Reciprocal seasonal variation in vitamin D status and tuberculosis notifications in Cape Town, South Africa

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Vitamin D deficiency is associated with susceptibility to tuberculosis (TB) in HIV-uninfected people in Europe, but it is not known whether such an association exists among HIV-infected people in subtropical Africa. We conducted a cross-sectional study to determine whether vitamin D deficiency was associated with susceptibility to active TB in HIV-uninfected (n = 196) and HIV-infected (n = 174) black Africans in Cape Town, South Africa. We also investigated whether there was evidence of seasonal variation in vitamin D status and TB notifications in this setting over an 8-y period. Vitamin D deficiency (serum 25-hydroxyvitamin D [25(OH)D] <50 nmol/L) was present in 232 (62.7%) of 370 participants and was associated with active TB in both HIV-uninfected (odds ratio = 5.2, 95% confidence interval: 2.8–9.7; $P < 0.001$) and HIV-infected (odds ratio = 5.6, 95% confidence interval: 2.7–11.6; $P < 0.001$) people. Vitamin D status varied according to season: The mean serum 25(OH)D concentration was highest in January through March and lowest in July through September (56.8 vs. 30.7 nmol/L, respectively; $P < 0.001$). Reciprocal seasonal variation in TB notifications was observed: The mean number of TB notifications per quarter for Cape Town in 2003 to 2010 was lowest in April through June and highest in October through December (4,222 vs. 5,080; $P < 0.001$). Vitamin D deficiency is highly prevalent among black Africans in Cape Town and is associated with susceptibility to active TB both in the presence and absence of HIV infection. Reciprocal seasonal variation in serum 25(OH)D concentration and TB notifications suggests that seasonal variations in vitamin D status and TB incidence in this setting are causally related.

immunity | micronutrient | *Mycobacterium tuberculosis*

South Africa has the third highest burden of tuberculosis (TB) in the world: 490,000 cases were estimated to have arisen in 2009 (1). Active TB can arise as a consequence of reactivation of latent *Mycobacterium tuberculosis* (MTB) infection following compromise of the antimycobacterial immune response (2). HIV infection is a major cause of such immunocompromise, and the high prevalence of HIV infection in South Africa drives its TB epidemic (3).

A growing body of evidence suggests that vitamin D deficiency may also impair the immune response to MTB. Most of the human vitamin D requirement is met by cutaneous synthesis of vitamin D during exposure to UV light; dietary sources, such as oily fish, are secondary (4). Vitamin D is metabolized in the liver to form 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite and measure of vitamin D status (5). 25(OH)D is metabolized by the 1-α-hydroxylase enzyme CYP27B1 to its biologically active metabolite, the steroid hormone 1,25-dihydroxyvitamin D [$1,25(OH)_2D$]. In vitro, MTB induces expression of the 1-α-hydroxylase enzyme CYP27B1 in the macrophage via ligation of Toll-like receptor 2/1 heterodimers to stimulate conversion of 25(OH)D to $1,25(OH)_2D$, which enhances the ability of the macrophage to restrict mycobacterial growth (6, 7).

We have previously reported that vitamin D deficiency is associated with susceptibility to TB in London and that this association is modified by polymorphisms in the vitamin D receptor and vitamin D binding protein (8, 9). We have also shown that in vivo vitamin D supplementation enhances immunity to mycobacteria both in healthy people (10) and in a genetically defined subgroup of patients with active TB (11). Reports of seasonal variation in the prevalence of vitamin D deficiency (12) and TB incidence (13) in the United Kingdom provide further evidence that low vitamin D status may compromise antimycobacterial immunity in this setting.

The prevalence of profound vitamin D deficiency among TB patients in tropical Africa is much lower than in Europe [reported in 0.3–11.2% of patients with TB in tropical Africa (14–17) vs. 64–84% of patients with TB in London (18–20)]. The prevalence of vitamin D deficiency in TB patients with and without HIV infection in subtropical Africa has not previously been reported. There is particularly good reason to investigate this question in Cape Town, South Africa, because TB incidence in Cape Town is higher than elsewhere in South Africa (21) and the ability of sunlight to synthesize vitamin D is compromised during the winter in Cape Town (latitude 33° south) but not in Johannesburg (latitude 26° south) (22). We therefore conducted an observational study to determine whether vitamin D deficiency is associated with susceptibility to active TB in HIV-infected and HIV-uninfected adults in Cape Town and to investigate whether there is evidence of seasonal variation in vitamin D status and TB notifications in this setting.

