}$ Supplementation Increases Peripheral WBC Count and Counteracts Anemia.** To investigate the functional consequence of seasonal serum 25(OH)D levels on the immune system, we next investigated seasonal differences in full blood count (FBC) and the effect of vitamin D$_{3}$ supplementation on FBC in Xhosa participants. Vitamin D$_{3}$ supplementation in the winter increased WBC count ($P = 0.0016$) and in particular lymphocyte count ($P = 0.032$), and there was a winter trend for decreased monocytes (Fig. 2A–C). In the winter, participant’s RBC parameters tended toward macrocytic anemia (evidenced by decreased RBC and RBC distribution width (RDW), increased mean corpuscular volume, and a trend toward decreased Hb), and this trend was reversed by supplementation ($P < 0.0007$) (Fig. 2D–F and Fig. S4A). The winter decline in RBC, RDW, and Hb also was seen in participants with Cape Mixed ancestry, albeit the effect of supplementation was not measured (Fig. S4B–D).

**Winter Vitamin D$_{3}$ Supplementation Decreases HIV-1 Replication in Peripheral Blood Mononuclear Cells.** Because supplementation modified peripheral WBC and RBC counts, we next investigated the functional consequences on response to HIV-1 infection. The active vitamin D metabolite, 1,25-dihydroxy-vitamin D$_{3}$, has been shown in vitro to inhibit HIV-1 replication in macrophages via induction of autophagy, mediated via cathelicidin induction (22), and 25(OH)D deficiency is associated with HIV-1 progression (1). Therefore we investigated the functional consequences of seasonal variation in serum 25(OH)D levels and winter vitamin D$_{3}$ supplementation on the extent of HIV-1 replication in freshly isolated peripheral blood mononuclear cells (PBMCs), at each study visit. PBMCs were cultured in the presence of fresh 20% autologous serum, isolated at the same time as PBMCs, to maintain the in vivo cellular environment as best possible, with regard to seasonal serum 25(OH)D levels, autologous DBP, and other circulating chemokines/cytokines. Two preparations of HIV-1 BaL, purified and unpurified, were used for infection. Unpurified HIV-1 preparations contain exosomes, microvesicles, and conditioned medium from propagation and were tested for the likelihood that activating cells might result in greater productive infection of unstimulated cells.

Infection of PBMCs in winter, compared with summer, resulted in greater productive HIV-1 infection on day 9, as measured by culture supernatant p24 antigen levels. This result was seen in PBMCs isolated from both Xhosa ($P = 0.0003$, Fig. 2G–J) and Cape Mixed ($P < 0.0001$, Fig. S4E and F) participants. This winter increase in HIV-1 infection was seen with both preparations of HIV-1, with ~1-log higher p24 measured from cells infected with unpurified virus (Xhosa median 7.038 pg/mL unpurified vs. 738 pg/mL purified) (Fig. 2H and I).

After 6 wk of vitamin D$_{3}$ supplementation in winter, the winter increase in HIV-1 p24 was attenuated, and Xhosa participants’ PBMCs showed a diminished capacity for productive HIV-1 infection: on day 9 p24 levels had dropped to the level as observed in summer (Fig. 2G). Again, this decrease occurred with infections using both purified and unpurified virus (Fig. 2H and I), demonstrating the robustness of oral vitamin D$_{3}$ supplementation in suppressing productive HIV-1 infection in peripheral blood cells ex vivo. Moreover there was no significant negative correlation between paired serum 25(OH)D and day 9 p24 concentrations from PBMCs infected with purified virus, across all time points (Spearman $r_s = -0.36$; $P < 0.0001$), indicating a direct correlation between serum 25(OH)D levels and the ability of peripheral blood cells to limit productive HIV-1 infection.