Results

The demographic and clinical characteristics of study participants are summarized in Table 1. In HIV-uninfected participants, a diagnosis of active TB was associated with greater age, male sex, lower body mass index (BMI), and fewer years of education ($P <$ 0.001 for all comparisons). Among HIV-infected participants, a diagnosis of active TB was associated with male sex ($P = 0.04$), lower BMI ($P < 0.001$), and lower CD4 cell count ($P < 0.001$).

Vitamin D deficiency was highly prevalent in all groups studied (Table 2) [serum 25(OH)D <50 nmol/L in 232 (62.7%) of 370 participants]. Active TB was associated with vitamin D deficiency in both HIV-uninfected and HIV-infected participants

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Table 1. Demographic and clinical characteristics of study participants (n = 370)

	HIV-uninfected (n = 196)			HIV-infected (n = 174)		
	Latent TB (n = 103)	Active TB (n = 93)	P	Latent TB (n = 75)	Active TB (n = 99)	P
Median age, y (IQR)	22.7 (19.7–26.0)	31.7 (25.8–42.3)	<0.001	30.0 (27.0–35.1)	32.0 (27.0–38.2)	0.38
Sex, n (%)						
Male	42 (40.8)	64 (68.8)	<0.001	19 (25.3)	40 (40.4)	0.04
Female	61 (59.2)	29 (31.2)		56 (74.7)	59 (59.6)	
Month of sampling*						
April–September	39	39	0.52	50	61	0.81
October–March	64	53		25	33	
Median BMI, kg/m^2 (IQR)	23.0 (21.4–29.0)	20.1 (18.1–22.5)	<0.001	24.8 (21.3–30.1)	21.1 (19.0–24.1)	<0.001
Median education, y (IQR)	11.0 (10.0–12.0)	10.0 (7.0–11.0)	<0.001	10.5 (8.0–12.0)	10.0 (8.5–11.5)	0.78
Ethnic group†						
Nguni, n (%)	103 (100)	89 (95.7)	0.05	72 (96.0)	98 (99.0)	0.58
"Colored," n (%)	0	4 (4.3)		2 (2.7)	1 (1.0)	
Median CD4 cell count (IQR)	Not determined	Not determined	—	393 (262–548)	167 (57–292)	<0.001

IQR, interquartile range.
*Month of sampling not known for six participants with active TB (1 HIV-uninfected, 5 HIV-infected).
†Ethnic group not classified in one HIV-infected participant with latent TB.

[for HIV-uninfected participants, odds ratio (OR) = 5.2, 95% confidence interval (CI): 2.8–9.7, $P < 0.001$; for HIV-infected participants, OR = 5.6, 95% CI: 2.7–11.6, $P < 0.001$]. A similar association was seen in both HIV-uninfected and HIV-infected people for the 20 nmol/L threshold for serum 25(OH)D concentration denoting profound vitamin D deficiency ($P \leq 0.002$). For the 75 nmol/L threshold for serum 25(OH)D concentration, proposed by some to denote optimal vitamin D status (23), a similar association was seen for HIV-infected participants (P = 0.001) but not for those without HIV infection (P = 0.45).

Serum 25(OH)D concentrations stratified according to the presence/absence of HIV infection and active TB are presented in Fig. 1. Active TB was associated with a lower mean serum 25 (OH)D concentration in both HIV-uninfected and HIV-infected participants (Table 2; for HIV-uninfected participants, mean difference = 14.7 nmol/L, 95% CI for difference: 9.0–20.3 nmol/L, $P < 0.001$; for HIV-infected participants, mean difference = 26.0 nmol/L, 95% CI for difference: 19.1–33.0 nmol/L, $P < 0.001$).

Because potential determinants of vitamin D status, such as season of sampling, were unequally distributed between study groups, a multivariate analysis of potential correlates of vitamin D status was conducted to determine which determinants were independently associated with lower serum 25(OH)D concentration. Results of this analysis are presented in Table 3. Lower vitamin D status was independently associated with the presence of active TB [mean difference in 25(OH)D concentration = 28.9 nmol/L, 95% CI: 18.2–39.7 nmol/L; $P < 0.001$], sampling from April to September inclusive [mean difference in 25(OH)D concentration = 10.3 nmol/L, 95% CI: 0.4–20.1 nmol/L; P = 0.04], and lower BMI [mean difference in 25(OH)D concentration = 0.50 nmol/L per unit of BMI, 95% CI: 0.06–0.93 nmol/L; P = 0.03]. The difference in vitamin D status between participants with vs. without active TB was greater in HIV-infected participants than in HIV-uninfected participants (P interaction = 0.03).

The influence of season on vitamin D status is illustrated by the sinusoidal pattern of variation in mean serum 25(OH)D concentration by month (Fig. 2), being highest in March (mean = 57.4 nmol/L, SD = 22.8) and lowest in July (mean = 22.9 nmol/L, SD = 18.5). When analyzed by quarter, mean serum 25(OH)D concentration was highest in January through March and lowest in July through September (56.8 vs. 30.7 nmol/L, respectively; 95% CI for difference: 20.6–31.6 nmol/L; $P < 0.001$). Our finding that lower vitamin D status in black Africans was associated both with susceptibility to active TB and with season prompted us to investigate whether there was any seasonal variation in new TB notifications for the City of Cape Town. A marked seasonal variation in the mean number of new TB notifications was observed, with a consistent dip in notifications seen in the second quarter of every year from 2003 to 2010 inclusive (Fig. 3). The mean number of new TB notifications per quarter for Cape Town over this period was lowest in April through June and highest in October through December (4,222 vs. 5,080 notifications per quarter; 95% CI for difference: 642–1,075 notifications per quarter; $P < 0.001$).

Discussion

We report that vitamin D deficiency is highly prevalent among black African adults living in Cape Town. Vitamin D deficiency is associated with susceptibility to active TB in both the absence and the presence of HIV infection, but the association is stronger

Table 2. Serum 25(OH)D concentrations of study participants stratified by HIV/TB status

	HIV-uninfected (n = 196)				HIV-infected (n = 174)			
	Latent TB (n = 103)	Active TB (n = 93)	OR/mean difference* (95% CI)	P	Latent TB (n = 75)	Active TB (n = 99)	OR/mean difference* (95% CI)	P
Serum 25(OH)D threshold								
<20 nmol/L, n (%)	1 (1.0)	11 (11.8)	6.7 (1.7–108.2)	0.002	3 (4.0)	37 (37.4)	9.0 (2.7–30.2)	<0.001
<50 nmol/L, n (%)	38 (36.9)	70 (75.3)	5.2 (2.8–9.7)	<0.001	39 (52.0)	85 (85.9)	5.6 (2.7–11.6)	<0.001
<75 nmol/L, n (%)	87 (84.5)	82 (88.2)	1.4 (0.6–3.1)	0.45	61 (81.3)	96 (97.0)	7.3 (2.0–26.6)	0.001
Mean serum 25(OH)D, nmol/L (SD)	55.2 (19.4)	40.5 (20.8)	14.7 (9.0–20.3)	<0.001	54.7 (27.4)	28.7 (19.1)	26.0 (19.1–33.0)	<0.001

*Mean difference applies to "Mean serum" data.

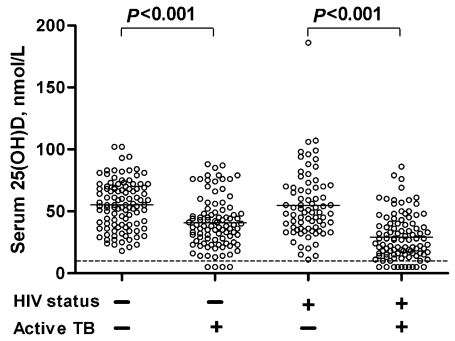

Fig. 1. Serum 25(OH)D concentration by HIV and TB status. Bars represent means. Dashed line represents limit of detection (10 nmol/L).