**Discussion**

The multitude of, and complex interactions among, variables that modify vitamin D status and their impact on immunological function are poorly understood, particularly in the context of disease prevention in healthy individuals. In an urban African location with seasonal UVB variability and high infectious disease prevalence, we found that personal UVB exposure habit is the most important determinant of vitamin D status in healthy adults with moderate to dark skin pigmentation. Moreover, we found that the reversal of vitamin D deficiency in winter through oral supplementation can modulate the number and function of circulating WBCs, prevent anemia, and decrease productive HIV-1 infection. Furthermore, we found that UVB exposure habit can counterbalance the effects of pigmentation, genetic variation, and poor dietary intake.

The skin of the indigenous people of the Cape, the Khoisan, is considerably lighter than that of either study group (23) and may represent a long-established adaptation to seasonal UVB. The darker skin of both study populations denotes a degree of
mismatch between skin pigmentation and environmental UVB resulting from their recent migration into the region; this effect is exacerbated by wearing concealing clothing and indoor living in the winter. The high prevalence of vitamin D deficiency in the winter in both groups indicates that people with moderate or dark pigmentation are at high risk of deficiency in the absence of significant dietary vitamin D intake when UVB radiation is limited by seasonal fluctuations.

We also noted significant polymorphic variation between the two populations for 5 of the 10 vitamin D-associated SNPs investigated, contributing further genetic insight into these understudied populations. Given the low MAF of the SNPs investigated, particularly in the Xhosa population, we identified only a minor effect of genetic variation on seasonal vitamin D status. This finding mirrors the recent genomewide association study investigating, contributing further genetic insight into these under-

Our findings of decreased winter WBC counts, particularly lymphocytes, and an increase in numbers following vitamin D supplementation corroborate a small longitudinal study in healthy Scandinavian adults with light pigmentation, which found a similar decrease in lymphocytes in winter, particularly CD4+ and CD8+ T cells, which was associated with reduced 25(OH)D (26). Higher serum 25(OH)D levels in children initiating antiretroviral therapy (ART) also were associated with higher CD4+ T-cell restoration (27). Further studies will investigate the detailed changes in innate and adaptive immune cell populations in our cohorts. Vitamin D deficiency also has been associated previously with anemia and low Hb in HIV-infected women (1). We found that vitamin D supplementation reversed winter-associated macrocytic anemia, suggesting that this adjunct therapy also may be effective in preventing anemia in HIV-infected individuals.

The demonstration that high-dosage oral vitamin D supplementation reversed serum 25(OH)D deficiency and attenuated the seasonal increase in ex vivo HIV-1 replication, similar to our previous finding that oral vitamin D reduces Mycobacterium tuberculosis replication in whole blood (28), provides strong evidence for the positive preventative effects of vitamin D supplementation for people with vitamin D deficiency and serious infectious diseases, conditions which apply to many cities in which the prevalence of vitamin D deficiency continues to rise. Furthermore, vitamin D may be a simple, cost-effective intervention, particularly in resource-poor settings, to prevent disease progression in persons infected with HIV-1 by suppressing viral replication, raising peripheral lymphocyte counts, and preventing anemia, potentially prolonging the time to ART initiation and enhancing the beneficial effects of ART once initiated.

Materials and Methods

Study Design. The study was conducted in accordance with the Helsinki 1964 declaration, including subsequent revisions, and the South African Guidelines for Good Clinical Practice and the Medical Research Council Ethical Guidelines for Research. Ethical approval was received from the Human Research Ethics Review Boards of the Faculty of Health Sciences, University of Cape Town (ref. 003/2013) and the Faculty of Medicine and Health Sciences, Stellenbosch University (ref. N12/100/06) and from the Institutional Review Board of The Pennsylvania State University (ref. 41940). Written informed consent was obtained from all participants.

The 104 participants were assessed for eligibility, and 100 HIV-1–uninfected individuals (Xhosa, n = 50; Cape Mixed, n = 50) were enrolled. The summer and winter visits began 6 wk postsolstice. At the winter visit, all participants receiving winter vitamin D supplementation. (A–F) Box plots show WBC (A), lymphocyte (B), monocyte (C), and RBC (D) counts, RBC distribution width (E), and mean corpuscular volume (F) measured at each study visit (summer, n = 42; winter, n = 22; winter + vitamin D, n = 30). The lines across the box plots indicate the median (minimum–maximum); Kruskal–Wallis with Dunn’s multiple comparison test. (G) HIV-1 p24 concentration in culture supernatant on day 9 longitudinally, median (IQR) with purified HIV-1 (H) or unpurified HIV-1 (I); repeated measures two-way ANOVA with Tukey’s multiple comparison test following log10 transformation. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. LOD, limit of detection (1 pg/mL); ns, not significant.

![Image](image-url)
participants received six capsules of 50,000 IU cholecalciferol (D3-50; Biotech Pharmaceutical), to be taken weekly, and Xhosa participants were followed up 6 wk (± 2 d; mean ± 3 d) after their winter visit (Fig. S1). Previous studies have shown that serum 25(OH)D plateaus after ~4–6 wk of supplementation (29); however, it is possible that the final equilibrated 25(OH)D level was underestimated at 6 wk. Ethics, recruitment, follow-up, and exclusion criteria are detailed in SI Materials and Methods. The procedures described below were conducted at each study visit.

UVR Exposure. Sun exposure was assessed at each visit by a retrospective questionnaire that captured duration and time of day of outdoor sun exposure and the amount of skin not covered by clothing or a hat at time of exposure. PNUVB was calculated as described in SI Materials and Methods. Direct measurements of UVR were made at the University of Stellenbosch Solar Resource and Weather Station.

Skin Reflectometry. Skin reflectance of all research subjects, expressed as E1 and M1, was measured using a portable reflectometry device (DSM II ColorMeter; Cortex Technology). Constitutive pigmentation was measured on the upper inner arm site, and facultative pigmentation was measured on the forehead. Three independent measures were taken from each site, and the mean was calculated.

Food Questionnaire. Intake of vitamin D (vitamin D3 and vitamin D2) was estimated using a 7-d quantitative food frequency questionnaire administered to every participant by a trained researcher at each study visit. The questionnaire (described in SI Materials and Methods) was adapted from a food frequency questionnaire shown to be valid in providing a reasonable estimation of dietary vitamin D intake in healthy young adults of diverse ancestry (30). Individuals taking vitamin D supplements were excluded.

Biochemistry and FBC. Peripheral blood collected in serum tubes was analyzed on the day of collection for 25(OH)D concentration by the chemiluminescent LIAISON 25 OH Vitamin D TOTAL Assay (DiaSorin). Serum also was stored at −80 °C and subsequently batch analyzed for DBP, acute phase markers, calcium, and albumin. FBCs were conducted within 2–3 h of collection. Assays are described in SI Materials and Methods.

SNP Genotyping. DNA was extracted from PBMCs using the QIAamp DNA Blood Mini Kit (QiAGEN), and 10 ng was analyzed by TaqMan Genotyping assay (Life Technologies), in duplicate, for the following SNPs in genes: DBP (rs7041 and rs4588), DHR7 (rs12785878), CYP2R1 (rs10741657), CYP2RA1 (rs6013897), CYP27B1 (rs10877012), and VDR (rs2544037, rs10738219, rs10738510, and rs731236), as described in SI Materials and Methods.

PBMC HIV-1 Infection and p24 Analysis. After the isolation of PBMCs, cells were infected immediately with HIV-1, as described in SI Materials and Methods. HIV-1 replication was measured by quantifying the HIV-1 p24 antigen concentration of lysed supernatant by Lumixin, as described in ref. 31, with slight modifications (SI Materials and Methods).

Statistical Analysis. All univariate statistics were conducted in GraphPad Prism 6.0 software with an alpha of 0.05 and two-sided testing. GLM was conducted using Glucore Omics Explorer 2.2 software and stepwise regression, and GLM on vitamin D optimality and deficiency was conducted in S-Plus. Full details are given in SI Materials and Methods.

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[Image 0x1 to 19x816]