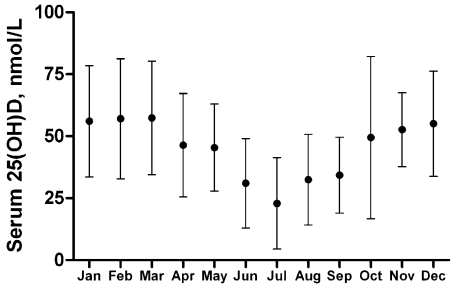

Fig. 2. Seasonal variation in mean serum 25(OH)D concentration, all study participants (n = 370). Error bars indicate SD.

in HIV-infected people. A seasonal variation in TB notifications and vitamin D status was observed: Peak vitamin D status in January through March directly preceded a trough in new TB notifications in April through June.

This study reports an association between vitamin D deficiency and susceptibility to active TB in HIV-infected people. Vitamin D supplementation enhances immunity to mycobacteria (10), and vitamin D metabolites induce antimycobacterial immunity in macrophages by pleiotropic mechanisms, including the induction of antimicrobial peptides (24), suggesting a potential direct causal relationship between vitamin D deficiency and susceptibility to active TB. In HIV-infected people, vitamin D might also indirectly enhance antimycobacterial immunity by slowing progression of HIV disease (25, 26), although studies investigating the effect of vitamin D metabolites on HIV replication in vitro report conflicting results (27–31). It is biologically plausible, therefore, that vitamin D deficiency impairs antimycobacterial responses in HIV-infected adults and that this phenomenon explains the association between vitamin D deficiency and susceptibility to active TB that we report here. However, our study is cross-sectional, and reverse causality cannot be excluded as a potential explanation of this association. Heightened immune activation in HIV-infected patients with active TB (32) might lead to up-regulation of macrophage vitamin D 1-α-hydroxylase, and thus increased consumption of 25(OH)D, for example, contributing to the particularly high rates of vitamin D deficiency observed in this group. Randomized controlled trials should be conducted to determine whether vitamin D supplementation can prevent active TB in HIV-infected people in this setting.

The need for such trials is pressing, given the very high incidence of TB in HIV-infected adults in Cape Town and the high prevalence of vitamin D deficiency that we report in this group, which is significantly higher than has been reported in tropical Africa (14–17). To our knowledge, the prevalence of vitamin D deficiency among people of Nguni descent living in Cape Town has not previously been investigated. However, a retrospective analysis of 216 laboratory requests at a tertiary hospital in Cape Town reported serum 25(OH)D concentrations of <45 nmol/L in 41% of samples tested; the ethnic origin of participants was not reported, and no seasonal variation in vitamin D status was seen (33). A cross-sectional study of 200 noninstitutionalized "colored" Cape Town residents aged ≥65 y reported a 17% prevalence of profound vitamin D deficiency, defined as serum 25(OH)D <25 nmol/L (34). Vitamin D status was determined in August through September in this study, precluding investigation of a seasonal effect on vitamin D status. A cross-sectional study of dietary intake among 148 black Capetonians aged ≥60 y found mean vitamin D intake below the recommended daily amount (35), and our finding of a significant seasonal variation in vitamin D status suggests that cutaneous synthesis is a major determinant of vitamin D status in this setting. The winter/spring trough in serum 25(OH)D concentrations that we report is likely to arise as a consequence of decreased ambient UVB light, decreased time spent out of doors, and increased clothing worn during these periods (22).

Seasonal variation in TB notifications has previously been reported among children aged <13 y in the Western Cape Province of South Africa, with an excess of cases in late winter and early spring attributed to increased transmission of TB in autumn and winter (36). This timing is consistent with the city-wide data presented here. The temporal relationship between the

Table 3. Correlates of vitamin D status in study participants (n = 370): univariate and multivariate analysis

		Univariate analysis		Multivariate analysis*	
	Correlates	Mean serum 25(OH)D, nmol/L (SD)/ Spearman's R (95% CI)†	P	β (95% CI)	P
TB status	Active	34.4 (20.8)	<0.001	−28.9 (−39.7 to −18.2)	<0.001
	Latent	55.0 (23.0)		Ref	
HIV infection	Yes	39.9 (26.4)	0.001	−8.3 (−17.7 to 1.1)	0.08
	No	48.3 (21.3)		Ref	
Sex	Male	41.6 (25.0)	0.06	—	—
	Female	46.5 (23.3)		—	
Month of sampling‡	April–September	34.5 (19.8)	<0.001	−10.3 (−20.1 to −0.4)	0.04
	October–March	55.3 (24.1)		Ref	
Age, years		−0.30 (−0.39 to −0.20)	<0.001	−0.25 (−0.50 to 0.00)	0.052
BMI, kg/m^2		0.21 (0.10–0.32)	<0.001	0.50 (0.06–0.93)	0.03

Ref, reference category; BMI, body mass index.
*Model incorporates only those correlates associated with serum 25(OH)D concentration with $P < 0.05$ on univariate analysis.
†Spearman's R applies to Age and BMI.
‡Month of sampling not known in six participants with active TB (1 HIV-uninfected, 5 HIV-infected).

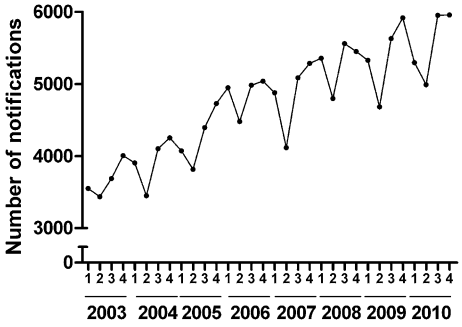

Fig. 3. New TB notifications by quarter, City of Cape Town, 2003 to 2010.

summer peak in vitamin D status and the ensuing autumn dip in TB notifications in Cape Town raises the possibility that seasonal variations in vitamin D status and TB incidence in Cape Town may be causally related. Interpretation of this observation is limited by the ecological nature of the association that we report (i.e., TB notification data reported are for the city as a whole and do not relate exclusively to individuals whose vitamin D status was determined for this study). Nevertheless, these data provide further evidence that vitamin D deficiency may be a significant contributory factor to the particularly high incidence of TB seen in the Western Cape Province of South Africa.

Materials and Methods

Populations Studied. Three hundred seventy participants were recruited to the study at the Ubuntu Clinic and Day Hospital, both at site B, Khayelitsha, and at G. F. Jooste Hospital, Manenberg, between April 2005 and January 2010. Of these, 103 were HIV-seronegative and had latent TB infection; 93 were HIV-seronegative and had active TB; 75 were HIV-seropositive and had latent TB infection; and 99 were HIV-seropositive and had active TB. Only 4 of 174 HIV-seropositive participants were taking antiretroviral therapy at the time of recruitment to the study.

The diagnosis of active TB was established on the basis of smear positivity for acid-fast bacilli and/or culture positivity for MTB. Blood was drawn before or within 3 d of commencing antitubercular medication. The diagnosis of latent TB infection was established on the basis of absence of TB symptoms, together with dual positivity for the tuberculin skin test (TST) and an in-house IFN-γ release assay (IGRA). Clinical assessment of HIV-infected patients also involved a normal chest radiograph and a single negative sputum culture for MTB. All HIV-infected patients with a TST induration >4 mm were offered a 6-mo course of isoniazid preventive therapy in the absence of contraindications. Self-classified ethnicity was recorded.

IGRA. A 7-d IGRA was performed at the laboratory at the Institute of Infectious Diseases and Molecular Medicine, University of Cape Town. Whole blood was diluted 1:10 in RPMI medium 1640 containing 1% L-Glutamine. A 24-well flat-bottomed plate (CR 3524; Corning) was set up with the antigens and controls. The antigens ESAT-6 and CFP-10 (both from Proteix) and ESAT-6/CFP-10 Fusion Protein [LUMC, produced as previously described (37)] were stored at −80 °C at a concentration of 1 mg/mL and used at a final concentration of 5 μg/mL. Phytohemagglutinin at a concentration of 5 μg/mL acted as a positive control, and RPMI medium 1640 with 1% L-Glutamine acted as a negative control. After gentle mixing, the plate was incubated at 37 °C with 5% CO_2 for 7 d. After incubation, the supernatants were harvested into sample storage tubes and stored at −80 °C until assayed for IFN-γ by ELISA as previously described (38).

TST. The TST was performed using 2 tuberculin units of purified peptide derivative RT23 injected intradermally into the volar aspect of the forearm after whole blood was collected for the IGRA.

Determination of 25(OH)D Concentrations. Serum 25(OH)D concentrations were determined by isotope-dilution liquid chromatography-tandem mass spectrometry (39) in the clinical biochemistry laboratory at Homerton University National Health Service Foundation Trust, which participates in the international Vitamin D external quality assessment program (http://www.deqas.org/). Sensitivity for 25(OH)D_2 and 25(OH)D_3 was 10 nmol/L, and 25(OH)D_2 was undetectable in all samples analyzed. Vitamin D deficiency was defined using a 25(OH)D threshold of 50 nmol/L (4).

Ethical Approval. This study was approved by the University of Cape Town Research Ethics Committee (REC REF 030/2010). All participants gave written informed consent to take part.

Statistical Analysis. Data were analyzed using SPSS (version 12.0.1, 2003; SPSS, Inc.) and Prism (version 4.03, 2005; GraphPad) software packages. Contingency tables were analyzed using χ^2 tests unless a cell in a table had an expected frequency of <5, when Fisher's exact tests were used. Median values were compared using Mann–Whitney tests, and means were compared using unpaired Student *t* tests and one-way ANOVA. Spearman's *R* was calculated to test for correlation between age, BMI, and serum 25(OH)D concentration. Multivariate analysis of correlates of serum 25(OH)D concentration was conducted using multiple linear regression. Reported *P* values are two-sided; *P* values <0.05 were considered to be statistically significant.

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1. WHO (2010) *Global Tuberculosis Control: WHO Report 2010* (WHO Press, Geneva).
2. Maartens G, Wilkinson RJ (2007) Tuberculosis. *Lancet* 370:2030–2043.
3. Lawn SD, Wood R, Wilkinson RJ (2011) Changing concepts of "latent tuberculosis infection" in patients living with HIV infection. *Clin Dev Immunol* 2011:980594.
4. Holick MF (2007) Vitamin D deficiency. *N Engl J Med* 357:266–281.
5. Institute of Medicine (2011) *Dietary Reference Intakes for Calcium and Vitamin D* (National Academy Press, Washington, DC).
6. Liu PT, et al. (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–1773.
7. Martineau AR, et al. (2007) IFN-γ- and TNF-independent vitamin D-inducible human suppression of mycobacteria: The role of cathelicidin LL-37. *J Immunol* 178:7190–7198.
8. Wilkinson RJ, et al. (2000) Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: A case-control study. *Lancet* 355:618–621.
9. Martineau AR, et al. (2010) Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. *Eur Respir J* 35:1106–1112.
10. Martineau AR, et al. (2007) A single dose of vitamin D enhances immunity to mycobacteria. *Am J Respir Crit Care Med* 176:208–213.
11. Martineau AR, et al. (2011) High-dose vitamin D_3 during intensive-phase antimicrobial treatment of pulmonary tuberculosis: A double-blind randomised controlled trial. *Lancet* 377:242–250.
12. Hyppönen E, Power C (2007) Hypovitaminosis D in British adults at age 45 y: Nationwide cohort study of dietary and lifestyle predictors. *Am J Clin Nutr* 85:860–868.
13. Douglas AS, Strachan DP, Maxwell JD (1996) Seasonality of tuberculosis: The reverse of other respiratory diseases in the UK. *Thorax* 51:944–946.
14. Wejse C, et al. (2007) Serum 25-hydroxyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. *Am J Clin Nutr* 86:1376–1383.
15. Friis H, et al. (2008) Hypovitaminosis D is common among pulmonary tuberculosis patients in Tanzania but is not explained by the acute phase response. *J Nutr* 138:2474–2480.
16. Tostmann A, et al. (2010) Serum 25-hydroxy-vitamin D3 concentrations increase during tuberculosis treatment in Tanzania. *Int J Tuberc Lung Dis* 14:1147–1152.
17. Banda R, Mhemedi B, Allain TJ (2011) Prevalence of vitamin D deficiency in adult tuberculosis patients at a central hospital in Malawi. *Int J Tuberc Lung Dis* 15:408–410.
18. Ustianowski A, Shaffer R, Collin S, Wilkinson RJ, Davidson RN (2005) Prevalence and associations of vitamin D deficiency in foreign-born persons with tuberculosis in London. *J Infect* 50:432–437.
19. Sita-Lumsden A, Lapthorn G, Swaminathan R, Milburn HJ (2007) Reactivation of tuberculosis and vitamin D deficiency: The contribution of diet and exposure to sunlight. *Thorax* 62:1003–1007.
20. Martineau AR, et al. (2009) Influence of a single oral dose of vitamin D(2) on serum 25-hydroxyvitamin D concentrations in tuberculosis patients. *Int J Tuberc Lung Dis* 13(1):119–125.
21. Yach D (1988) Tuberculosis in the Western Cape health region of South Africa. *Soc Sci Med* 27:683–689.
22. Pettifor JM, et al. (1996) The effect of season and latitude on in vitro vitamin D formation by sunlight in South Africa. *S Afr Med J* 86:1270–1272.
23. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B (2006) Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 84(1):18–28.
24. Liu PT, Stenger S, Tang DH, Modlin RL (2007) Cutting edge: Vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J Immunol* 179:2060–2063.
25. Mehta S, et al. (2010) Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. *PLoS ONE* 5:e8770.

26. Viard JP, et al.; EuroSIDA Study Group (2011) Vitamin D and clinical disease progression in HIV infection: Results from the EuroSIDA study. *AIDS* 25:1305–1315.
27. Skolnik PR, et al. (1991) Enhancement of human immunodeficiency virus 1 replication in monocytes by 1,25-dihydroxycholecalciferol. *Proc Natl Acad Sci USA* 88: 6632–6636.
28. Nevado J, Tenbaum SP, Castillo AI, Sánchez-Pacheco A, Aranda A (2007) Activation of the human immunodeficiency virus type I long terminal repeat by 1 alpha,25-dihydroxyvitamin D3. *J Mol Endocrinol* 38:587–601.
29. Connor RI, Rigby WF (1991) 1 alpha,25-dihydroxyvitamin D3 inhibits productive infection of human monocytes by HIV-1. *Biochem Biophys Res Commun* 176:852–859.
30. Pauza CD, Kornbluth R, Emau P, Richman DD, Deftos LJ (1993) Vitamin D3 compounds regulate human immunodeficiency virus type 1 replication in U937 monoblastoid cells and in monocyte-derived macrophages. *J Leukoc Biol* 53(2):157–164.
31. Campbell GR, Spector SA (2011) Hormonally active vitamin D3 (1alpha,25-dihydroxycholecalciferol) triggers autophagy in human macrophages that inhibits HIV-1 infection. *J Biol Chem* 286:18890–18902.
32. Vanham G, et al. (1996) Generalized immune activation in pulmonary tuberculosis: Co-activation with HIV infection. *Clin Exp Immunol* 103(1):30–34.
33. Haarburger D, Hoffman M, Erasmus RT, Pillay TS (2009) Relationship between vitamin D, calcium and parathyroid hormone in Cape Town. *J Clin Pathol* 62:567–569.
34. Charlton KE, Labadarios D, Lombard CJ, Louw ME (1996) Vitamin D status of older South Africans. *S Afr Med J* 86:1406–1410.
35. Charlton KE, Bourne LT, Steyn K, Laubscher JA (2001) Poor nutritional status in older black South Africans. *Asia Pac J Clin Nutr* 10(1):31–38.
36. Schaaf HS, et al. (1996) A decade of experience with Mycobacterium tuberculosis culture from children: A seasonal influence on incidence of childhood tuberculosis. *Tuber Lung Dis* 77(1):43–46.
37. Hill PC, et al. (2005) ESAT-6/CFP-10 fusion protein and peptides for optimal diagnosis of mycobacterium tuberculosis infection by ex vivo enzyme-linked immunospot assay in the Gambia. *J Clin Microbiol* 43:2070–2074.
38. Martineau AR, et al. (2007) Neutrophil-mediated innate immune resistance to mycobacteria. *J Clin Invest* 117:1988–1994.
39. Maunsell Z, Wright DJ, Rainbow SJ (2005) Routine isotope-dilution liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of the 25-hydroxy metabolites of vitamins D2 and D3. *Clin Chem* 51:1683–1690.